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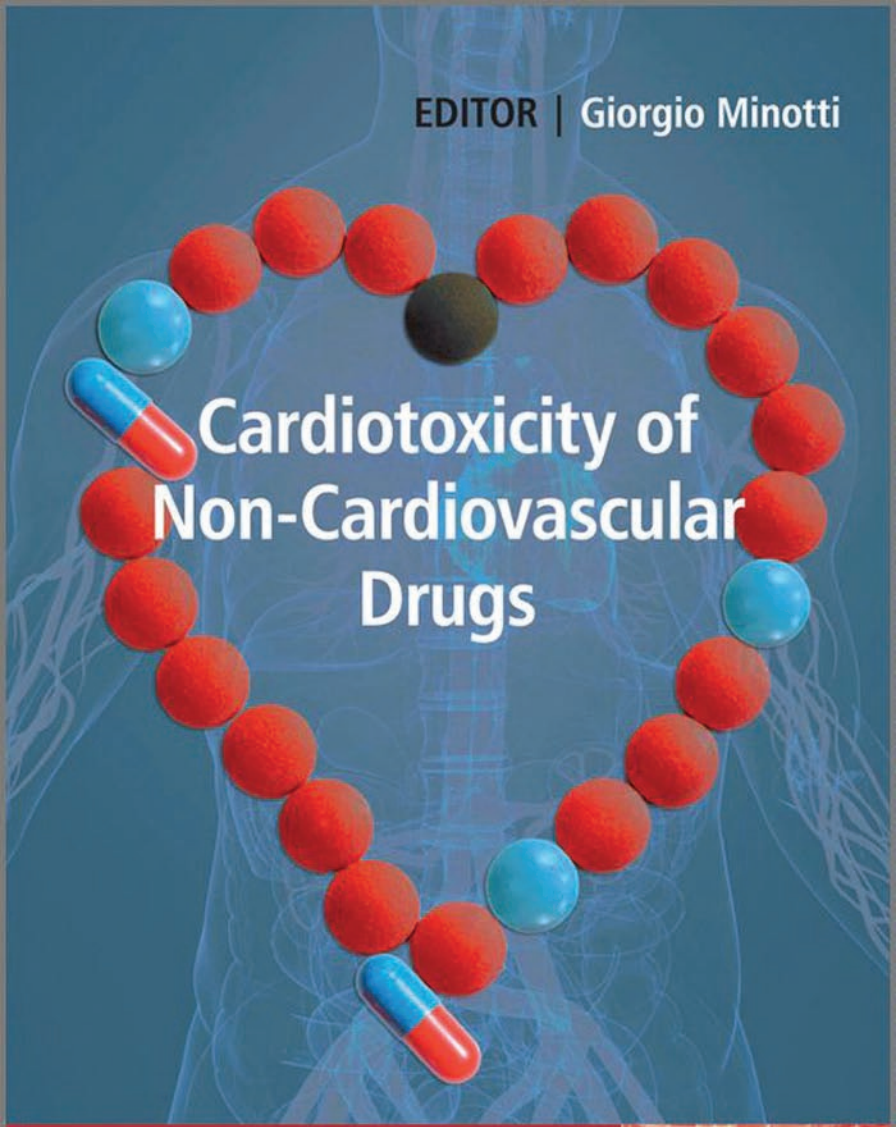
Cardiotoxicity of Non-Cardiovascular Drugs



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Editor

Giorgio Minotti
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To Federica, Anna Martina, and Lorenzo, for supporting my work with understanding and loving tolerance.

Contents

Preface

Some drugs that have been developed and marketed to treat noncardiovascular diseases have been known to induce a number of untoward cardiovascular effects (arrhythmias, blood pressure disorders, cardiomyopathy, thromboembolism, or combinations of one effect with another). There are cases where such effects can be conceptually reconciled with the known mode of action of the drugs at their target tissues; in other cases, however, the causative link between the molecular signature of the drugs and the clinical manifestations of cardiovascular toxicity proves much less obvious. Both on-target and off-target mechanisms contribute to generate cardiovascular toxicities, and such complexity reflects on the lack of guidelines on how the cardiovascular sequelae of noncardiovascular drugs should be prevented or treated.

Further complexity is introduced by uncertainties about the actual prevalence of cardiovascular effects induced by noncardiovascular drugs: some effects are common, but others are felt to be rare, unproven, or suspected at best. The actual incidence of expected or unexpected cardiovascular events also seems to vary from low to high depending on whether cardiovascular safety signals are detected in prospective or retrospective analyses. And finally, there is a concern that the canonical process of drug development might prove unable to anticipate the cardiotoxic potential of new chemical entities. The study of preclinical toxicology may suffer from unavoidable pitfalls in wild type or engineered cells or laboratory animals; moreover, the conspiring effects of polymorphisms, comorbidities, and drug-drug interactions cannot be adequately explored in phase II or III trials as they are designed at the moment. It follows that defining the cardiovascular safety of a given noncardiovascular drug may only become possible when that particular drug is used to treat hundreds of thousands patients, with each patient introducing variables like favourable or unfavourable lifestyle, preexisting cardiovascular or metabolic risk factors, concomitant medications,

and other culprits that we cannot foresee at this time. In cases where a non-cardiac blockbuster drug is withdrawn from the market or subjected to restrictive relabelling because of unexpected or higher than expected cardiotovascular toxicity, we cannot escape the conclusion that its development and approval were flawed by the limited predictiveness of preclinical and clinical trials.

This book provides a collection of contributions on the basic, epidemiologic, and clinical aspects of cardiovascular toxicity induced by some popular classes of noncardiovascular drugs (antihistamines, psychoactive drugs, antibiotics, antineoplastics, nonsteroidal anti-inflammatory drugs, antiretrovirals). Some sections address the concept of cardiovascular liability in regulatory settings and that of mitochondrial dysfunction as a common pathway to cardiotoxicity induced by drugs in general. In moving from one section to another the reader may develop a sense that some information is redundant, or that there is a lack of consistency at other points. This is explained by the different backgrounds of the contributors and the consequent overlap or diversification in their points of view. Whilst possibly detracting from an easy-to-read overview of the problem, such limitations may help the reader to capture the ups and downs of a field that is still moving in the search for firm conclusions and guidelines. Likewise, the book is unavoidably incomplete in its coverage of topics. Reviewing all of the drugs that surfaced in the literature because of unconfirmed warnings or anecdotical reports would have been beyond the aims of the editors: here we wished to call the reader's attention to those preclinical and clinical paradigms that best identified cardiovascular toxicity of noncardiovascular drugs as a standalone discipline.

In the next few years our perception of drug-induced cardiovascular toxicity might easily change because of the approval of safer drugs or refinements of techniques for predicting, managing, or preventing such toxicity. We therefore hope that this book will be received by both experts and non experts in this field as a genuine attempt to define where we are now, and what should be done in the near future in the interest of patients' health and scientific progress.

I personally thank the team at John Wiley & Sons, Ltd for encouraging the editing of this book, and the contributors for their invaluable efforts which made it all possible.

Giorgio Minotti
Editor

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Abbreviations

AIF	apoptosis inducing factor
AKR	aldehyde reductase
APC	adenoma prevention with celecoxib
APPROVE	adenomatous polyp prevention on Vioxx
$[Ca^{2+}]_m$	concentration of intramitochondrial Ca^{2+}
$[Ca^{2+}]_i$	concentration of intracellular Ca^{2+}
CABG	coronary artery bypass grafting
CoASH	unesterified coenzyme A
COX	cyclooxygenase; prostaglandin H synthase
COXIBs	class of COX-2 selective inhibitors
CHF	congestive heart failure
CLASS	celecoxib long-term arthritis safety study
Δp	protonmotive force
$\Delta\psi_m$	mitochondrial membrane potential
DCT	docetaxel
DNR	daunorubicin
DOX	doxorubicin
DOXOL	doxorubicinol
EPI	epirubicin
EPIOL	epirubicinol
ETC	electron transfer chain
GI	gastrointestinal
GSH	reduced glutathione
H_2O_2	hydrogen peroxide
IDA	idarubicin
IMM	inner mitochondrial membrane
IPC	ischemic preconditioning
LVEF	left ventricular ejection fraction
MAO	monoamine oxidase

MEDAL	multinational etoricoxib and diclofenac arthritis long-term
MNNG	*N*-methyl-*N*′-nitro-*N*-nitrosoguanidine
NO	nitric oxide
NSAIDs	non-steroidal anti-inflammatory drugs
OMM	outer mitochondrial membrane
$O_2^{\cdot -}$	superoxide anion
PARP	poly(ADP-ribose) polymerase
PG	prostaglandin
PGE_2, PGD_2, $PGF_{2\alpha}$, PGI_2, and TxA_2	prostaglandin products from PGH_2
PGEM	major urinary metabolite of PGE_2
PGIM	major urinary metabolite of PGI_2
Pi	inorganic phosphate
PK	pharmacokinetics
PreSAP	prevention of colorectal sporadic adenomatous polyps
PTP	mitochondrial permeability transition pore
PTX	paclitaxel
ROS	reactive oxygen species
SHR	spontaneously hypertensive rat
SOD	superoxide dismutase
TKI	tyrosine kinase inhibitor
Tn	troponin
VIGOR	Vioxx gastrointestinal outcomes research
5-LO	5-lipoxygenase

1

Mitochondrial Dysfunction in Cell Injury and Cardiotoxicity

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1.1 INTRODUCTION

Cardiotoxicity represents an adverse side-effect of drugs and a potentially life-threatening response to toxic compounds and pollutants [1]. Due to the abundance of mitochondria in cardiomyocytes and the close relationship linking oxidative metabolism with myocardial function and viability, mitochondrial dysfunction should always be considered as a prime suspect in cardiotoxicity, especially when cardiomyocyte survival is compromised.

Toxicants that affect mitochondrial structure and function have been analyzed in excellent and comprehensive reviews [2–4]. This chapter reviews the general mechanisms causing mitochondrial dysfunction and the wide array of consequences that altered mitochondria generate in the heart. These mechanisms will be exemplified by reviewing mechanisms of toxicity dependent on mitochondrial dysfunction as compared to the profound derangements induced by curtailing oxygen availability.

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1.2 MITOCHONDRIAL RELEVANCE DUE TO CARDIOMYOCTE DEPENDENCE ON OXYGEN SUPPLY

Myocardial function and viability depend strictly on an incessant supply of oxygen that is necessary for the oxidative degradation of substrates within mitochondria. In a complex series of reactions substrate oxidation is coupled with the reduction of coenzymes (i.e., pyridine and flavin nucleotides) that are then reoxidized by a series of elements located within the inner mitochondrial membrane. Through this functional assembly, termed as respiratory or electron transfer chain (ETC), substrate-derived electrons move from reducing coenzymes towards the final acceptor, that is, oxygen. This thermodynamically favorable process releases a conspicuous amount of energy that is immediately coupled to the endoergonic movement of protons against a concentration and an electrical gradient across the inner mitochondrial membrane (IMM). Therefore, the energy derived from the nutrients is stored by creating a proton electrochemical potential (negative inside) that is commonly termed a protonmotive force (Δp). The utilization of this force, composed of membrane potential ($\Delta\psi_m$) and pH gradient (ΔpH_m) components, directly drives energy-requiring reactions in mitochondria and, indirectly, every endoergonic process in cells. Among mitochondrial reactions using Δp a crucial role is played by ADP phosphorylation to ATP at the level of F_1F_o ATP synthase. On this basis the entire process of ATP synthesis should be referred to as energy conservation or conversion rather than the more common, yet incorrect, energy production.

To further emphasize the tight relationship between energy-dependent reactions and oxygen, it is worth pointing out that in the heart more that 90% of the intracellular O_2 is utilized by a single reaction (i.e., its reduction to water) catalyzed by the terminal portion of the ETC, namely cytochrome oxidase.

1.3 MYOCARDIAL ISCHEMIA EXEMPLIFIES MITOCHONDRIAL RELEVANCE IN CARDIOMYOCYTE INJURY

Ischemia initiates as an extracellular event that becomes a major biological and clinical problem because of intracellular alterations. In the heart, ischemia results from coronary artery occlusion. This dramatic

event curtails the supply of nutrients and oxygen to cardiomyocytes. The lack of exogenous substrates does not pose an immediate threat to cell viability since, though to a limited extent, glucose and fatty acids are made available by utilizing intracellular stores of glycogen and triacylglycerols. Conversely, the lack of oxygen represents the immediate and most relevant cause of cell injury that is set in motion by mitochondrial dysfunction. In fact, a given cell becomes anoxic or ischemic when the amount of oxygen is less than that required to fuel the activity of cytochrome oxidase. Therefore, the very first consequence of a critical reduction in cellular oxygenation is ETC inhibition, which inevitably impairs both energy conservation and oxidative metabolism. The following sections summarize the major consequences of ischemia-induced ETC inhibition. As detailed below, primary and direct consequences of the lack of oxygen, such as inhibition of oxidative phosphorylation and impairment of oxidative metabolism, result in secondary, or indirect, changes, among which the most relevant appears to be the generation of a vicious circle linking alterations in intracellular Ca^{2+} homeostasis with oxidative stress. Finally, all of the above changes create ideal conditions for promoting the opening of the mitochondrial permeability transition pore (PTP) that can be considered as the final effector of any significant perturbation of mitochondrial function. In the field of myocardial ischemia a general consensus exists that PTP opening is the mitochondrial executioner in cell death. The same concept appears to be valid for a wide variety of cell types and pathological conditions [5]. Notably, as discussed in the following sections and illustrated in Figure 1.1, many, if not all, of the mitochondrial derangements caused by ischemia are also part of the toxic effects elicited by various xenobiotics.

1.4 MAJOR CONSEQUENCES OF ISCHEMIA-INDUCED ETC INHIBITION

1.4.1 Direct Consequences

1.4.1.1 Impairment of Energy Metabolism

By inhibiting electron flow the lack of oxygen prevents the maintenance of the electrochemical gradient across the IMM, so that the driving force for ATP synthesis at the level of F_1F_o ATP synthase is no longer available. Notably, however, not only is ATP synthesis hampered, but ATP hydrolysis

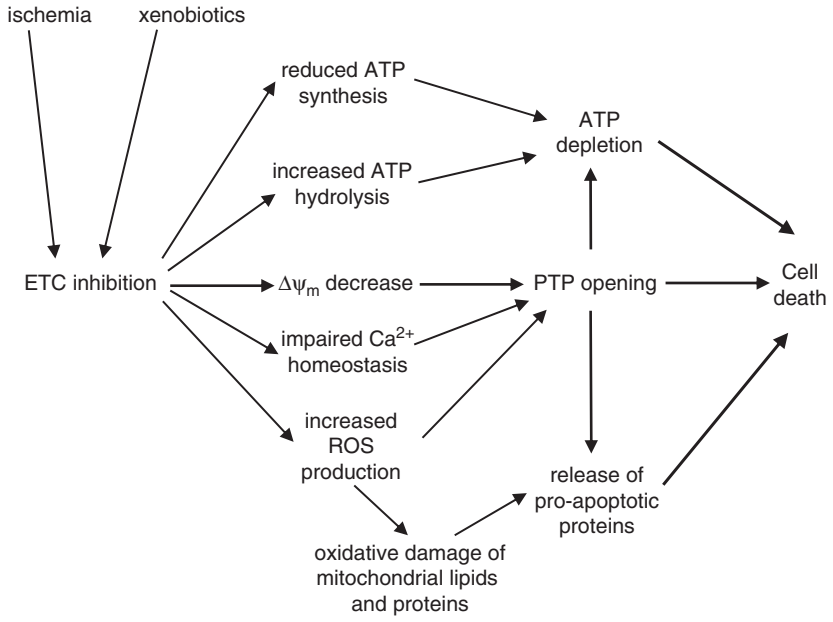

Figure 1.1 Mechanisms linking ETC inhbition induced by ischemia or toxicants with cell death

is also stimulated, resulting in its rapid depletion. In fact, F_1F_o ATP synthase catalyzes a reversible process of coupling between proton movements across the inner mitochondrial membrane and ATP synthesis or hydrolysis. If the proton gradient is not generated by the respiratory chain, F_1F_o ATP synthase couples ATP hydrolysis with proton pumping to maintain the mitochondrial membrane potential. This process explains why this enzyme is also termed ATPase, and this reverse operation resembles the activity of other cellular ATPases that convert ATP hydrolysis into active transport of ions against concentration and/or electrical gradients. The net result is that mitochondria cease to represent the main source of intracellular ATP and become a powerful system for hydrolyzing glycolytically-produced ATP [6–9]. This concept explains why the inhibition of ATP hydrolysis is considered a major component of myocardial protection elicited by both self-defense mechanisms and pharmacological treatments [9].

1.4.1.2 Impairment of Oxidative Metabolism

Oxidative metabolism relies on the immediate reoxidation of coenzymes reduced by means of substrate oxidation. Since the main pathway for

$NADH(H^+)$ and $FADH_2$ reoxidation is the respiratory chain, its inhibition hampers the oxidation of every substrate. In the absence of oxygen, $NADH(H^+)$ oxidation is made possible mostly by lactate dehydrogenase. This allows cytosolic (anaerobic) glycolysis that is practically the only pathway for ATP (re)synthesis under anoxic conditions, especially considering that phosphocreatine is rapidly depleted at the onset of ischemia [10,11]. However, due to the absence of washout, lactic acid accumulates intracellularly, contributing to the large drop in pH occurring in ischemic hearts. On the other hand, the lack of adequate coenzyme reoxidation hampers the arrest of fatty acid oxidation, resulting in the accumulation of potentially toxic intermediates, such as long chain acyl-CoAs and long chain acylcarnitines [12–14]. In addition, the formation of these metabolites exacerbates metabolic impairments by reducing the availability of unesterified coenzyme A (CoASH) and carnitine. As a general rule that holds valid in the context of cardiotoxicity, any ETC inhibition (i.e., even in the presence of oxygen) inevitably results in a severe decrease, if not arrest, of fatty acid oxidation along with the stimulation of anaerobic glycolysis and lactate formation. When these changes occur in large organs (the heart is less than 5% of the total body mass) the massive stimulation of glycolysis results in lactic acidosis.

1.4.2 Secondary Consequences

1.4.2.1 Alterations in Intramitochondrial and Intracellular $[Ca^{2+}]$

Since both mitochondrial Ca^{2+} uptake and release depend on Δp[15,16], respiratory chain inhibition results in profound alterations of mitochondrial Ca^{2+} homeostasis that can trigger and be further exacerbated by opening of the mitochondrial permeability transition pore (PTP) [5,16–19]. It is generally accepted that, during ischemia and/or reperfusion, mitochondrial $[Ca^{2+}]$ ($[Ca^{2+}]_m$) increases, yet it must be pointed out that quite different mechanisms underlie $[Ca^{2+}]_m$ rise or overload. In fact, during ischemia Δp is abolished because of respiratory chain inhibition and $[Ca^{2+}]_m$ may passively follow the occurrence of elevations of cytosolic Ca^{2+}. On the other hand, upon reperfusion, Δp is recovered together with the capacity of mitochondria to take up huge amounts of Ca^{2+}. Therefore, only under the latter condition can active accumulation of Ca^{2+} take place within the matrix. In any case, $[Ca^{2+}]_m$ elevation is the consequence of a preceding rise in

cytosolic [Ca^{2+}] ([Ca^{2+}]$_c$). Therefore, the abrupt release of Ca^{2+} from an overloaded matrix is not likely to determine *per se* intracellular Ca^{2+} overload. The lack of adequate pharmacological or genetic tools to inhibit mitochondrial Ca^{2+} uptake *in situ* has so far prevented a clear assessment of its role in myocardial physiology and pathology. Nevertheless, strategies aimed at decreasing cytosolic Ca^{2+} overload are likely to prevent or delay alterations in mitochondrial structure and function.

1.4.2.2 Mitochondria-Derived Oxidative Stress

Reactive oxygen species (ROS) are formed within mitochondria under physiological and pathological conditions, especially during post-ischemic reperfusion [20–22]. The common estimate that 2–4% of oxygen utilized by the respiratory chain undergoes univalent reduction becoming superoxide anion ($O_2^{\bullet -}$) is probably too high, and a correct estimate might be one order of magnitude lower [23,24].

ROS formation and toxicity is counterbalanced by a complex defense system. The most efficacious strategy is the enzymatic removal of ROS catalyzed by superoxide dismutases (SODs) and peroxidases [25,26]. Through SOD reaction $O_2^{\bullet -}$ is transformed into H_2O_2 that is then reduced to water by peroxidases. Reduction of oxidized molecules can also be catalyzed by thioredoxin and peroxyredoxin [27]. The enzymatic defenses are paralleled by non-enzymatic mechanisms that rely upon antioxidants, such as vitamins A, E and C, ubiquinone, urate, lipoic acid and glutathione [28].

The mitochondrial formation of ROS might be modulated by $NO^{\bullet}$ [29,30] as a consequence of the inhibition of cytochrome oxidase [31–35]. This reversible process can be transformed into irreversible alterations of the respiratory chain when $NO^{\bullet}$ formation is sustained. Indeed, $NO^{\bullet}$ reacting with $O_2^{\bullet -}$ generates peroxynitrite, which can produce the irreversible nitration of proteins [36].

The imbalance between formation and removal of ROS is termed oxidative stress and plays a major role in every cardiac disease. In this respect, major attention has been focused on the relationship between oxidative stress and ischemia/reperfusion injury [28,37–39]. Nevertheless, an increased formation of ROS is suggested to be involved in heart failure [28,40–42] as well as in atherosclerosis [43–47].

Besides altering every cell component, oxidative stress increases the occurrence of cell death. To this end, a relevant consequence of ROS accumulation is the increased susceptibility to PTP opening, as discussed below. On the other hand, although major emphasis has been put on the

pathological consequences resulting from ROS-induced derangements of every macromolecule, ROS, especially H_2O_2, also contribute to several physiological processes [28] by modulating signaling pathways [48,49].

ETC inhibition favors ROS formation, lending support to the concept that respiratory complexes, especially Complex I, are the main intracellular sites for ROS formation [35,50]. However, mitochondrial sites other than the inner mitochondrial membrane are capable of generating H_2O_2 at significant rates [51]. A relevant role in this respect is likely played by monoamine oxidases (MAOs). These outer membrane flavoproteins catalyze electron transfer from various amine compounds (including catecholamines) to O_2, thus producing large amounts of H_2O_2 [52,53]. MAO inhibition has been shown to afford a significant degree of protection against myocardial injury caused by ischemia and reperfusion [54,55].

p66Shc, which catalyzes electron transfer from cytochrome c to oxygen, also contributes to mitochondrial H_2O_2 formation [56]. Experiments performed on $p66^{Shc-/-}$ mice, and on redox-defective mutants of $p66^{Shc}$, demonstrated that this protein, which localizes in part within mitochondria, is required for inducing the elevation of intracellular oxidants, cytochrome c release and apoptosis [56–58]. On the other hand, the genetic deletion of this protein has been shown to protect against oxidative stress generated by several experimental protocols, including myocardial ischemia and reperfusion [48,55].

Mitochondria-induced oxidative stress and the increase in mitochondrial and intracellular $[Ca^{2+}]$ favor each other in a vicious relationship that amplifies damaging processes [59] (Figure 1.2). An increased availability of Ca^{2+} within mitochondria is likely to increase ROS accumulation by means of the following processes: (i) increased delivery of electrons to the ETC (feed forward) by activating several matrix dehydrogenases [60]; (ii) reduced removal of H_2O_2 resulting from a decrease in mitochondrial content of reduced glutathione (GSH) due to the inhibition of glutathione reductase [59]; (iii) direct ETC inhibition at the level of complex I [61]; (iv) increased formation of $NO^{\bullet}$ resulting in a decreased activity of cytochrome oxidase [62]; (v) binding to cardiolipin that might cause cytochrome c detachment from the IMM, thus hampering electron flow between Complex II and IV [63]; (vi) PTP opening [5]. The latter two processes are likely to create a mitochondrial vicious circle, since the increased generation of ROS will exacerbate ETC inhibition, resulting in an increased probability of PTP opening. This vicious circle might also be extended to the rest of the cell through the interaction between cytochrome c and IP_3 receptors of the endo/sarcoplasmic reticulum, causing a further rise in intracellular $[Ca^{2+}]$ (Figure 1.3).

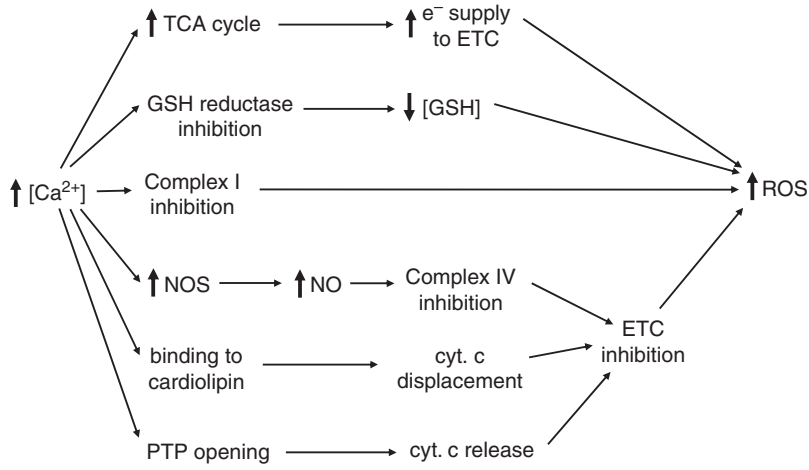

Figure 1.2 Relationships between $[Ca^{2+}]_i$ increase and increased ROS formation

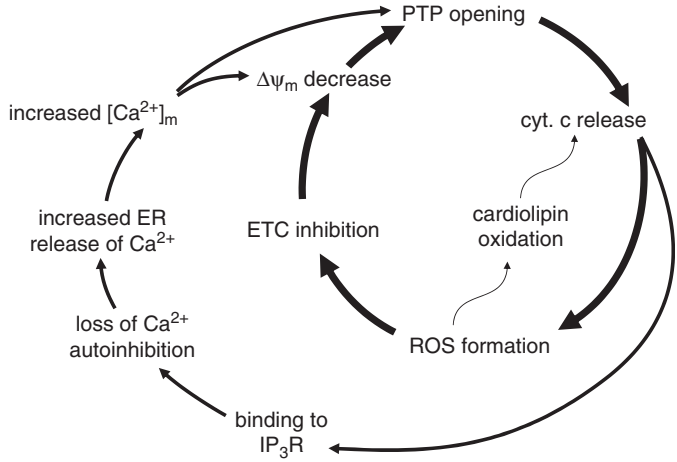

Figure 1.3 Amplification loops linking $[Ca^{2+}]_m$ with ROS formation and PTP opening

1.4.3 PTP Opening as the Final Effector of Mitochondrial Dysfunction

A central role in cell death is attributed to PTP opening as related to a wide range of pathologies extending far beyond myocardial injury ([5] and references therein). The permeability transition describes a sudden

increase in the inner membrane permeability to solutes with molecular weights up to 1500 Da caused by the opening of a voltage-dependent, high-conductance channel located in the inner mitochondrial membrane, defined as the permeability transition pore (PTP) (reviewed in [15,16,64,65]). PTP opening can be induced by all the factors analyzed above, that is, mitochondrial de-energization, $[Ca^{2+}]_m$ increase, and oxidative stress, and represents a major cause of mitochondrial dysfunction, creating conditions hardly compatible with cell survival. In fact, especially in the case of prolonged events [66] PTP opening causes (i) collapse of $\Delta\psi_m$, (ii) depletion of ATP and NAD^+, (iii) oxidative stress, (iv) matrix swelling that may cause rupture of the outer membrane followed by release of proteins of the intermembrane space, such as cytochrome *c* [18,19].

PTP opening is favored by $[Ca^{2+}]_m$ elevation, depolarization, increases in ROS and inorganic phosphate (Pi) [67]. These factors are counteracted by physiological PTP antagonists, such as elevated values of $\Delta\psi_m$ and high concentrations of H^+, Mg^{2+} and adenine nucleotides, especially ADP [16,18]. This explains the evidence obtained in many laboratories that PTP opening occurs during post-ischemic reperfusion. In fact, during ischemia, intracellular acidosis together with high levels of Mg^{2+} and ADP overrides the PTP promoting conditions established by $\Delta\psi_m$ decrease and increases in Ca^{2+} and Pi levels. Conversely, upon reperfusion the recovery of intracellular pH together with a burst in ROS formation in the presence of high matrix concentrations of Ca^{2+} and Pi create the perfect scenario for PTP opening, despite the antagonizing effect of $\Delta\psi_m$ recovery.

In isolated mitochondria PTP opening is usually obtained by additions of Ca^{2+} exceeding 0.1 mM. Such high Ca^{2+} concentrations are hardly achieved in viable cells, suggesting that Ca^{2+} does not cause PTP opening directly, and that PTP is sensitized to Ca^{2+} by several processes, such as formation of ROS or metabolites generated by Ca^{2+}-dependent enzymes. For instance, a recent report suggests that PTP onset is due to a deadly mixture of ROS generation and pH normalization, whereas intracellular Ca^{2+} overload would be the consequence and not the cause of bioenergetic failure [68]. On the other hand, the activation of Ca^{2+}-dependent enzymes could signal the occurrence of cell damage to mitochondria by converting and amplifying initial Ca^{2+}-related signals into Ca^{2+}-dependent derangements. Among the possible signaling pathways we have characterized the activation of cytosolic phospholipase A_2 resulting in the formation of arachidonic acid which induces apoptosis by promoting PTP opening [69]. Several reports indicate the relevance of calpains. The

relationship between these Ca^{2+}-dependent proteinases and cell death involves multiple processes and cellular sites [63,70]. The involvement of mitochondria is suggested by evidence of intramitochondrial localization, especially of the μ isoform [71], and by the reports that calpains may be involved in PTP opening [72], mitochondrial release of the apoptosis inducing factor (AIF) [73] and cleavage of Bid, a proapoptotic member of Bcl-2 family [74].

1.5 MITOCHONDRIA-CENTERED MECHANISMS OF CELL INJURY

1.5.1 ETC Inhibition

The information provided by ischemia injury clearly indicates that ETC inhibition is followed by profound derangements that jeopardize the viability of any cell and, especially, of cardiomyocytes. Besides anoxia or ischemia, ETC can be inhibited by a vast number of compounds [2,3]. Three major possibilities can be envisaged:

1. Direct inhibitors with no other site of action. This is the case for most of those compounds that have been instrumental in elucidating ETC organization and function. In fact, besides other approaches, electron flux has been investigated by analyzing redox spectra of ETC components (such as flavins, ubichinone and cytochromes) or assessing O_2 consumption upon addition of compounds that inhibit electron transport at specific sites. Paramount examples of these compounds are rotenone, antimycin and cyanide that inhibit electron transport at the level of complex I, III and IV, respectively. Complex I appears to be the most sensible target, being affected practically by any molecule bearing an aromatic head and a hydrophobic tail [75]. Complex I inhibition has been suggested to be involved in Parkinson's disease [76,77], and complex II inhibition has been linked to Huntington's disease [78]. However, to the best of our knowledge cardiotoxicity has not been linked to compounds acting only as ETC inhibitors.
2. Direct inhibitors acting also at other sites and/or eliciting additional mitochondrial modifications. Several compounds add ETC inhibition with other alterations of energy-linked functions. From a theoretical

standpoint it must be pointed out that the decrease in $\Delta\psi_m$ induced by ETC inhibition favors PTP opening [5]. On the other hand, this latter process causes mitochondrial NAD^+ release and depletion [79], eventually resulting in ETC inhibition [80]. However, in some cases the close relation between respiration and permeability transition can be addressed by identifying direct effects on the PTP without concomitant alterations of respiratory complexes [81,82]. For instance, adenine dinucleotides generated from NAD^+ were shown to affect mitochondrial function by causing PTP opening with complex I inhibition at higher concentrations [81]. While these effects have not yet been documented in cardiomyocytes, a similar recruitment of mitochondrial derangements has been clearly demonstrated in myocytes treated with the local anesthetic bupivacaine [82]. Although multiple mechanisms of cytotoxicity have been attributed to bupivacaine, mitochondria appear especially relevant in myotoxicity since bupivacaine toxicity affected only highly oxidative fibers (i.e., soleus) and was prevented by inhibiting PTP opening [82]. Based on the strict myocardial dependence on oxidative metabolism, it is tempting to speculate that mitochondrial dysfunction induced by PTP opening might also play a crucial role in cardiac toxicity associated with bupivacaine administration [83].

3. ETC inhibition as an indirect consequence of primary effects at other cellular sites. In this respect among countless possibilities oxidative stress and PTP opening are by far the most relevant processes. Complex I and cardiolipin are especially susceptible to ROS generated at any cellular site [84,85]. In addition, as already mentioned, ROS favors PTP opening that can then affect the ETC by causing NAD^+ depletion and cytochrome c release. On the other hand an increased susceptibility to PTP opening can result from an elevation in $[Ca^{2+}]_i$, generation of metabolites, such as arachidonic acid [69], or stimulation of Ca^{2+}-dependent enzymes, such as calpain.

It is worth pointing out that, especially *in vitro*, non-specific ETC inhibition might be elicited by administering high doses of lipophilic molecules that disturb mitochondrial function and structure by binding to or inserting into the IMM.

When the final effect of a given substance is cell death, ETC inhibition and/or mitochondrial dysfunction should always be considered. This might not be immediately evident when studies are performed in intact cells. Since oxygen consumption can hardly be measured in cells attached to plastic or glass supports, mitochondrial function is commonly assessed by

monitoring $\Delta\psi_m$ with fluorescent probes. Besides technical issues related to this technique [86], in cells, including cardiomyocytes, that undergo ETC inhibition, $\Delta\psi_m$ can be maintained by F_1F_o ATP synthase [7]. The latent defect in ETC function can be unmasked by using oligomycin. In fact, this prototype inhibitor of F_1F_o ATP synthase will produce an immediate collapse of $\Delta\psi_m$ by curtailing the possibility of using glycolytically-produced ATP.

1.5.2 Uncoupling of Oxidative Phosphorylation

Under physiological conditions the largest fraction of oxygen consumption is coupled to ATP synthesis. In fact, the proton pumping activity of respiratory complexes determines the driving force (i.e., Δp) that is used not only for phosphorylating ADP to ATP, but also for exporting ATP into the cytosol by means of adenine nucleotide translocase. This vital process of energy conversion requires an absolute lack of IMM permeability to protons that must gain access to the matrix only at the level of F_1F_o ATP synthase. Oxidative phosphorylation becomes uncoupled when IMM permeability to protons is increased. While the favorable movement of protons through F_1F_o ATP synthase is converted into ATP synthesis, the re-entry of protons into the matrix through a leaky IMM cannot be utilized in useful reactions and is dissipated as heat. At least in part, this is a useful and necessary process that greatly contributes to maintaining our body temperature at 37 °C. In fact, mitochondria also contain uncoupling proteins that regulate the degree of uncoupling in the various organs, being maximal in brown adipose tissue.

IMM permeability to protons is increased by a wide variety of substances that are termed protonophores or uncouplers [2]. In most cases, the uncoupling effect is achieved by means of the proton shuttling ability of lipophilic weak acids that associate protons within the more acidic intermembrane space and, after crossing the IMM, release protons into the more alkaline matrix space. Proton re-entry inevitably results in a decrease in the proton electrochemical potential that is re-established by ETC proton pumping. Therefore, in contrast to ETC inhibition that determines a more or less profound decrease in O_2 consumption, uncouplers cause an increase in respiration while, in both cases, ATP synthesis is hampered and its hydrolysis stimulated. ROS formation is also affected differently by uncoupling and ETC inhibition. While the latter alteration generally promotes ROS generation, the increased flow of electrons induced by uncouplers reduces

oxidative stress. Therefore, especially under mild uncoupling conditions, the threat related to the reduced ATP synthesis might be counterbalanced by the potential benefit stemming from a decrease in ROS formation. This might explain why uncoupling is hardly listed among mechanisms underlying cardiotoxicity [1,3,4].

1.5.3 Alternate Redox Reactions

As described above, in going from reduced coenzymes (i.e., $NADH(H^+)$ and $FADH_2$) toward oxygen, electrons move along the ETC according to the redox potential of the various respiratory complexes. This orthodox pathway can be replaced, at least in part, by alternate routes created by compounds that divert electrons from the ETC for their own reduction. This process usually generates radical intermediates that are then reoxidized by either univalent reduction of oxygen (i.e., generating $O_2^{\bullet -}$) or by returning electrons to the ETC. The former possibility is more dangerous, since the ensuing oxidative stress is associated not only with useless consumption of oxygen and nutrients, but also with $\Delta\psi_m$ decrease. This combination of factors prompts a sequence of events that might undermine cell survival. In fact, oxidative stress alters respiratory complexes, especially complex I, further exacerbating mitochondrial de-energization that is then likely to be disrupted by PTP opening as a result of ROS accumulation and critical $\Delta\psi_m$ decrease. This dramatic scenario underlies cellular injury caused by several substances, termed alternate electron acceptors, among which doxorubicin is by far the most prominent example and the most investigated substance in the field of cardiotoxicity [87–89]. Multiple targets and mechanisms have been proposed to explain doxorubicin-induced cardiotoxicity and these are thoroughly analyzed in other chapters of this book. Nevertheless, redox cycling [90,91] and mitochondrial dysfunction [92–95] appear pivotal also because of cardiolipin accumulation in these organelles [96], favored by their binding to cardiolipin [97]. In addition, PTP involvement, that was originally proposed based upon pharmacological approaches [92], has recently been supported by an elegant genetic study using mice devoid of cyclophilin D (CyP-D) [98]. The absence of this mitochondrial protein, that is targeted by PTP inhibitors, decreases the susceptibility to PTP opening [99,100]. Cyp-$D^{-/-}$ mice have been reported to display a significant resistance against doxorubicin-induced myocardial failure as compared to wild type littermates [98].

1.5.4 Inhibition of Metabolic Pathways

The variety of changes altering energy-linked functions of the IMM is far smaller than the multiplicity of pathological consequences caused by inhibiting metabolic pathways involved in the oxidative degradation of nutrients, especially glucose and fatty acids. Besides specific alterations produced by any enzyme inhibition, the analysis of common findings further highlights the relationship between myocardial metabolism and function. In fact, the majority of inborn errors of mitochondrial metabolism result in severe cardiomyopathies [101–103]. Generally, myocardial hypertrophy evolves into maladaptive remodeling, leading to myocardial dilation and contractile failure. The mechanisms linking mitochondrial dysfunction with myocardial hypertrophy and failure are far from being conclusively elucidated, yet oxidative stress is likely to provide a relevant contribution [22,104].

Regarding the action of toxicants and drugs, besides the effects of compounds such as fluorocitrate and malonate that were exploited to elucidate Krebs' cycle, undesired inhibitory effects afforded by pharmaceutical compounds have been described for fatty acid and pyruvate oxidation [105–108]. Rather than affecting specifically single enzymes, toxicity is seldom produced by generating nonphysiological acyl esters of coenzyme A. In order to re-establish an optimal acyl-CoA/free CoA ratio that is required for the activity of key metabolic steps [109,110], the undesired acyl moieties are esterified to carnitine, releasing free CoA (CoASH) through the action of carnitine acyltransferases [110]. Unfortunately, this scavenging process might result in a critical decrease in carnitine availability. Such a condition, termed secondary carnitine deficiency or carnitine insufficiency, affects skeletal and cardiac muscles. This concept is exemplified by the toxic effects elicited by pivalate and valproate [105–108] that, by generating pivaloyl- and valproyl-carnitine, reduce the intracellular content of free carnitine.

The maintenance of an optimal acyl-CoA/CoASH ratio indicates a general role of carnitine in both energy metabolism and the maintenance of cell viability. This cytoprotective role is likely to be contributed to by inhibitory effects on ceramide synthesis [111] and caspase activities [112]. In cardiac myocytes exposed to doxorubicin, carnitine administration reduced the degree of apoptotic death by preventing the increase in intracellular levels of ceramide, a powerful endogenous promoter of apoptosis [111]. The inhibition of ceramide production is the result of two different actions of carnitine. In fact, the subtraction of palmitoyl CoA, which is diverted from

ceramide synthesis to oxidative metabolism [113], is reinforced by the inhibitory effect on acid sphyngomyelinase, which generates ceramide in response to a host of apoptotic stimuli [111]. In addition, carnitine has been shown to inhibit the activity of caspases 3 and 8 [112], which act as initiator and executioner of apoptosis, respectively. Since the action of carnitine is reversed by palmitoylcarnitine, the free/esterified carnitine ratio has been suggested to play a relevant role in the cell commitment to apoptosis [112].

1.6 CONCLUDING REMARKS

This article is not meant to convey the concept or the impression that mitochondrial dysfunction is required for, or involved in, any mechanism of cell injury or cardiotoxicity. Effects at the plasma membrane disturbing ionic homeostasis and signaling pathways and/or alterations of transcription and translation due to changes in nucleic acids are likely to be crucial derangements in many pathological states. Nevertheless, cell death can hardly be independent of mitochondrial dysfunction, and energy-linked functions of the IMM should always be carefully investigated when loss of cell viability and/or myocardial hypertrophy and failure ensue as unexpected side-effects of a given compound.

Examples of unsuspected or overlooked mitochondrial contribution to pathological conditions are not rare. For instance, a suicide hypothesis has been proposed whereby DNA damage causes cell death by means of NAD^+ and ATP depletion due to an uncontrolled repair activity of poly(ADP-ribose) polymerase (PARP) within the nucleus. Unfortunately, without any known exception, compounds and procedures used for altering DNA, such as UVA irradiation, hypoxia and oxidants, are powerful PTP agonists. This is also the case with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG), the reference compound used for causing PARP activation. In fact, this DNA-intercalating agent causes PTP opening in isolated mitochondria, and cell death that is prevented by PTP inhibition [114]. In addition, and in any case, intracellular NAD^+ cannot be depleted because of PARP activation unless PTP opening occurs, since 80% of cellular NAD^+ is compartmentalized within the mitochondrial matrix [80] (Figure 1.4).

Finally, rephrasing the catchy title of a recent review [115], sometimes bad things become good. In fact, paradoxically, strong cardioprotection can be afforded by mild mitochondrial dysfunction. This concept is

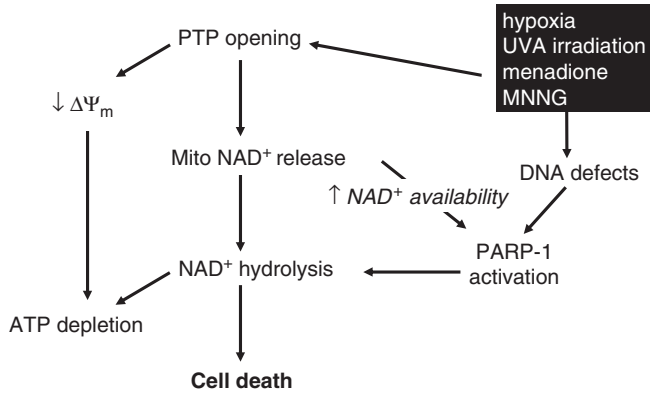

Figure 1.4 Relevance of mitochondrial dysfunction due to PTP opening in cell death following treatment with agents causing DNA damage and PARP activation

strikingly exemplified by ischemic preconditioning (IPC) [116], whereby short episodes of ischemia protect from the otherwise lethal injury caused by a subsequent prolonged period of ischemia. Although the mechanisms underlying this endogenous defense are not elucidated yet, it is generally accepted that a mild oxidative stress caused by mitochondrial dysfunction during the initial ischemic episodes prevents the occurrence of massive ROS formation upon reperfusion after the prolonged ischemic period [117,118]. In this respect, evidence has been provided that, at concentrations of $H_2O_2 \leq 1$ μM, the susceptibility to PTP opening is decreased, in contrast to the increased susceptibility induced by high H_2O_2 concentrations (≥ 0.1 mM) [119].

IPC protection is mimicked by various procedures and substances in phenomena sharing the common definition of pharmacological preconditioning [116]. Interestingly, the rather long list of compounds with preconditioning effects includes volatile anesthetic agents, such as halothane, isoflurane, and sevoflurane [120,121]. Their preconditioning-like action has been attributed to a mild mitochondrial dysfunction involving a slight oxidative stress, as in the case of the classical IPC. Based upon these results, that appear in contrast with bupivacaine-induced myotoxicity, it is possible that subtle changes in intracellular conditions might change toxic substances into protective agents. The validation of this hypothesis requires a better understanding of the mitochondrial role in cell injury and protection. Elucidating this role might help change undesired toxic effects in novel or ameliorated therapeutic interventions.

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2

Cardiovascular Liabilities of Drugs: Regulatory Aspects

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2.1 INTRODUCTION

Over recent decades, the variety and range of available pharmaceutical agents has grown exponentially, and pharmaceutical treatments are now available for almost all diseases. One unavoidable consequence, and undesirable effect, is the increase in drug-induced adverse reactions, resulting very often in illnesses including cardiovascular diseases. In a retrospective study on the causes of drug failure, Schuster *et al.* [1] found that, of 16 drugs withdrawn from the market from 1992 to 2002, seven (>43%) were withdrawn because of cardiovascular adverse reactions.

Cardiovascular diseases are the number one cause of death globally. In 2005, an estimated 17.5 million people worldwide died from cardiovascular disease, representing 30% of all global deaths (http://www.who.int/cardiovascular_diseases/en/). Given this high incidence of cardiovascular disease and the prevalence of recognized cardiovascular risk factors in the population, it is sometimes difficult to clearly ascribe an individual patient's cardiovascular condition to the use (or abuse) of a particular drug. It is not surprising, therefore, that Regulatory Authorities worldwide have paid particular attention to the compilation of specific

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guidelines dealing with the conduct of pre-clinical studies aimed at identifying potential cardiovascular liabilities of new chemical entities before they enter clinical testing. The scope of the guidelines is to protect participants in clinical trials, and patients receiving marketed products, from potential adverse effects of pharmaceuticals.

Drugs can cause cardiovascular disease by a variety of mechanisms: from direct myocyte injury to the alteration of biochemical processes, to the induction of allergic reactions [2]. The results of these mechanisms encompass changes in blood pressure (both hypertension and hypotension), depolarization/repolarization duration (shortening or lengthening, which can trigger ventricular arrhythmias), left ventricular systolic dysfunction, congestive heart failure, myocardial infarction, stroke. These cardiovascular adverse effects can broadly be divided into two categories: pharmacological or toxicological [3]. Pharmacological side-effects are normally caused by interference of the drug with hemodynamic and/or electrical activity of the heart. These are physiological processes mediated by receptors and ion channels and are subject to neural regulation through neuromediators. They usually appear already after the first administration of the drug, are characterized by a clear dose-response and their assessment is based on pharmacodynamic endpoints. Toxicological adverse events involve changes in the function of the heart through a direct cytotoxic effect on myocytes or as a consequence of prolonged pharmacological activity (i.e., hypertension sustained in time, which can trigger left ventricular hypertrophy, left ventricular systolic dysfunction and, ultimately, heart failure). Their appearance usually requires some time (from weeks to months or years) and their assessment is based on histopathological endpoints as normally used in traditional toxicity studies. This distinction was already emphasized by Zbinden in 1984 [4]. Table 2.1 reports some examples of cardiovascular adverse events elicited by drugs.

Table 2.1 Examples of cardiac adverse events elicited by drugs

Cardiovascular event	Drug	Reference
Hypertension	erythropoietin, ciclosporin, corticosteroids, NSAIDs, COX-2 inhibitors, kinase inhibitors	[2] [5,6]
Delayed ventricular repolarization	cisapride, terfenadine, astemizole	[7–9]
Heart failure	anthracyclines, antidiabetic drugs (glitazones)	[10,13]
Myocardial infarction, stroke	COX-2 inhibitors	[11,12]

Examples of pharmacological side-effects include hypertension and delayed ventricular repolarization. Hypertension is an uncommon side-effect of drugs, but some medications are recognized to induce it: among these are erythropoietin, ciclosporin, corticosteroids, NSAIDs, COX-2 inhibitors [2] and, most recently, kinase inhibitors developed for the treatment of cancer [5,6]. Delayed ventricular repolarization is a more common side-effect of drugs, and some have been removed from the market for this (cisapride, terfenadine, astemizole, among others) [7–9]. The best known example of a toxicological adverse event is the congestive heart failure induced by anthracyclines, which was first reported in 1967 [10]. Most recently, COX-2 inhibitors have been demonstrated to increase the risk of myocardial (MI) infarction and stroke [11,12], and were therefore withdrawn from the market. An adverse cardiovascular effect (heart failure) has also been demonstrated for the new class of antidiabetic drugs, the glitazones [13].

Preclinical investigations on possible cardiovascular liabilities of new chemical entities follow this distinction between pharmacological and toxicological adverse effects. The former are investigated by means of *ad hoc* safety pharmacology studies, while the latter are normally evaluated by adding measurements and observations to the existing pivotal toxicology studies [14]. International guidelines also follow this distinction; safety pharmacology studies are the subject of specific regulatory documents (ICH S7A and S7B for preclinical studies and ICH E14 for clinical trials) [15–17], while no specific guideline has been published for toxicological end points of cardiac investigations, but recommendations are given in particular guidance documents (for example, a draft Guidance for Industry published in 2008 by the FDA regarding the development of PPAR agonists in the treatment of diabetes mellitus) [18]. We will follow the same distinction here, addressing regulatory aspects of safety pharmacology and toxicology studies separately.

2.2 REGULATORY ASPECTS OF CARDIOVASCULAR SAFETY PHARMACOLOGY

Safety pharmacology has been defined as a separate and distinct safety evaluation discipline only recently [19]; it is situated between toxicology and pharmacology and examines changes in organ/system functions with emphasis on acute and functional effects, whereas toxicology is centered on histopathological changes in tissue structure resulting from chronic use

of drugs. While pharmacology studies and defines the primary effect of a drug, safety pharmacology is interested in off-target pharmacological interactions, and was thus originally referred to as 'general pharmacology'. The first attempt at rationalizing and giving a precise structure to this testing was made by the Japanese Ministry of Health in 1995 with the Japanese Guidelines for Nonclinical Studies of Drugs Manual 1995 [20]. This guideline included requirements for cardiovascular testing and specifically stated that 'Effects of the test item on respiration, blood pressure, blood flow, heart rate and electrocardiogram should be assessed'. The conduct of these studies was mandatory for execution of clinical trials and registration of new drugs in Japan. At about the same time, in the late 1980s/early 1990s, spontaneous reports of a particular type of arrhythmia (torsade de pointes, TdP) and sudden death related to the use of terfenadine, a non-sedating H1-antihistamine, and of other non-cardiovascular drugs, began to appear in the literature [21–24]. This arrhythmia was usually associated with prolongation of the QT interval of the ECG, ascribed to the prolongation of cardiac repolarization following inhibition of potassium channels, namely the delayed rectifier potassium channel I_{Kr} encoded by the hERG gene (human ether-a-go-go related gene) [25].

Before addressing the Regulatory turmoil elicited by these reports, it can be useful to review the mechanism of drug-induced alterations in the cardiac depolarization–repolarization cycle that might cause life-threatening arrhythmias such as TdP. The QT interval of the surface ECG represents action potential changes of cardiac cells during depolarization and repolarization (Figure 2.1). Changes in the polarization state of the cell membrane are mediated by movements of ions (sodium, calcium and potassium) across the cell membrane through a multitude of ion channels. Rapid inward movements of sodium ions (the so-called I_{Na} current) are responsible for the depolarization that causes the phase 0 upstroke of the action potential, corresponding to the QRS complex on the surface ECG. Following the quick inactivation of sodium channels, a transient outward repolarizing potassium current (I_{to}) causes the phase 1 notch of the action potential and signals the beginning of cellular repolarization. The plateau characterizing the phase 2 is due to a balance between inward depolarizing calcium currents (I_{Ca}) and the delayed rectifier outward (repolarizing) potassium current (I_{Kr}). This balance between inward and outward currents results in the flat S–T segment on the surface ECG. The progressive inactivation of the calcium currents and the activation of other repolarizing currents (among which is the slow rectifier potassium current, I_{Ks}) lead to the phase 3 of the action potential, which completes the repolarization of the cardiac cells and corresponds to the T wave on the surface ECG, and to

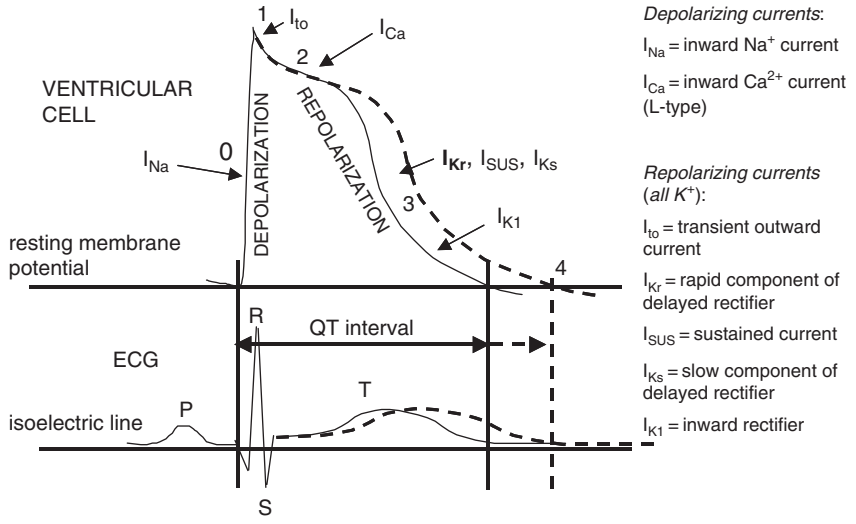

Figure 2.1 Ventricular cell action potential and surface ECG (schematic, not to scale). Increase of inward currents (I_{Na}, I_{Ca}) or reduction of outward currents (such as through channel block) can prolong the action potential and the QT interval (dotted line). Reprinted with kind permission from Springer Science + Business Media. Copyright 2007

phase 4, in which the cell membrane is at its resting potential. As depicted in Figure 2.1, a prolongation of the phase 3 of the action potential results in QT prolongation on the surface ECG (dotted lines).

Congenital long QT syndromes (LQTS) have been known for some decades and are characterized by prolongation of the QT interval caused by genetic mutations. People affected by this syndrome are subject to cardiac events such as arrhythmia and syncope, leading in some cases to sudden death. At present, mutations at seven distinct genes have been identified as responsible for LQTS [26] (Table 2.2). Of these genes, three

Table 2.2 Gene mutations causing long QT syndromes [26]. Adapted with permission from Elsevier, Copyright 2005

Type	Gene	Channel
LQT1	KCNQ1 (KvLQT1)	I_{Ks}
LQT2	KCNH2 (hERG)	I_{Kr}
LQT3	SCN5A (hH1)	Na
LQT4	ANK2 (ankyrin B)	--
LQT5	KCNE1 (minK)	I_{Ks}accessory subunit
LQT6	KCNE2 (MiRP1)	I_{Kr}accessory subunit
LQT7	KCNJ2 (Kir2.1)	I_{K1}

encode potassium channels, one encodes the sodium channel and two encode potassium channels accessory subunits (the last, most recently described gene encodes a scaffolding protein) [27–30]. LQTS2 is due to a mutation in the gene that encodes the potassium channel known as hERG (KCNH2). The combination of the product of this gene with the product of another gene (KCNE2, MiRP-1) produces the I_{Kr} potassium channel (the fast component of the delayed rectifier current) [31]. Antiarrhythmic drugs with class III action (sotalol, amiodarone, dofetilide) selectively block the I_{Kr} channel. The same effect has been demonstrated for several non-cardiovascular drugs shown to prolong the QT interval and, in some cases, to cause TdP [32].

As a consequence of such an unwanted effect, several drugs were removed from the market or had their label revised to reflect this risk. These agents come from a wide variety of chemical and pharmacological classes, as reported in Table 2.3. No regulatory guideline existed at that time for studying the potential effect of drugs on the cardiac repolarization.

Since these arrhythmias were often elicited by drugs taken for the treatment of rather trivial, non-life-threatening non-cardiovascular indications, Regulatory Authorities became very interested in how to predict which drugs might cause alterations in ventricular repolarization. The European Agency for the Evaluation of Medicinal Products (EMEA) was the first Regulatory Agency to produce such a document through an ad hoc panel of experts convened in 1996 by the Committee for Proprietary Medicinal Products (CPMP, an arm of the EMEA). The resulting 'Points to Consider' document [33] recommended the conduction of non-clinical as well as clinical studies in order to assess the potential for QT prolongation by non-cardiovascular drugs. The non-clinical part of the document strongly supported the integration between the results of *in vitro* and

Table 2.3 Examples of marketed drugs causing QT prolongation

Pharmacological Class	Drug	Reference
Antibiotics	erythromycin, grepafloxacin	[60,61]
H1 antihistamines	terfenadine, diphenhydramine, astemizole	[62,63]
Psychotropic agents	phenothiazine derivatives, haloperidol, sertindole, risperidone	[64,65]
Antifungals	fluconazole, ketoconazole	[66]
Gastrointestinal prokinetics	cisapride	[67,68]

in vivo models. Therefore, results from action potential duration (APD) studies in isolated cardiac tissues such as Purkinje fibers or papillary muscle were considered complementary to the careful evaluation of electrocardiographic parameters (QT duration and T-wave morphology) in animal models. Even more emphasis was given to the description of clinical studies to be implemented in case the new drug showed TdP liabilities (e.g. QT and/or APD prolongation) in pre-clinical studies. After reporting normal, borderline and prolonged QTc values for males and females, threshold values for absolute QT and changes in QTc were given: changes up to 30 ms were considered of no concern, prolongation between 30 and 60 ms was considered to raise concern and an increase of more than 60 ms was definitely considered to pose a serious risk; absolute QT values above 500 ms were also considered to indicate a clear concern for the induction of TdP. It is noteworthy that the Bazett formula was indicated for QT correction in clinical trials, while no indication was provided for pre-clinical *in vivo* studies.

A direct link between QT prolongation and arrhythmogenesis was not (and still is not) clear. This fact, and the specification in the 'Points to Consider' document of methods and magnitude of changes, opened a debate between Industry and Regulators on the scientific basis and relevance of the recommendations given. The debate resulted in an International Conference on Harmonization (ICH, www.ich.org) process and in the publication of two ICH guidelines. The first (ICH S7A) [15] covers the requirements for Safety Pharmacology testing, which differentiate between "safety pharmacology core battery", which includes cardiovascular testing, and "follow-up and supplemental studies" to be conducted in case concerns are raised by the core battery studies or findings in clinical studies that cannot be explained by the existing body of information. It also covers test systems (species, *in vitro* systems, sample size, use of controls, route of administration), dose levels or concentrations for *in vivo* and *in vitro* studies, and the duration of the studies, and addresses the evaluation of metabolites, isomers and finished products. It does not, however, mention any specific QT prolongation testing; this is deferred to the second guideline (ICH S7B) [16], which deals specifically with pre-clinical testing on QT prolongation liabilities. At the same time, a third ICH guideline was published, covering clinical investigations on the same subject (ICH E14) [17]. The S7B guideline describes a core cardiovascular study battery that, as in the 'Points to Consider' document, includes an *in vitro* test and an *in vivo* experimental model. In almost all reported cases, drugs that prolong the QT interval do so by inhibition of the I_{Kr} potassium channel. For this reason, as *in vitro* model the S7B recommends

patch clamp experiments on this channel as expressed in cells (HEK293 or CHO cell lines) which have been stably transfected with the encoding gene, or in native cardiac cells. The recommended *in vivo* model is a cardiovascular study conducted by means of telemetric techniques in conscious, freely moving non-rodents. As in the CPMP document, emphasis is given to the collection of ECG interval data and to the morphological analysis of the T-wave. Rats and mice are not indicated as useful species because their cardiomiocytes lack the I_{Kr} channel (in these species the primary ionic current controlling repolarization is I_{to}). Conscious, freely moving animals are preferred over anesthetized preparations and tethered or restrained animals for several reasons. Anesthetic agents can prolong QT and thus mask a drug's effect [34]. Anesthetized animals normally have higher heart rates than conscious animals, and this is reflected in a shorter QT, therefore QT prolongation by a drug could be balanced and hidden by the shortening caused by the anesthetic and by the change in sympathetic balance. Similarly, restraint or tethering can increase the stress of the animals, thereby increasing heart rate, although in such cases training of the animals before performing the experiment can reduce greatly the change in cardiac frequency. And, last but not least, the adoption of telemetry allows the re-use of animals, therefore reducing their unnecessary use.

S7B takes a further step in comparison to the CPMP 'Points to Consider' document with the introduction of the 'integrated risk assessment' concept (Figure 2.2). Data from the two core studies (*in vitro* I_{Kr} and *in vivo* QT study) should be interpreted together with other relevant information such as primary and secondary pharmacology, chemical/pharmacological class

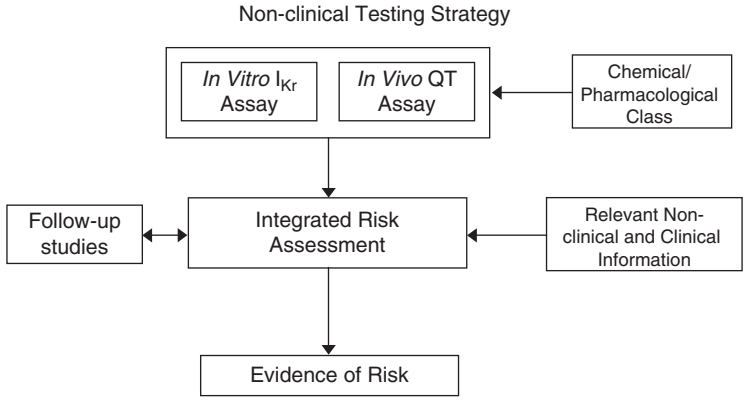

Figure 2.2 Integrated risk assessment strategy from ICH S7B

(whether or not at risk of delayed ventricular repolarization), results from supplemental and/or follow-up studies (when conducted), potency of the test substance relative to reference compounds, relationship between exposures associated with repolarization effects (both *in vitro* and *in vivo*) and those eliciting the primary pharmacological effect in pre-clinical species (or the projected human therapeutic exposure), contribution of metabolites and metabolic differences between pre-clinical species and man. The overall conclusion from the risk assessment constitutes the 'evidence of risk' for the test substance to delay ventricular repolarization and prolong the QT interval in man. The conduct of these studies prior to first administration in man (suggested by the guideline) and the resulting integrated risk assessment should support the planning and interpretation of subsequent clinical studies (see ICH E14).

Supplementary and follow-up studies are also described in the S7B guideline. These studies, both *in vitro* and *in vivo*, should be conducted when inconsistent results are obtained from the core studies or between these and clinical findings. *In vitro* studies such as APD in Purkinje fibers or papillary muscle, ventricular wedge preparations and Langendorff isolated heart, can give information on the effects of compounds on ion channels other than the I_{Kr}. In cases where inhibition of the I_{Kr} current does not result in QT prolongation in animal models, very often an inhibition of the cardiac L-type Ca^{++} channel can also be demonstrated (e.g. verapamil). In such a case, the prolongation due to the inhibition of the repolarizing potassium current is balanced by the concurrent inhibition of the calcium current sustaining the plateau phase of the action potential. Anesthetized *in vivo* preparations can be useful when side effects in conscious animals (such as emesis or tremors) do not allow testing of sufficiently high doses (and therefore plasma levels). In such experimental models, the increase in heart rate can be overcome by cardiac pacing; sustained plasma levels can be achieved by intravenous infusion; electrophysiology parameters (monophasic action potential, MAP) can be obtained in the same animals, thus allowing comparison of effects on action potentials and surface ECG.

S7B also gives indications on how to design and conduct an appropriate study. Doses/concentrations tested should exceed the anticipated human exposure, so that possible overdosing or extremely high plasma levels in patients and clinical trial participants (such as those attainable in low metabolizers or by inhibition of metabolic pathways by concurrently administered drugs) are taken into account. The guideline does not give any ratio between experimental levels and projected therapeutic levels, but requires, in the case of negative results, to test up to the highest

achievable concentration *in vitro* and up to doses which are known (from toxicology studies) to cause moderate toxicity *in vivo*. This requirement (especially the part concerning *in vitro* testing) has raised some concern among pharmaceutical companies, which assert that any compound will show a sort of effect if tested at such high concentrations. A recent paper [32] has compared published data on I_{Kr} activity, APD and QT prolongation in animals against QT effects and reports of TdP in humans for 100 drugs, reporting that drugs with no QT liabilities show a >30-fold separation between I_{Kr} activity and unbound therapeutic plasma concentrations. This could, therefore, appropriately represent the upper limit of concentrations/doses to be tested in the case of negative results.

In the presence of a major active metabolite, this chemical entity should be tested separately, at least *in vitro*, where metabolism does not occur. The same is required if in man a metabolite not present in a laboratory species is produced. In this case, an *in vivo* test should also be performed. Positive controls are always required in *in vitro* tests, while for *in vivo* telemetry models an ad hoc experiment for scientific validation is deemed sufficient. This experiment should be conducted with a compound known to prolong QT (e.g. sotalol, astemizole, E4031) and should demonstrate the adequacy of the experimental model to detect QT prolongation with good sensitivity. If the compound belongs to a chemical or pharmacological class known to possess I_{Kr} blocking properties, a reference compound from the same class should be tested (at least *in vitro*) in order to rank potency. Good laboratory practice (GLP) compliance is required for the core battery tests, while supplementary or follow-up studies, due to their investigative nature, do not need to comply strictly (although compliance to the greatest extent feasible is expected). Core battery studies are expected to be conducted before first-in-man (FIM) studies, while supplemental and follow-up studies (which can be triggered by clinical findings) need to be conducted in a timely fashion when needed.

Analysis of the QT interval must take into account the rate-dependence of this variable, as QT is inversely proportional to the heart rate. S7B suggests that QT data obtained after administration of the test substance be compared with control and baseline data at similar heart rates. When cardiac frequency is increased for any reason (effect of the test drug or other environmental variables), correction formulae should be used to normalize QT duration on the heart rate (the so-called corrected QT, QTc). Investigators have devised various correction formulae, among which the most commonly used are Bazett's [35] and Fredericia's [36]. These two formulae, however, besides having been developed for man, have other drawbacks. Bazett's, for example, is inaccurate for high and

low heart rates, resulting in over and underestimation of the QT, respectively [37]. In recent years, other more accurate formulae have been developed to correct the QT interval, including linear regression formulae, analysis of covariance equations [38–41] and, more recently, probabilistic methods [42]. The Guideline does not suggest a particular correction formula (as was the case with the EMEA 'Point to Consider' document, which suggested Bazett's formula for clinical studies), but requires justification of the chosen formula based on historical data and invites investigators to use individual correction formulae and to conduct QT/RR analysis.

2.2.1 QT Correction and QT/RR Analysis

Due to the inverse relationship between QT and heart rate, the need for correcting QT duration with respect to cardiac frequency for a careful analysis of possible effects on cardiac repolarization is widely acknowledged. However, no consensus has been achieved, either among Regulatory Authorities or in the pharmaceutical industry or academia, on the best correction formula. Bazett's and Fredericia's are still widely used, notwithstanding the recognition of their inadequacy at high and low heart rates (they both overestimate corrected QT at high heart rates and underestimate it at low heart rates).

Using canine toxicity study data, Spence *et al*. [41] demonstrated that, for large studies, pre-test data can be used to derive a correction model based on an analysis of covariance (ANCOVA) method, which provides a near-zero slope for the QTc–HR relationship. Limitations are that the study sample size is usually insufficient to accurately describe the QT–HR relationship, especially at high and low heart rates, and the correction formula varies from study to study, hindering comparisons between studies and drugs. An alternative is to use the ANCOVA method after compiling a large historical database of QT–HR data from many animals from previously conducted studies to fully describe the normal QT and HR relationship within a species [43]. According to this approach, the log–log formula for correcting the QT interval for heart rate is one of the best models for describing the QT–HR relationship and the best at achieving a zero slope for the QTc–HR relationship, in both beagle dogs and cynomolgus monkeys (Figure 2.3). The log–log correction has the advantages of using the ANCOVA method as described by Spence, and further, since corrections are derived from a large historical database,

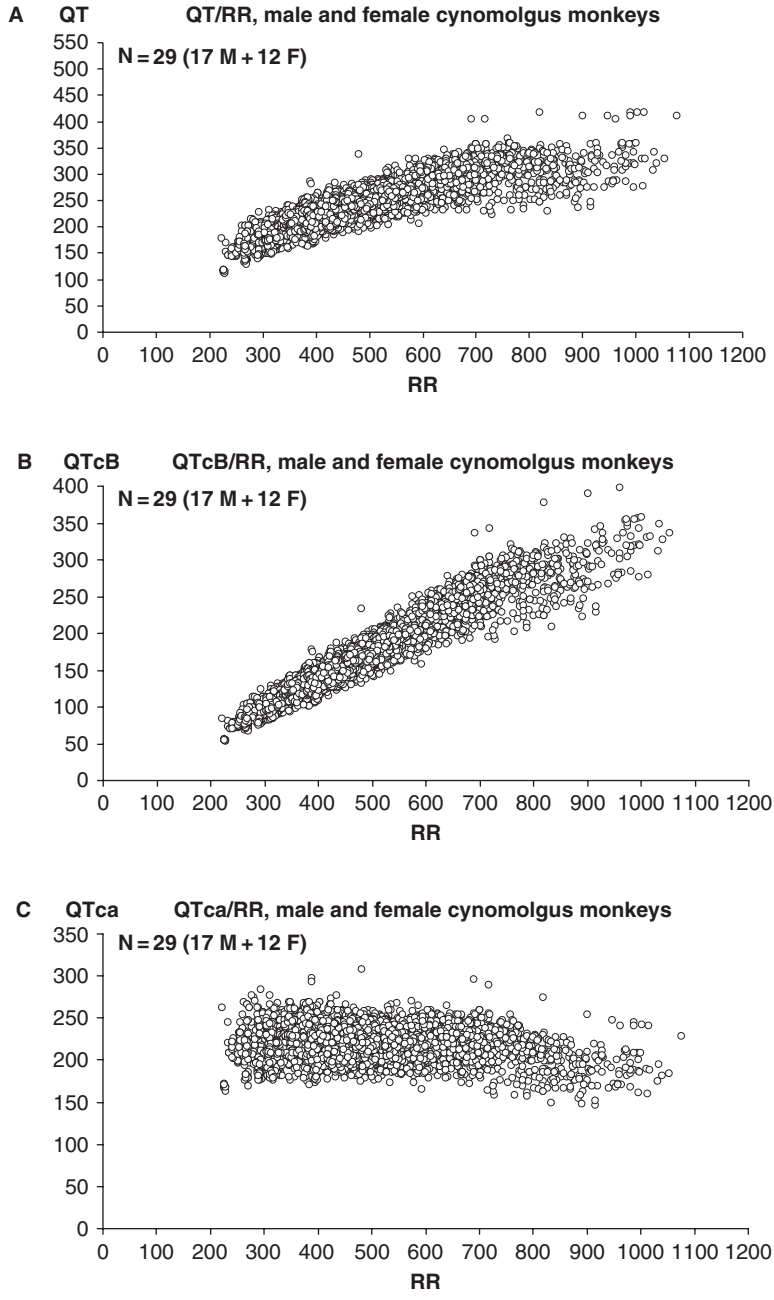

Figure 2.3 QT correction formulae in cynomolgus monkeys. A: QT/RR plots show inverse relationship between QT and RR. B: Correction with Bazett's formula does not solve the inverse relationship. C: Analysis of covariance correction normalizes QT and gives a near zero slope.The same correction can be achieved in beagle dogs

Table 2.4 Population distribution of QT values within discrete HR bins in male and female beagle dogs; internal database

HR	QT		
	Males	**Females**	**Both sexes**
41–60	169–276	180–281	169–281
61–80	160–264	156–296	156–296
81–100	143–269	156–304	143–304
101–120	151–260	150–268	150–268
121–140	138–247	146–261	138–261
141–160	141–243	146–241	141–243
161–180	131–222	142 – 214	131–222
181–200	143–199	137–253	137–253

allowing this method to be used with smaller studies such as those used in common safety pharmacology studies.

The historical database can also be used to describe the population distribution of QT values within discrete HR bins (Table 2.4), so that an outlier analysis similar to that proposed by Osborne and Leach [44] can be used in data review, allowing interpretation of QT effects independent of any correction method.

Correction-free interpretation of QT data can also be obtained by means of beat-to-beat QT/RR analysis [9], as illustrated in Figure 2.4.

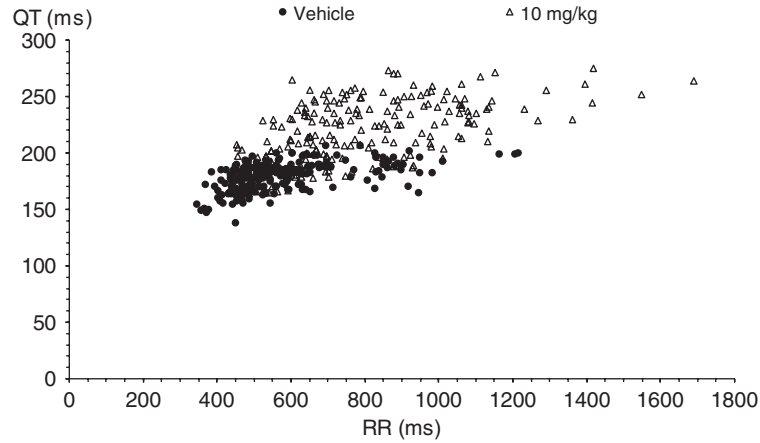

Figure 2.4 QT/RR interval relationship on a beat-to-beat basis in an individual dog following oral administration of sotalol. QT prolongation is shown by the upward shift of QT/RR points

By plotting absolute, non-corrected QT and RR values on a graph, QT prolongation results in an upward shift of the QT/RR points.

The best QT analysis can therefore be achieved by combining heart rate correction of QT, QT/RR analysis and outlier analysis.

2.3 REGULATORY ASPECTS OF CARDIOVASCULAR TOXICOLOGY

The conduct of toxicology studies is regulated by several international guidelines, dealing with the timing, duration and type of study. No particular guideline, however, describes specific testing for cardiovascular liabilities. This is understandable if one considers the type of toxicological cardiovascular adverse events; they appear following repeated exposure to the drug and are characterized by morphological changes that are picked up by clinical pathology and histopathology investigations, as conducted in normal toxicology studies. Therefore, the conduct of *ad hoc* studies would only be a duplication of activities and results, resulting in unnecessary use of animals. Exceptions to this general concept are represented by specific studies that could be performed with cytotoxic compounds developed for the treatment of cancer and by two "Guidance for Industry" documents published by the FDA in 2008 regarding the development and registration of new chemical entities for the treatment of diabetes mellitus [18,45]. In addition to these two classes of compounds, in recent years, other drug classes have been shown to cause cardiovascular toxicity: COX-2 antagonists [11,12,46] and tyrosine kinases inhibitors developed for the treatment of cancer [5,6,47], but for these two classes of drugs no specific preclinical studies are available and no specific regulatory documentation has been produced. In the next sections we will examine cytotoxic agents and drugs for the treatment of diabetes and the regulatory implications of their cardiovascular toxicity. A more comprehensive list of compounds causing cardiovascular toxicity, together with their mechanism, can be found in the literature [2,13,14,48].

2.3.1 Cytotoxic Drugs

One of the best recognized instances of chemotherapy-induced cardiotoxicity is the heart failure associated with the use of anthracyclines.

Cardiotoxic effects caused by anthracyclines can be divided into two categories: an acute or sub-acute form, which can occur days or weeks after treatment, and a chronic progressive cardiotoxicity, which may become manifest years (or even decades) after exposure [13,48]. Short term effects (arrhythmias or pericarditis) are not considered to be dose-related, can be reversible and, due to the life-threatening nature of cancer which makes a higher degree of risk acceptable in comparison to other diseases, do not limit further use of anthracyclines. On the contrary, long-term effects (resulting in heart failure) are thought to be strictly correlated with the cumulative dose of anthracyclines received; in a retrospective study, Von Hoff *et al*. [49] found that up to the cumulative dose of 400 mg m^{-2}, the incidence of heart failure was 0.14%, increasing to 7% at cumulative doses between 400 and 550 mg m^{-2} and reaching 18% in patients receiving 700 mg m^{-2}. To give an idea of the impact of anthracycline-mediated cardiac toxicity, it is reported that cancer survivors today have a higher risk of cardiovascular disease than of tumor relapse [50]. This is especially true for children: the incidence of cardiac dysfunction among pediatric patients receiving anthracyclines may be as high as 57% about 6 years after treatment [51]. The histomorphology of anthracycline-induced cardiotoxicity has been well characterized in several animal species [52–55] and closely resembles that in humans [56]. Animal models have been developed which can predict the cardiotoxicity of this class of compounds [57–59]. Although not specifically mentioned in any guideline, these models could be useful in the definition of cardiotoxic liabilities of new anthracyclines under development, allowing definition of possible advantages over existing drugs.

2.3.2 Drugs for the Treatment of Diabetes Mellitus

In the USA alone, 23.6 million people (8% of the population) have diabetes, with an increase in prevalence of 13.5% from 2005 to 2007 (www.diabetes.org/diabetes-statistics.jsp). Although several treatments are currently available, there is a recognized need for new drugs for the prevention and treatment of diabetes. Among the complications of diabetes, heart disease and stroke account for about 65% of deaths in affected people (www.diabetes.org/diabetes-statistics/complications.jsp). It is, therefore, important that new drugs at least do not exacerbate the cardiovascular effects of the disease. A new class of drugs, the

thiazolidinediones, introduced in the market in the late 1990s, have been shown to increase cardiovascular risk in patients taking this medication either alone or in combination with other therapies [69,70]. The mechanism for this increased cardiovascular risk is not clear and could be multifactorial, from direct cardiotoxicity to effects on metabolism and fluid accumulation. One potential factor could be the adverse effect of the drug on lipid metabolism [70]. A major role is probably played by their peroxisome proliferator-activated receptor (PPAR) agonistic activity, which is thought to be responsible for increasing plasma volume by as much as 6–7% and causing peripheral oedema [13]. At present, two PPAR agonists are available for the treatment of diabetes (rosiglitazone and pioglitazone), while several reaserch programs on this class of compounds have been stopped for safety reasons. In February and December 2008, the FDA issued two Guidance for Industry documents on the preclinical and clinical testing of new potential drugs for the treatment of diabetes [18,71]. The most recent document [71] deals exclusively with the clinical development and clearly states that 'To establish the safety of a new antidiabetic therapy to treat type 2 diabetes, sponsors should demonstrate that the therapy will not result in an unacceptable increase in cardiovascular risk'. For this purpose, a detailed description of the expected Phase 2 and 3 clinical trials studies is given. The document also gives indications on how to manage studies that have already been completed. The February document [18] covers also preclinical studies and in Appendix A (Preclinical Considerations for Peroxisome Proliferator-Activated Receptor Agonists) gives very clear requirements for the preclinical development of PPAR agonists. In particular, with respect to cardiovascular risk, it requires that chronic studies in non-rodents be of 12 months (instead of the normal 9 months as per ICH guidelines). Cardiac effects should be evaluated by means of ECGs, clinical chemistry and cardiac histopathology (including electron microscopy). If the compound is recognized to possess alpha or delta agonistic activity, biomarkers of direct cardiac toxicity (such as troponin I and T) should be monitored [www.fda.gov/cder/present/DIA2004/Elhage.ppt]. If the compound has gamma activity, since heart weight increases of 25% or greater in 13-week studies have been predictive of excess cardiac mortality with longer-term chronic dosing (greater than or equal to 12 months) in all animal models, a dose that results in 20 to 25% increase in heart weight will have to be used as the maximum dose for use in the 2-year carcinogenicity studies [www.fda.gov/cder/present//DIA2006/Elhage_Safety.ppt].

2.4 SUMMARY

Cardiovascular adverse effects of drugs can be divided into two categories: pharmacological and toxicological. Pharmacological adverse effects usually appear after a single drug administration, are due to interference with receptors and/or ion channels and are investigated by means of safety pharmacology studies. Toxicological adverse effects usually appear following repeated administrations of the drug (from weeks to months or even years), can be caused by a variety of mechanisms (including a direct cytotoxic effect) and are investigated during classical repeated-dose toxicology studies.

Safety pharmacology studies are regulated by two ICH guidelines (S7A and S7B) [15,16], which identify the cardiovascular system as one of the vital organs for which preclinical studies are required before the start of any clinical development program. In particular, S7B deals exclusively with the investigation on the potential of new drugs to cause delays in ventricular repolarization, which could lead to arrhythmias such as Torsades de Pointes. Such investigations must be conducted in one *in vitro* (K^+ current through the I_{Kr} channel in cells expressing the channel) and one *in vivo* test (QT analysis in non-rodents). The results of these studies, together with information from primary pharmacology studies, from follow-up or supplemental studies (as suggested by the guideline) and with other relevant information (such as chemical/pharmacological class), must be used to make an "integrated risk assessment", which will result in an overall conclusion of "evidence of risk". Another guideline (ICH E14) [17] covers the clinical testing for the same potential.

Investigation of cardiac toxicity in general toxicology studies is not covered by specific guidelines, as it is part of the usual toxicological investigation on new drugs, which is regulated by ICH guidelines dealing with the timing, duration and design of preclinical studies. The only exception is represented by two Guidance for Industry documents issued by the FDA in 2008 [18,71], dealing with the conduct of preclinical and clinical studies for the development of new drugs for the treatment of diabetes. This exception can be explained by the fact that cardiovascular complications are very common in patients affected by diabetes and some recently introduced drugs (thiazolidinediones) have been proven to exacerbate these complications. Appendix A of the February 2008 document [18] gives clear indications on the endpoints to be examined in toxicology studies (such as biomarkers of cardiac toxicity, troponin I and T, and heart weight), techniques to be used (electron microscopy) and

duration of chronic studies (12 months for non-rodents). The December 2008 document [71], deals exclusively with the conduct of clinical studies.

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3

Cellular Mechanisms, Molecular Targets, and Structure–Function Relationships in Drug-Induced Arrhythmias: Antihistamines, Psychoactive Drugs, and Antimicrobial Agents

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3.1 INTRODUCTION

During the last few years, rare but serious cardiotoxic events following the assumption of a wide variety of drugs belonging to diverse therapeutic classes have been major causes of concern among patients and clinicians, as well as drug developers and regulators. In an attempt to increase the safety profile of currently available drugs, the International Conference

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on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), a supranational organization composed of representatives from drug regulatory agencies from Europe, USA, and Japan, has developed guidelines and recommendations at both preclinical and clinical level to recognize drug arrhythmogenic potential early during compound development. The need for such global efforts is mainly justified by the existence of validated preclinical and clinical predictors of drug-induced pro-arrhythmic potential in humans; therefore, establishing clear-cut strategies of preclinical and clinical testing appears mandatory to improve safety of newly introduced drugs. The field has undergone an amazing development, boosted by discoveries from disciplines as diverse as genetics, molecular and cellular pharmacology, pharmacovigilance, and clinical cardiology.

In this chapter, we will review the most relevant and recent findings in the field, describing from an historical perspective the current status of the strategies undertaken to optimize the risk–benefit ratio of non-cardiovascular drugs potentially endowed with proarrhythmic proclivity. Particular attention will be given to drugs belonging to three broad therapeutic areas (antihistamines, psychotropics, and antimicrobials) where considerable progress has been made in understanding the cellular mechanisms, the molecular target(s), and the structure–function relationships underlying such an important cardiovascular adverse effect.

3.2 IONIC MECHANISMS OF THE CARDIAC ACTION POTENTIAL

Hemodynamically-efficient contraction of the cardiac muscle is due to the ordered propagation of the action potential within the atria, the electrical conduction system (i.e. Purkinje cells), and the ventricular myocytes (i.e. sub-endocardial myocytes, mid-myocardial M-cells, and sub-epicardial myocytes). In each of these cells, the action potential is shaped by the sequential opening of distinct classes of ion channels. The electrical activity of a normal Purkinje cell is classically sub-divided into five distinct phases (from 0 to 4); phases 0 to 3 correspond to the systole, and phase 4 corresponds to the diastole (Kao and Furbee, 2005) (Figure 3.1A). When the cell is depolarized above threshold by the electrical connections with neighboring cells, the rapid influx of sodium ions through voltage-gated Na channels (I_{Na}) generates an influx of positive charges into the cytoplasm (phase 0); the abrupt increase in cell

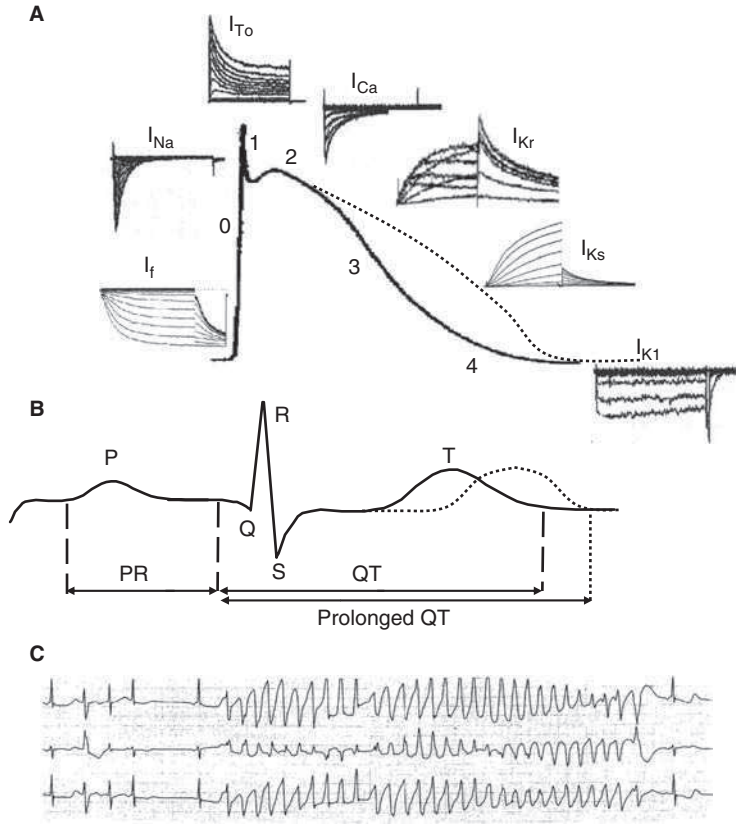

Figure 3.1 A. Illustration of a human cardiac action potential and of the currents underlying each of its phases; dotted line indicates an action potential whose repolarisation phase is delayed (Crumb and Cavero, 1999, adapted with permission from Elsevier, Copyright 1999). B. A schematization of a human ECG recording corresponding to the action potential profile shown in A; the dotted line shows the same recording during a cardiac repolarization. C. ECG recording showing a torsade de pointes arrhythmia, preceded by the characteristic short-long-short sequence (Chiang and Roden, 2000, adapted with permission from Elsevier, Copyright 2000)

membrane permeability to Na ions allows the membrane potential to approximate to the equilibrium potential for Na ions (≈+50 mV), thereby causing a strong depolarization of the cell interior. This depolarization is subsequently followed by the activation of a rapidly activating and inactivating potassium current, defined as transient outward (I_{to}), a phenomenon which generates the initial repolarization of phase 1. However, during phase 1, the cell membrane does not return to the resting membrane potential because of the rapid inactivation of I_{to}; moreover,

the activation of voltage-gated calcium channels (I_{Ca}), together with the Ca-induced Ca release from the sarcoplasmic reticulum, allows a massive influx of positive Ca ions which maintains the myocyte membrane potential in a depolarized state and allow contraction to occur (phase 2). After a few hundred milliseconds, the membrane potential gradually repolarizes because of the inactivation of voltage-gated Ca channels and the activation of the delayed rectifier repolarizing current (I_K) made up of both rapid (I_{Kr}) and slow (I_{Ks}) components (phase 3). Late in phase 3, the inward rectifier I_{K1} current, which was previously silenced by a voltage-dependent block by intracellular cations such as magnesium and polyamines (Ficker *et al.*, 1994), reactivates, thereby contributing to the final phases of repolarization. In phase 4, the cell membrane is mainly permeable to K ions, and, therefore, the membrane potential approximates to the K equilibrium potential (≈–90 mV) Figure 3.1.

These phases also occur in other cardiac cells, although each cell type expresses a specific pattern and level of ion channels which give rise to unique electrophysiological properties. As an example, the electrical properties of the diastolic phase (phase 4) are considerably divergent among cardiac cell types; in spontaneously active cells of the sino-atrial node, the expression of a hyperpolarization-activated non-selective current, called I_f, allows a slow influx of Na ions which gradually depolarizes the cell membrane toward the activation threshold of voltage-gated Na channels, thereby triggering a subsequent action potential. This current is not expressed in the ventricular myocardium, which is, therefore, not endowed with intrinsic excitability. Moreover, the three predominant cell types that make up the ventricular myocardium (epicardial, midmyocardial or M-cells, and endocardial cells) display marked differences in the time course of repolarization. In particular, M-cells display a relatively weak I_{Ks} current density and a relatively stronger Na–Ca exchanger (I_{NaCa}) and I_{Na} current densities compared to endocardial or epicardial cells (Antzelevitch, 2005). These functional properties sensitize the M cells to drugs that prolong the action potential duration (APD) (see below).

3.3 ELECTROCARDIOGRAPHIC MANIFESTATIONS OF DRUG-INDUCED CARDIOTOXICITY

The surface electrocardiogram (ECG) records a summation of the electrical activity across the entire myocardium, and is, therefore, widely used

to provide an objective assessment of the cardiotoxic manifestations prompted by drugs (Figure 3.1B).

Drug-induced syncopal episodes and sudden deaths involving the class I antiarrhythmic quinidine were first described in the 1960s; these events were attributed to a previously un-recognized ventricular dysrhythmia (Selzer and Wray, 1964). The electrocardiographic basis for this ventricular arrhythmia was soon after dissected by Dessertenne (Dessertenne, 1966), who described a self-sustained polymorphic ventricular tachicardia with "torsade de pointes" (TdPs) ventricular fibrillation (Figure 3.1C). A TdP episode consists of a progressive modification of the amplitude and polarity of the QRS complexes (the ECG manifestation of the ventricular spread of the action potential), which appear to be twisting around an imaginary isoelectric baseline. TdPs generally occur in the setting of a marked bradycardia with prolongation of the QT interval, the time interval between the onset of the QRS complex and the end of the T wave. The QT interval includes both the ventricular depolarization and repolarization intervals. Although non-sustained TdPs may be asymptomatic or may manifest clinically as palpitations or syncope, sustained TdPs may degenerate into ventricular fibrillation, leading to cardiac arrest.

Under normal circumstances, the QT interval duration is strongly dependent on the heart rate. Therefore, several correction methods have been developed to take such a possibly confounding factor into account; the "corrected" QT is therefore defined as QT_c. Accepted normal values of QT_c are generally <440 ms, with slightly higher values in females (<450–460 ms).

Although delayed repolarization (QT lengthening on the ECG) does not inevitably lead to TdPs, a QT interval longer than 500 ms is believed to increase the risk for TdP. As recently described by Hancox *et al.* (2008), excessively delayed repolarization is linked to arrhythmogenesis in two ways. First, in experimental models such as the canine cardiac Purkinje fibers, drug-induced excessive ventricular action potential prolongation leads to the development of early after-depolarizations (EADs). EADs are exacerbated at slow rates (i.e. AP prolongation is greater at slower than at faster rates). EADs are considered to result largely from reopening of voltage-gated Na and Ca channels during AP repolarization. EAD generation may be a trigger event for initiation of TdP. Purkinje fibers, which have comparatively weak delayed rectifier currents and, hence, limited 'repolarization reserve', appear particularly sensitive to drug-induced AP prolongation and EADs. A second way in which drug assumption, by delaying repolarization, may facilitate TdP arrhythmia is via exacerbation of transmural dispersion of repolarization (TDR), which

results from the heterogeneous expression of ion channels across the ventricular wall (Antzelevitch, 2005). As previously-mentioned, M-cells repolarize later than endo- or epi-cardial cells and tend to have less I_{Ks}; therefore, a given reduction in I_{Kr} has a greater effect on repolarization in the mid-myocardium than in other regions of the ventricular wall, leading to increased TDR. Enhanced TDR generates an increased heterogeneity of tissue refractoriness, which provides a substrate for re-entrant arrhythmia. Consideration of these factors highlights how a reduction in magnitude of I_{Kr} can lead to electrophysiological changes at the cell and tissue levels that facilitate initiation and maintenance of the arrhythmia.

This short description of drug-induced ECG modifications emphasizes the role that QT prolongation and TdP occurrence have in drug-induced arrhythmogenesis. However, before we proceed to reviewing the specific drugs involved and analyzing the molecular basis for these cardiotoxic manifestations, it should again be specified that drugs that prolong the QT interval are not always associated with TdPs; on the other hand, medications causing TdPs do not always cause QT prolongation. This may be, at least in part, the result of the many pharmacological actions shown by each compound; as an example, the antiarrhythmic drug amiodarone is often associated with QT prolongation, but TdP episodes are rare, possibly because the drug also blocks Na and Ca channels, leading to a reduced excitability which impedes TdP occurrence. On the other hand, quinidine prolongs the QT interval and may cause TdPs at therapeutic plasma concentrations; however, at supratherapeutic concentrations, it may also block Na channels, resulting in a decreased repolarization heterogeneity and a relatively low TdP incidence. Finally, tryciclic antidepressants such as amitriptyline (see below), in addition to causing delayed repolarization and QT prolongation, also display antimuscarinic properties, leading to a significant enhancement of the heart rate; therefore, TdPs are relatively uncommon during tryciclic antidepressant overdose.

3.4 hERG/I_{Kr} AS A MOLECULAR TARGET FOR DRUG-INDUCED ARRHYTHMIAS

Although the concept that two distinct currents (denoted as I_{X1} and I_{X2}) participated in repolarizing the cardiac action potential during phase 3 was proposed by Noble and Tsien (1969), it took more than 20 years before the rapid (I_{Kr}) and slow (I_{Ks}) components of I_K were separated,

thus allowing a precise description of their biophysical properties (Sanguinetti and Jurkiewicz, 1990). Availability of the experimental class III antiarrhythmic drug E-4031, a methanesulfonanilide compound which behaves as a selective inhibitor of I_{Kr}, was crucial in achieving this goal. Although these experiments were originally performed in guinea pig myocytes, qualitatively similar results were also achieved in several species, including humans.

The molecular basis of I_{Kr} was defined a few years later, when Curran *et al.* (1995) revealed that patients affected by one form of long QT syndrome (LQTS-2), a life-threatening genetically transmitted arrhythmia characterized by a marked prolongation of the QT interval on the ECG and by frequent episodes of syncope or cardiac arrest occurring mainly during conditions of psychological or physical stress, carried mutations in a gene defined as human ether-a-gogo-related gene (hERG). The hERG gene had been previously cloned from a human hippocampal cDNA library (Warmke and Ganetski, 1994), using its sequence homology to another gene (the ether-a-gogo or EAG) identified in the fruit-fly *Drosophila Melanogaster* (Warmke *et al.*, 1991). The hERG gene encodes for K channel subunits, and is abundantly expressed in cardiac tissue; heterologous expression of the hERG protein gives rise to voltage-gated K currents (Sanguinetti *et al.*, 1995; Trudeau *et al.*, 1995). Altogether, these observations, in one with the peculiar biophysical properties of heterologously-expressed hERG currents (I_{hERG}), strongly suggested that hERG encoded for the main component of I_{Kr} involved in cardiac repolarization.

As will be extensively discussed later, the largest group of drugs endowed with pro-arrhythmic potential act as blockers of I_{Kr}; therefore, these discoveries allowed the establishment of a fundamental link between drug-induced (acquired) and genetically-determined (LQTS-2) cardiac arrhythmias, both conditions characterized by delayed action potential repolarization, phase 3 prolongation, and increased susceptibility to TdPs.

3.5 BIOPHYSICAL PROPERTIES OF hERG/I_{Kr}

hERG currents (I_{hERG}), similarly to I_{Kr}, are characterized by distinct biophysical properties which allow them to exert their specific role in action potential repolarization. In fact, I_{hERG} is inactive at resting (negative) membrane potential values, and is activated upon membrane

depolarization. The size of I_{hERG} increases within the membrane potential range –60/0 mV, but, despite the increase in the driving force for K ions, declines at potentials positive to ~0 mV (Figure 3.2A). This unusual voltage-dependence, which introduces a negative-slope region in the hERG current–voltage relation at positive voltages (Figure 3.2B), results from a uniquely fast, voltage-dependent inactivation process (Smith *et al*., 1996; Spector *et al*., 1996). One characteristic feature of I_{hERG} is the appearance of large 'tail' currents on repolarization to more negative potentials, whose amplitude exceeds that of the currents evoked by the depolarizing voltage command (Sanguinetti *et al*., 1995; Trudeau *et al*., 1995). These large tail currents are due to fact that the fast inactivation process is rapidly relieved at negative potentials; moreover, the recovery from inactivation has kinetics much faster than channel closing and the channels mainly recover passing through the open state; therefore, hERG channels recover quickly from inactivation, before closing occurs. The physiological consequences of hERG's rapid voltage-dependent inactivation kinetics are evident under action potential voltage-clamp of mammalian cell lines expressing recombinant hERG channels (Zhou *et al*., 1998; Hancox *et al*., 1998a; Hancox *et al*., 1998b; Lu *et al*., 2001; Lu *et al*., 2003) (Figure 3.2C). At the peak of the ventricular AP, relatively little I_{hERG} flows because the majority of hERG channels are in an inactivated state. However, as the voltage becomes less positive, some channels transit out of the inactivated state into the open state and allow current flow, leading to a progressive increase in outward (repolarizing) current, with maximal I_{hERG} occurring before the final rapid declining phase of the AP. Therefore, I_{hERG} has ideally evolved to regulate AP repolarization from plateau voltages and, thereby, to control the duration of the ventricular AP and QT interval of the ECG. Any condition (acquired or genetically determined) which limits current flow through I_{hERG}/I_{Kr} reduces the physiological function of this current, thereby leading to delayed action potential repolarization, prolongation of the QT interval on the surface ECG, and to an increased propensity to TdPs arrhythmic manifestations.

Although a direct comparison is often limited by technical issues, I_{hERG} recapitulates most of the major functional properties of I_{Kr}, namely inward rectification, slow deactivation, and blockade by micromolar concentrations of lanthanum and class III methanesulfonanilide antiarrhythmics such as E-4031; moreover, studies in which native ventricular I_{Kr} has been recorded under AP clamp showed that native I_{Kr} had a biophysical profile similar to I_{hERG} during the AP (Mitcheson and Hancox, 1999; Gintant, 2000; Varro *et al*., 2000; Rocchetti *et al*., 2001).

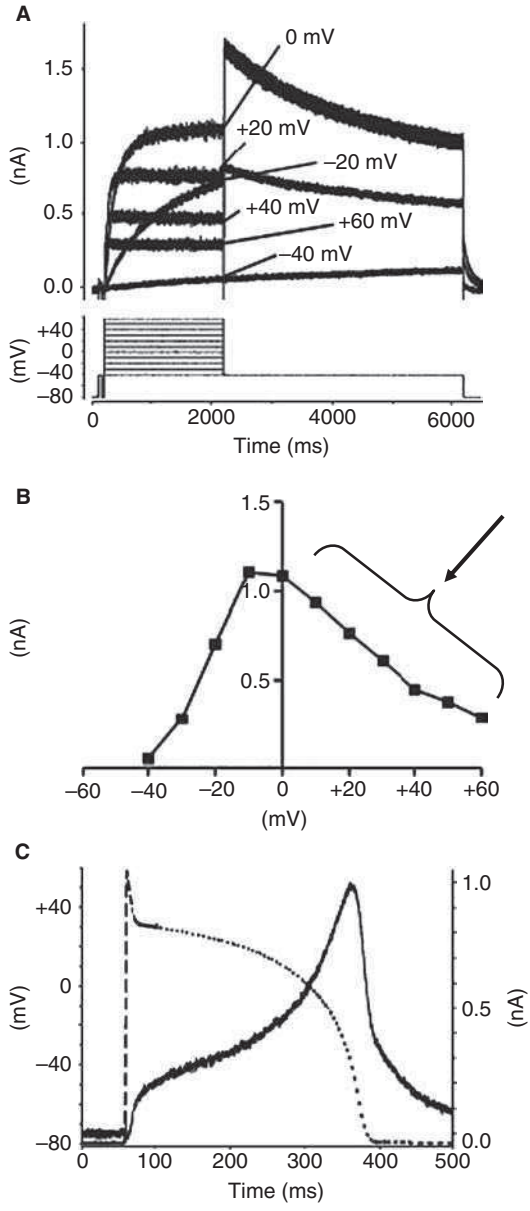

Figure 3.2 Biophysical properties of I_{hERG}. A. Representative trace family of I_{hERG} recorded from a hERG-expressing Chinese Hamster Ovary (CHO) cell at 37 °C in response to the voltage protocol shown in the lower panel. B. Current/voltage curve (*I/V*) for I_{hERG}; the arrow indicates the I_{hERG} characteristic negative-slope region in the *I/V* at positive potentials. C. Profile of I_{hERG} at 37 °C (solid line) in response to an action potential voltage command (dotted line) (from Hancox *et al.*, 2008, reproduced with permission from Elsevier, Copyright 2008)

Nevertheless, several differences exist between native I_{Kr} and the currents expressed heterologously by hERG channels, particularly in their gating properties and regulation by external K ions; splice variants heterogeneity (Kupershmidt *et al.* 1998) and assembly with accessory subunits (Abbott *et al.*, 1999) might contribute significantly to generating these functional differences.

3.6 ANTIHISTAMINES AND CARDIAC TOXICITY

3.6.1 Clinical Evidence

Following the milestone discovery that an 'H-substance' (histamine) played a crucial role as a mediator of the allergic responses, several clinically useful drugs able to antagonize the effects of histamine at the level of H_1 receptors were developed in the fourth and fifth decades of the last century (reviewed by Soldovieri *et al.*, 2008). These included chlorpheniramine, brompheniramine, triprolidine, hydroxyzine, and diphenhydramine; more recently, to improve selectivity for histamine receptors and to minimize the well-known sedative effects of these 'first generation' molecules, the so-called 'second generation' antihistamines were introduced in the mid 1980s. These more recently introduced drugs, including acrivastine, astemizole, azelastine, cetirizine, desloratadine, ebastine, emedastine, fexofenadine, ketotifen, levocarbastine, levocetirizine, loratadine, mizolastine, oxatomide, rupatadine, and terfenadine, do not accumulate significantly in the brain tissue because they possess lower lipid solubility and act as substrates for several transporters, including the P-glycoprotein of the blood–brain barrier. The pharmacodynamic and pharmacokinetic profile characterizing second generation antihistamines bears an obvious clinical advantage over first generation drugs in the therapy of allergic diseases in most (though not all) instances; therefore, second generation antihistamines are a first-choice treatment for many symptoms (including rhinorrhea, contraction of bronchial and gastrointestinal smooth muscle, and many forms of itch) associated with allergic diseases such as allergic rhinitis, chronic urticaria, and atopic dermatitis.

About 10 years after the introduction of second generation antihistamines in clinical practice, several reports appeared indicating the rare occurrence of TdPs, after the administration of astemizole (Craft, 1986; Snook *et al.*, 1988) or terfenadine (Davies *et al.*, 1989; Monahan *et al.*, 1990), two of the most widely used second generation antihistamines on

the market at that time. If one excludes intentional overdosing, additional risk factors could be detected in most cases of cardiotoxicity associated with terfenadine or astemizole; these mainly involved an impediment of drug metabolism. In fact, under standard therapeutic settings and conventional doses, terfenadine can be considered a pro-drug since it undergoes hepatic first-pass extraction and extensive metabolization into an acid metabolite, called fexofenadine or terfenadine carboxylate, by the CYP3A4 isoenzyme of the P450 oxidative pathway. In most normal subjects, and if prescribed at the reccommended doses (60 mg p.o. b.i.d), terfenadine levels in the plasma are below the detection limits (<10 nM) (Honig *et al.*, 1993); however, during conditions of impaired metabolic conversion into fexofenadine, such as in patients affected by pre-existing hepatic dysfunction or during the simultaneous exposure to inhibitors of the CYP3A4 enzyme (ketoconazole, itraconazole, macrolide antibiotics, cimetidine and ranitidine, and grapefruit juice), plasma levels of the parental compound terfenadine become easily detectable, increasing up to 100–200 nM. The increase in terfenadine plasma levels is associated with a significant prolongation of the QT interval (from 408 to 490 ms), raising the hypothesis that terfenadine itself was directly responsible for such a pharmacological effect (Honig and Baranjuk, 1996). This also seemed to be confirmed by the direct correlation between plasma levels of unmetabolized terfenadine and QT duration in conscious cynomolgus monkeys (Ohmura *et al.*, 1999). Altogether, these observations pointed to an impaired liver metabolism as one of the main predisposing factors for the occurrence of terfenadine cardiotoxicity. Fexofenadine retains the H_1 receptor antagonistic and nonsedative properties of the parent compound (Sorkin and Heel, 1985), does not undergo phase I hepatic metabolism (Markham and Wagstaff, 1998), and does not affect the cardiac QT interval in preclinical and clinical studies (Woosley *et al.*, 1993). Because of this favorable pharmacokinetic and pharmacodynamic profile, fexofenadine was introduced for therapeutic use in 1996; a few months later, terfenadine marketing authorizations were suspended in several European countries and in the USA, leading to its progressive disappearance from most pharmaceutical markets.

Astemizole, which was withdrawn from the market worldwide in 1998, is a potent and very long-acting antihistamine also undergoing hepatic metabolism to generate the active metabolite desmethylastemizole; the elimination half-life of desmethylastemizole is about 12 days, making astemizole intoxications difficult to treat (Hoppu et al., 1991; Paakkari, 2002).

Serious cardiotoxic events occur very rarely during assumption of antihistamines; in a UK study involving approximately 200 000 people who received 500 000 prescriptions, it has been estimated that the absolute effect is quite low, requiring 57 000 prescriptions, or 5300 person-years of use, for one case to occur (de Abajo and Rodríguez, 1999). The crude incidence of idiopathic ventricular arrhythmias was 1.9 per 10 000 person-years, and the relative risk in those receiving antihistamines compared with non-users was 4.2; among antihistamines, astemizole presented the highest relative risk (RR = 19) of all study drugs. It should be underlined, however, that not all studies agree on the higher propensity of astemizole, when compared to other non-sedating antihistamines, to cause ventricular arrhythmias and death (Lindquist and Edwards, 1997).

As a consequence of the above considerations about antihistamine safety depending crucially on drug–drug interference at the level of metabolism, the lack of significant hepatic metabolism probably contributes to the freedom from significant cardiotoxic potential shown by some other second generation antihistamines, such as desloratadine, cetirizine, levocetirizine, and acrivastine (Soldovieri *et al*., 2008).

Beside the clinical conditions causing an increase in the antihistamine plasma levels well above therapeutic values, it should be emphasized that cases have also been described in which the arrhythmic episodes occurred at 'therapeutic' drug concentrations, therefore in the absence of significant interference with drug metabolism. These cases often reveal the existence of additional predisposing factors to the development of cardiac arrhythmias, such as ischemic heart disease, congestive heart failure, and electrolyte imbalance, particularly hypokalemia (defined as K serum levels lower than 3.5 mmol l^{-1}) and/or hypomagnesemia (defined as magnesium serum levels lower than 0.6 mmol l^{-1}) (Woosley, 1996). Moreover, few documented cases of drug-induced arrhythmias have been related to the occurrence of predisposing genetic conditions (mutations or polymorphisms) in drug target genes, including hERG or hERG-associated subunits, although the clinical relevance of these genetic variations remains poorly defined at the moment (Roden, 2003).

The increased awareness about the cardiotoxic events prompted by non-sedative antihistamines has also raised concern about the cardiovascular safety of older first generation antihistamines; these molecules are often sold in most countries as 'over the counter' (OTC) or prescribed medications, and are frequently implicated in accidental or intentional poisoning. Therapeutic doses of diphenhydramine (DPH) lengthen cardiac repolarization *in vivo* in normal volunteers and in patients undergoing angioplasty (Khalifa *et al*., 1999). Moreover, pharmacosurveillance

studies have shown that the incidence of ventricular arrhythmic events and cardiac arrests was higher in patients receiving OTC antihistamines (mainly DPH), than in those receiving terfenadine or a non-cardiotoxic, non-antihistaminic reference drug (ibuprofen) (Pratt *et al.*, 1994). Other first generation antihistamines, including hydroxyzine (the metabolic precursor of cetirizine), orphenadrine, promethazine, pheniramine, and chlorpheniramine, are also endowed with a certain degree of cardiotoxic potential, although the risk factors precipitating cardiac events by these drugs are difficult to identify (Taglialatela *et al.*, 2000b).

3.6.2 Cardiotoxic Antihistamines Block hERG/I_{Kr}

In theory, drug interaction with any ion channel that contributes to cardiac repolarization could be arrythmogenic; however, clinically relevant drug-induced QT prolongation invariably results from hERG/I_{Kr} blockade (Perrin *et al.*, 2008). This concept was first proven by antihistamines; in fact, soon after the description of the cardiotoxic manifestations by astemizole and terfenadine in the late 1980s, followed by the cloning of the hERG channels (Warmke and Ganetski, 1994) and the identification of hERG as the main molecular determinant of the I_{Kr} repolarizing current (Curran *et al.*, 1995; Sanguinetti *et al.*, 1995), it was elegantly shown that both cardiotoxic antihistamines block hERG currents (Roy *et al.*, 1996; Suessbrich *et al.*, 1996). The IC_{50s} for hERG blockade by astemizole and terfenadine was in the low nanomolar range of concentration, consistent with the plasma concentrations reached in clinical situations in which patients experienced cardiotoxic manifestations (Honig and Baranjuk, 1996). Using a similar approach, fexofenadine was shown to be ineffective in blocking hERG K channels, whereas desmethylastemizole was highly effective (Vorperian *et al.*, 1996).

These studies prompted several others in attempt to correlate the cardiotoxic propensity of a given antihistamine with its ability to interfere with hERG/I_{Kr}; in fact, hERG/I_{Kr} blockade has been increasingly regarded as a 'surrogate marker' for cardiac toxicity. These studies demonstrated that desloratadine (Kreutner *et al.*, 2000), cetirizine (Taglialatela *et al.*, 1998), the H_1 and platelet-activating factor receptors antagonist rupatadine (Izquierdo *et al.*, 2003), and epinastine (Chachin *et al.*, 1999) were all devoid of a significant interference with hERG/I_{Kr}, a result again supporting their observed lack of significant cardiotoxic potential in humans. On the other hand, ebastine was shown to interact with

mammalian K channels including hERG/I_{Kr} (Ko *et al.*, 1997), although the clinical relevance of these results is still debated (Paakkari, 2002); carebastine, the CYP3A4 metabolite of ebastine, does not seem to affect cardiac K channels (Woosley, 1996).

Conflicting results have also been obtained with loratadine; although most studies suggested that loratadine only marginally interfered with hERG/I_{Kr} (Taglialatela *et al.*, 1998), results obtained in isolated cardiac myocytes at 37 °C have suggested that loratadine and terfenadine display similar IC_{50}s for hERG K channel blockade (Crumb, 2000). These preclinical discrepancies, whose causes are yet unknown, also translated in a significant degree of uncertainty regarding the cardiac safety profile of loratadine, particularly when administered at doses higher that those recommended (10 mg/day) or in association with metabolic inhibitors (Abernethy *et al.*, 2001). Also the potent and selective second generation H_1 receptor blocker mizolastine shows hERG/I_{Kr} blocking activity, although the potency of this interaction seems considerably lower than that of terfenadine or astemizole (Taglialatela *et al.*, 2000a).

With regard to the first generation 'conventional' antihistamines, hydroxyzine (the cetirizine metabolic precursor) and diphenhydramine, at micromolar concentrations, both exerted inhibitory actions on heterologously-expressed hERG K channels; hERG inhibitory concentrations, which are similar to those found to block I_{Kr} in guinea-pig ventricular myocytes (Salata *et al.*, 1995) and to delay cardiac repolarization in feline hearts (Wang *et al.*, 1998), are also within the plasma concentration range found in patients undergoing poisoning with these first generation antihistamines (Sakaguchi *et al.*, 2008). Thus, experimental, clinical, and pharmaco-epidemiological data support the idea that first-generation H_1 receptor antagonists provided of hERG1-blocking ability exert cardiotoxic manifestations under specific clinical settings (Taglialatela *et al.*, 2000b). Intriguingly, given the widespread use of older antihistamines, cardiotoxic manifestations often occur within the context of significant pharmacokinetic or pharmacodynamic interactions with other potentially QT-prolonging drugs belonging to different therapeutic categories; a clear example of this is the inhibition of CYP2D6-mediated metabolism of the antidepressant venlafaxine (see below) by therapeutic doses of diphenhydramine in humans (Lessard *et al.*, 2001).

Having briefly reviewed some of the more relevant data on hERG/I_{Kr} blockade by first and second generation antihistamines, it seems worth pointing out that a fully detailed definition of the cardiotoxic potential of a specific drug cannot rely exclusively on results on hERG blocking affinity, but should always be evaluated within the context of a more

integrated approach involving additional studies on the potential interaction of the molecule with other cardiac ion channels, direct measurements of QT interval duration and dispersion, transmural dispersion of repolarization, T-wave morphologies, drug metabolic pathways, and other biomarkers of drug-induced arrhythmogenesis (Lagrutta and Salata, 2006; Killeen, 2009). Nevertheless, it is useful to define, for each molecule, a provisional safety margin expressed by the ratio between the IC_{50} for hERG/I_{Kr} blockade and the effective therapeutic drug concentration (ETDC). Pooling together available data, it seems possible to divide drugs into five categories with respect to their cardiotoxic potential. In particular, although false positive (amiodarone) and false negative (verapamil) exist, a >30-fold separation between hERG/I_{Kr} activity and $ETPC_{unbound}$ values seems adequate to ensure an acceptable degree of safety from arrhythmogenesis. Noticeably, these data also reveal that molecules belonging to the same therapeutic class do not show the same cardiotoxicity propensity; this is very clearly illustrated by the case of antihistamines. In fact, the astemizole safety margin is only around threefold, and was withdrawn after approximately 16 years on the market due to an unacceptable risk of TdP; by contrast, cetirizine displays a margin approaching 2000-fold, and has been used clinically for the same period of time without a single report of TdP (Redfern *et al*., 2003).

3.7 PRO-ARRHYTHMIC ACTIONS OF PSYCHOTROPIC DRUGS

3.7.1 Clinical Evidence

It has long been recognized that many psychotropic drugs (such as antipsychotics, antidepressants, psychostimulants, analgesics, and mood stabilizers) are associated with risks of cardiac arrhythmia and sudden death (Witchel *et al*., 2003). Among *antipsychotic agents*, phenothiazines appear at higher risk of causing ECG changes including QT prolongation associated with TdPs (Ban *et al*., 1965; Alvarez-Mena *et al*., 1973). In fact, the first report of sudden arrhythmic death with the phenotiazine thioridazine, an antipsychotic drug introduced in 1959 showing a lesser tendency to cause extrapyramidal reactions when compared to other antipsychotics available at that time, dates back to 1963 (Kelly *et al*., 1963); following severe restrictions on the maximum allowable dose of

the drug because of concerns about the drug adversely affecting eyesight, the incidence of thioridazine-induced cardiotoxicity has probably decreased. Nevertheless, dose-dependent QTc prolongation and broad complex tachycardias, including TdPs, have been consistently observed with thioridazine and mesoridazine, as well as with other phenothiazines such as chlorpromazine, perphenazine, trifluoperazine and fluphenazine (Reilly *et al*., 2000); although these effects, in conjunction with other adverse cardiovascular effects like tachycardia and hypotension, were originally linked to the potent antimuscarinic actions exerted by the drugs, studies on the long QT syndrome and the terfenadine story in the early 1990s have increased our understanding of the underlying mechanisms and created a target-based rational explanation for those early observations (Glassman and Bigger, 2001); as a result, thioridazine was discontinued worldwide in 2005. Butirrophenones, like haloperidol and droperidol, have also been shown to cause QT prolongation when used at therapeutic doses (Goodnick *et al*., 2002), and several reports of TdPs caused by these drugs have appeared in the past literature; as a consequence, droperidol has been given a 'black box' warning by the FDA and has been withdrawn from the UK market. The diphenylpiperidine neuroleptic agent pimozide also carries a distinct risk for cardiovascular side effects and QT prolongation; TdPs have been documented after acute poisoning, leading to sudden deaths (Flockhart *et al*., 2000). Therefore, severe use restrictions have been introduced for this drug.

Among newer atypical antipsychotics, molecules showing greater efficacy and reduced extrapyramidal side-effects when compared to older congeners, sertindole and ziprasidone appear to carry the highest risk for cardiotoxic events. In particular, because of the cardiotoxic potential documented in the approval documentation for sertindole, the molecule was never introduced in the USA, and was on the market in Europe for only a few months, before sales were suspended in 1998 because of the increasing evidence of unexpected deaths and serious but not fatal arrhythmias associated with its use (Glassman and Bigger, 2001; Witchel *et al*., 2003). Ziprasidone, on the other hand, was approved by the FDA in 2001; the drug has a modest (apparently smaller than sertindole or thioridazine) but unequivocal effect on cardiac repolarization and prolongs the QT interval, although post-marketing studies revealed no cases of TdP or sudden death at therapeutic doses in either adults or children (McNally *et al*., 2007). Noticeably, the drug exhibits oxidative metabolism mediated only in part by the CYP3A4 pathway, but also including a major aldehyde oxidase pathway, the latter being much less prone to pharmacokinetic interactions (induction or inhibition). Other clinical

reports on atypical drugs possibly showing QT prolongation include both usual and supra-therapeutic doses of risperidone (Ravin and Levenson, 1997), clozapine, and quetiapine (McNally *et al*., 2007), although the role of these drugs as triggers for ventricular arrhythmias and TdPs is still debated.

Quantification of the cardiotoxic risk upon antipsychotic drug exposure presents several technical limitations. Retrospective epidemiological studies on the risks of adverse cardiovascular events in patients taking neuroleptics have revealed that treatment with moderate doses of antipsychotics carried a 2.39 times higher risk of sudden cardiac death compared to patients currently prescribed low doses of antipsychotics, formerly prescribed antipsychotics, or non-users (Ray *et al*., 2001), suggesting a dose-dependent correlation between drug use and cardiac side effect. An increased incidence in cardiac arrests and ventricular arrhythmias was also documented when comparing schizophrenic patients treated with clozapine, haloperidol, risperidone, and thioridazine with non schizophrenic subjects (Hennessy *et al*., 2002). Moreover, it has been reported that conventional antipsychotic use is associated to a two-fold increase in the risk of hospitalization for ventricular arrhythmias and cardiac arrest in nursing home residents (Liperoti et al., 2005); in the same study, it was shown that no increased risk was instead associated with atypical antipsychotics such as olanzapine, clozapine, risperidone, and quetiapine.

The cardiovascular side-effects of *triciclic antidepressants (TCAs)*, including orthostatic hypotension, atrioventricular conduction delay, reduced heart rate variability, and tachycardia are very well known (Witchell *et al*., 2003). Prolongation of the QTc interval can be associated with the assumption of therapeutic doses of desipramine, amitryptiline, nortriptiline, and imipramine, although, among TCAs, maprotiline appears to carry the greatest risk for QT prolongation (Vieweg and Wood, 2004). In those cases in which TdPs were associated with TCA use, concurrent cardiovascular diseases (Coupland *et al*., 1997), conduction abnormalities (Roose *et al*., 1994), or overdosing (Henry *et al*., 1997) could be demonstrated. These considerations explain the recommendation to use alternative drugs in patients with reported history of cardiac diseases (Glassman *et al*., 1993). Considering currently available data, it appears safer to treat depressed cardiac patients with selective *serotonine reuptake inhibitors (SSRIs)* such as fluoxetine, citalopram, paroxetine, sertraline, or fluvoxamine. At therapeutic dosages, these drugs are very rarely associated with QTc prolongation (Pacher *et al*., 1998), have fewer cardiotoxic side effects (Pacher *et al*., 1999), and exhibit a higher margin of safety than TCAs (Barbey *et al*., 1998); nevertheless, other antidepressants such as

bupropion (Isbister and Balit, 2003), venlaflaxine (Peano *et al.*, 1997), and trazodone (Levenson, 1999) can prolong the QTc interval when assumed well above therapeutic doses.

When psychotropic drugs are classified according to their relative risk to cause TdP, most TCAs are classified as category 4 ('drugs weakly associated with TdPs'), implying a small risk; in the same classification, antipsychotics carry a greater torsadogenic potential, with many congeners being classified as category 1 ('drugs generally accepted by authorities to have a risk of causing TdP') or 2 ('drugs that in some reports may be associated with TdP but lack at this time substantial evidence for causing TdP'). A comprehensive list of drugs grouped by risk of TdPs, possible risk of TdPs, and conditional risk of TdPs can be found at www.torsades.org.

Additional psychotropic drugs which are known to inhibit cardiac repolarization leading to a prolonged QT and TdPs in predisposed patients include cocaine (Bilman, 1990), methadone (both after i.v. and oral use) (Kornick *et al.*, 2003; Maremmani *et al.*, 2005), and, possibly, lithium (Mamiya *et al.*, 2005).

As already discussed with antihistamines, drug-induced TdP is rare because blockade of hERG channels is only one of many factors that sum together to prolong ventricular repolarization and make the heart vulnerable to an event that triggers the arrhythmia. A recent study, using a database of 249 patients who developed TdPs while taking psychotropic drugs, showed that the most frequent identifiable risk factors for TdP occurrence were: female gender (71%), pre-existing heart diseases (34%), concomitant use of QT-prolonging drugs (31%), overdosing (27%), history of long QT syndrome (18%), and hypokalemia (14%) (Justo *et al.*, 2005). Thus, similarly to antihistamines, drug–drug interactions (either at the pharmacodynamic and/or pharmacokinetic level) also play a relevant role in determining the cardiotoxic manifestations by psychotropic drugs; therefore, administration of psychotropic drugs that have a QT-prolonging potential is not recommended together with class III antiarrhythmics (that prolong APD by blocking I_{Kr}) or with class I antiarrhythmics (that slow conduction by blocking Na^+ channels), or with metabolizing enzyme inhibitors (Idle, 2000).

3.7.2 hERG/I_{Kr} as Target for Psychotropic Drugs

The clinical data briefly reviewed suggest that wide differences exist among antipsychotics with respect to their propensity to induce QT

prolongation, ventricular arrhythmias and cardiac deaths. As previously introduced, hERG/I_{Kr} appears as a preferred target for most cardiotoxic molecules, and this general rule also largely applies to psychotropic drugs. It is, therefore, likely that the drug-induced QT prolongation observed with antipsychotics involves the inhibition of the hERG cardiac K channel. Thus, following the results on antihistamines, several studies have been performed to investigate the interaction of antipsychotics with hERG/I_{Kr}. After preliminary studies with cisapride (Rampe *et al*., 1997), thioridazine (Drolet *et al*., 1999), and pimozide (Kang *et al*., 2000), all molecules showing blocking potencies in the nanomolar range of concentrations, Kongsamut *et al*. (2002), using patch-clamp electrophysiology on hERG transfected CHO cells, demonstrated a differential affinity for hERG channel blockade by a number of antipsychotic drugs. The resulting hERG-blocking IC_{50} values (expressed in nM) were: 2.7 for sertindole, 18 for pimozide, 167 for risperidone, 169 for ziprasidone, 191 for thioridazine, 5765 for quetiapine, and 6013 for olanzapine. More interestingly, when the affinities of each drug for hERG channel blockade were compared with those for dopamine D_2 receptors and for serotonin 5-HT_{2A} receptors (mediating at least some of the therapeutic efficacy of these drugs), it was demonstrated that sertindole, pimozide and thioridazine displayed little selectivity for hERG (<10-fold) while olanzapine displayed the most selectivity of all the drugs tested (≈200-fold). These data appear in reasonable agreement with the described cardiotoxic propensity for each of these molecules; nevertheless, in addition to hERG channel/receptor affinities, pharmacokinetic considerations will also determine the potential for a drug to prolong the QT interval. In fact, when the IC_{50} values for hERG channel blockade were correlated to QT interval prolongation and plasma drug levels for each drug, it was found that the ratio of total plasma concentration to hERG IC_{50} appeared to correspond well with the observed changes in QT_c. This ratio ranged from approximately 11 for thioridazine (which displayed an almost 30 ms prolongation in QT_c) down to 0.03 for olanzapine (1 ms prolongation in QT_c). More recent data confirmed the low affinity blockade of hERG/I_{Kr} by olanzapine, and suggested that hERG/I_{Kr} blocking concentrations for olanzapine are similar to those measured in patients under conditions of impaired drug elimination, such as renal or hepatic insufficiency, during co-administration of other CYP1A2 substrates/inhibitors, or after drug overdose (Morissette *et al*., 2007). In a direct comparison between hERG blockade and QT prolongation in isolated perfused rabbit hearts for 14 compounds (including several antipsychotics), it has been suggested that drugs could generally be segregated into four groups based

on their potency and mechanism of I_{HERG} inhibition (Katchman *et al.*, 2006). Haloperidol was classified among the most potent I_{HERG} blockers ($IC_{50} < 100$ nM), whereas sertindole and thioridazine were within a second category of compounds blocking I_{HERG} with moderate potency ($IC_{50} = 0.1–1$ μM) and showing some use dependence (see below).

Among antidepressants, similar studies also showed hERG/I_{Kr} inhibition by imipramine and amitriptyline (Teschemacher *et al.*, 1999), fluvoxamine (Milnes *et al.*, 2003), trazodone (Zitron *et al.*, 2004), maprotiline (Ferrer-Villada *et al.*, 2006), and doxepin (Duncan *et al.*, 2007). In all these studies, the IC_{50} for hERG/I_{Kr} inhibition appeared to be in the low micromolar range, significantly higher than that calculated for most potent antipsychotics; this result is in line with the different cardiotoxic potential described for these two drug classes. Noticeably, fluoxetine, despite its relative safety from drug-induced long QT induction, appears to show both direct hERG channel inhibition and a decreased membrane expression of the channels (Hancox and Mitcheson, 2006; Dennis *et al.*, 2007) (see below).

3.7.3 Interaction of Psychotropic Drugs with Other Ion Channels: the Brugada Syndrome

QT prolongation and TdPs are not the only expression of psychotropic drug toxicity at the cardiac level, and psychotropic drugs interaction with other ion channels might explain some distinctive features of specific compounds.

In fact, psychotropic drugs, and particularly TCAs, have been frequently implicated in the induction of another cardiotoxic ECG manifestation called the Brugada syndrome (BrS), an arrhtythmia believed to be responsible for at least 20% of sudden arrhythmic deaths in patients with structurally normal hearts. The BrS is characterized by right bundle branch block and ST segment elevation in the right precordial leads, relatively normal QTc interval, coupled with syncope and sudden death due to ventricular tachycardia/ventricular fibrillation (Yapp and Camm, 2003; Sicouri and Anzelevich, 2008). Although fatalities due to TCA overdose may arise from QT prolongation and TdPs, PR and QRS widening on the EEG have also been observed during overdose with amytriptiline, desipramine, clorimipramine, and nortryptiline.

Similarly to LQTS, genetically-determined forms of the Brugada syndrome also exist; loss-of-function mutations in the SCNA5 gene,

encoding for the main pore-forming subunit of the cardiac voltage-gated Na channel, have been detected in Brugada patients (Chen *et al.*, 1998). The reduction in I_{Na} in Brugada patients would allow epicardial cells (where I_{to} is more prominent) to repolarize much faster than endocardial or midmyocardial cells, thus generating an accentuated intramural dispersion of repolarization, which determines the characteristic ST elevation on the ECG and which may be responsible for triggering an arrhythmogenic re-entry circuit. More importantly, pharmacological blockade of I_{Na} (with flecainide, ajmaline, or procainamide) can unmask concealed forms of Brugada syndrome, and is, therefore, used for diagnostic purposes. In addition to Na channel defects, mutations in the genes encoding for the main (CACNA1C) and accessory (CACNB2b) subunits of the cardiac Ca channel, have also been detected as less common causes of genetically determined Brugada syndrome (Yap *et al.*, 2009). At variance with LQTS, the Brugada phenotype is more prevalent in males (8:1) (Table 3.1).

The genetic data linking I_{Na} defects with the Brugada syndrome, and the fact that this phenotype can be triggered upon exposure to I_{Na} blockers, possibly clarify the mechanism for the drug-induced form of the syndrome by psychotropics; in fact, earlier data indicate that psychotropic drugs, in addition to hERG/I_{Kr}, can also block cardiac I_{Na} (Ogata and Narahashi, 1989), resulting in pro-arrhythmic or antiarrhythmic effects depending on various factors, including the vascularization state of the

Table 3.1 Risk factors for drug-induced long QT syndrome (LQTS)/TdPs and for Brugada syndrome (BrS). (Modified from Sicouri and Antzelevitch, 2008. Reprinted by permission of Taylor & Francis Group, http://www.informaworld.com)

Risk factor	Increased risk for LQTS/TdPs	Increased risk for BrS
Gender	Female (2:1)	Male (8:1)
Bradycardia	+	+
Hypokalemia	+	+
Hypomagnesemia	+	−
Pharmacodynamic drug interaction with QT prolonging agents	+	−
Pharmacodynamic drug interaction with sodium or calcium channel blockers, parasympathetic agonists	−	+
Pharmacokinetic drug interaction with slow CYP inhibitors 2D6, 1A2, 3A4	+	+
Hepatic disfunction (increased drug concentration)	+	+
Genetic predisposition	Congenital LQTS	Congenital BrS

myocardium (Witchel *et al.*, 2003). Noticeably, in these experiments, chlorpromazine was more potent than imipramine and much more potent than haloperidol; this rank order of potency was clearly distinct from that previously mentioned for hERG/I_{Kr} inhibition. In addition, imipramine also interferes with the Ca current (I_{Ca}) flowing during phase 2 of the action potential plateau (Delpon *et al.*, 1991); this possibly explains the fact that the APD may be shortened (rather than prolonged) by imipramine; similar data are also available for the SSRI citalopram (Witchel *et al.*, 2002) and for the typical antipsychotic pimozide (Enyeart *et al.*, 1990).

In addition to TCAs, fluoxetine has also been shown to depress I_{Na} and I_{Ca}, to shorten the action potential, and to induce the Brugada ECG pattern (Yap *et al.*, 2009). Other psychotropic drugs causing the Brugada syndrome also include lithium, a compound commonly used for affective bipolar disorders which, at therapeutic plasma levels, strongly depresses I_{Na} (Darbar *et al.*, 2005), centrally-acting antihistamines, like diphenhydramine and dimenhydrate (during intravenous administration or intentional overdose), and cocaine, a well-known local anesthetic with I_{Na} blocking activity. In general, these rare cases occur within the context of drug combination (antipsycotics and antidepressants), or of drug overdose.

As with LQTS, drug assumption can precipitate the Brugada phenotype within the context of a specific genotype. Cases have in fact been described in which the Brugada phenotype was revealed in desipramine-treated patients carrying SCNA5 mutation (Chow *et al.*, 2005), arguing in favour of a significant role for the genetic background in this form of drug-induced arrhythmogenesis.

3.8 CARDIAC TOXICITY OF ANTIMICROBIAL AGENTS

Several antimicrobial agents have been associated with QT prolongation and/or TdP in clinical reports; as recently reviewed (Simkó *et al.*, 2008), these include macrolides, fluoroquinolones, antifungals, antimalarials, and others. However, it should be underlined that, in most cases, the existence of a clearcut causative relationship between the assumption of a specific drug and the occurrence of the arrhythmic episode is lacking. In the present section, we will review the clinical evidence showing cases of antimicrobial agent-induced cardiac toxicity. Although 'QT liability' associated with most antimicrobials appears primarily caused by an intrinsic capacity to interfere with the function of hERG/I_{Kr}, it should be mentioned that several

of these compounds also act as substrates and/or inhibitors of cytochrome P450 enzymes; thus, in most of these clinical reports, the 'metabolic liability' of these compounds should also be taken into account. In fact, among the risk factors identified in cases of antibiotic-induced arrhythmias, concomitant administration of another QT-prolonging drug or of an inhibitor of drug metabolism was present in 50–60% of the cases (Justo and Zeltser, 2006; Schaffer *et al.*, 2002); at least for fluoroquinolones, additional risk factors include congenital prolonged QT_c (LQTS), bradycardia (particularly in females), hypokalaemia or hypomagnesaemia, and organic heart disease (particularly congestive heart failure) (Rubinstein and Camm, 2002).

3.8.1 Macrolides

Macrolides are characterized by a macrocyclic lactone ring (usually containing from 14 to 16 atoms) to which sugars are attached. The prototype of this class is erythromycin, whereas clarithromycin differs structurally only for the presence of a hydroxylmethyl group; to reduce side-effects and the inhibition of CYP450 isoenzymes, newer molecules have been synthesized, such as azitromycin, spiramycin, dirythromycin, josamycin, erythromycylamine, oleandomycin, and roxithromycin. Numerous reports have been published showing the cardiac adverse effects of macrolides, although different molecules within this therapeutic class appear to display different torsadogenic potential. Based on data collected from 1987 to 2000 from the FDA Adverse Event Reporting System (AERS), erythromycin and clarythromycin appear to be responsible for most of the cases of macrolide-induced TdPs (53% and 36%, respectively) (Schaffer *et al.*, 2002; Kundu *et al.*, 1997; Hensey *et al.*, 2008).

The majority of arrhythmias described with erythromycin have been associated with i.v. administration of the drug at high doses (3–4 g/day), which produces plasma levels of approximately 30 μg ml^{-1} (corresponding to about 40 μM); similar doses have been reported to produce QT prolongation and the induction of ventricular tachycardia, including TdPs, in patients that did not exhibit signs of significant heart disease, or other apparent risk factors that may have contributed to the genesis of the cardiac arrhythmias (McComb *et al.*, 1984; Brandriss *et al.*, 1994). Nevertheless, oral administration of erythromycin (1.5–2 g/day, at a single dose of 500 mg, resulting in serum levels of 2.7–5.5 μM) has also been associated with drug-induced LQTS and with an increased risk of

sudden death (Ray *et al*., 2004; Owens and Nolin, 2006). It is noteworthy that about 70% of cases in which erythromycin was responsible for QT prolongation and TdPs involved females, and the male–female difference was particularly evident when episodes occurred during reproductive age (15–50 years); animal studies also confirmed that females show more pronounced erythromycin-induced QT prolongation compared to males (Drici *et al*., 1998). The reason for such gender difference is as yet unknown, although the 'facilitating' role of estrogens and the 'protective' role of androgens have obviously been called into play.

Because of the side-effects observed with erythromycin and clarithromycin, newer macrolides have been developed. Azithromycin, one of these new compounds, lacks significant interactions with CYP450 enzymes. Although older studies report azithromycin-associated ventricular arrhythmias in patients taking amiodarone (Samarendra *et al*., 2001), dysopiramide (Granowitz *et al*., 2000), or affected by congenital long QT syndrome (Arellano-Rodrigo *et al*., 2001) or with concomitant hypokalemia (Kim *et al*., 2005), more recent reports describe the occurrence of a significant QT prolongation with TdPs in patients taking azithromycin but apparently without additional precipitating risk factors (Huang *et al*., 2007; Kezerashvili *et al*., 2007). Despite these sporadic reports, in view of the widespread worldwide use of azithromycin, this molecule is currently regarded as the safest macrolide available with respect to cardiovascular liability (Owens and Nolin, 2006). Finally, QT interval prolongation with TdPs (in the absence of additional risk factors), has also been reported in neonates during toxoplasmosis prophylaxis with spiramycin (Stramba-Badiale *et al*., 1997), and in both pediatric (Promphan *et al*., 2003) and geriatric (Justo *et al*., 2004) patients treated with roxithromycin.

Preclinical studies reported that concentrations of erythromycin several fold higher than peak plasma levels (>100 μM) can prolong the action potential duration in both guinea pig and canine ventricular myocytes by selective blockade of the rapid component of the delayed rectifier K current (I_{Kr}) (Daleau *et al*., 1995; Antzelevitch *et al*., 1996). More recently, in order to better understand the molecular basis of the different pro-arrhythmic potential of distinct macrolides, Milberg *et al*. (2002) compared the effects prompted by erythromycin, clarithromycin and azithromycin on the cardiac action potential in Langendorff-perfused rabbit hearts. The results obtained revealed that all macrolides tested caused similar QT interval lengthening and dispersion of repolarization; nevertheless, erythromycin and clarithromycin induced a similar number of EADs associated with TdP, whereas no TdP occurred in the presence of

azithromycin, despite the fact that this latter drug showed the largest QT interval increase (another indication that the QT prolongation alone may not serve as a surrogate marker of cardiotoxicity!). Furthermore, azithromycin suppressed erythromycin-induced TdPs, showing instead an anti-arrhythmic proclivity. In order to explain these differences in proarrhythmic-potential among macrolides, these authors also observed that erythromycin and clarithromycin prolonged phase 3 and conferred a triangular shape to the action potential, whereas azithromycin lengthened phase 2, with the action potential assuming a rectangular shape. Phase 3 prolongation may trigger I_{Ca} reactivation, thus leading to EADs and TdPs, whereas this is much less likely to occur during phase 2 prolongation, which impedes I_{Ca} reactivation. These observations may provide a plausible explanation for the different torsadogenic potential of macrolides.

Interaction with hERG/I_{Kr} seems to be crucial for macrolide-induced cardiac toxicity; this was directly tested by Volberg *et al.* (2002), who showed that macrolides all caused a concentration-dependent inhibition of I_{hERG}, although with rather different potencies. Moreover, the ratio between the IC_{50} values for hERG block and C_{max} of the free drug for different macrolides appeared to be smaller for clarithromycin, roxithromycin and erythromycin, all endowed with cardiotoxic potential in humans, when compared to safer drugs such as josamycin, erythromycylamine, and oleandomycin. Qualitatively similar conclusions were also reached by other studies (Stanat *et al.*, 2003). These results indicate that blockade of hERG K channels by cardiotoxic macrolides contributes to their ability to prolong the QT interval and, therefore, to cause TdPs.

It is important to underline that, except for azithromycin, all macrolide antibiotics are potent inhibitors of the cytochrome P4503A4, leading to serious consequences in cases of co-assumption of other QT-prolonging drugs metabolized through the same pathway, such as nitroimidazole antifungals, diltiazem, verapamil, and others, as previously described for psychotropic drugs (Ray *et al.*, 2004).

3.8.2 Fluoroquinolones

The quinolones, especially new 6-fluoroquinolones, have a broad antibacterial spectrum of activity against Gram-positive, Gram-negative and mycobacterial pathogens. They show good oral absorption and tissue penetration in humans, resulting in high clinical efficacy in the treatment of many kinds of infections. Inhibition of bacterial DNA gyrase and

topoisomerase IV enzymes mediate antibacterial effects. The fluoroquinolones' history starts from nalidixic acid, a molecule developed in 1962 showing modest activity against Gram-negative bacteria and low oral absorption. Introduction of a piperazinyl group in nalidixic acid improved activity against Gram-negative organisms (Pseudomonas aeruginosa); moreover, the presence of a fluorine atom at the C6-position of the quinolone structure (such as flumequine) increased the activity against Gram-positive bacteria. Both these structural features were introduced in norfloxacin, which shows significant improvement in antibacterial activity and phamacokinetics in humans. After norfloxacin, many other molecules have been synthesized, including enoxacin, ofloxacin, ciprofloxacin, lomefloxacin, fleroxacin, tosufloxacin, levofloxacin, sparfloxacin, gatifloxacin, prulifloxacin and pazufloxacin. Nevertheless, several postmarketing surveillance studies have revealed unexpected adverse reactions, such as CNS reactions, drug–drug interaction, phototoxicity, hepatotoxicity and cardiotoxicity during assumption of these new molecules. In particular, pro-arrhythmic side-effects of fluoroquinolones have been documented, particularly for grepafloxacin and sparfloxacin (Dupont *et al*., 1996); these observations have led to their withdrawal from the market. QT interval prolongation and TdP were also reported with other fluoroquinolones, such as levofloxacin (Paltoo *et al*., 2001), ciprofloxacin (Knorr *et al*., 2008), gatifloxacin (Fteha *et al*., 2004), moxifloxacin (Altin *et al*., 2007, Sherazi *et al*., 2008). Although cardiotoxicity data from registration clinical trials and spontaneous adverse reaction reports are rather scarce and confounded in most cases by co-administration of other QT-prolonging drugs (as well as other risk factors, such as female gender, co-morbidities, renal failure, and/or electrolyte imbalance), it has been estimated that the rate of sparfluoxacin-induced TdPs is around 14.5 per million, as compared to 1 per million with ciprofloxacin and, probably, levofloxacin; for comparison, the clarithromycin rate is believed to be around 3 per million (Katritsis *et al*., 2003). Thus, most studies suggest that QT prolongation is a fluoroquinolone class effect, but that individual molecules display different arrhythmogenic potential; this also seems to be confirmed by preclinical studies on the interaction of molecules from the fluoroquinolone series with hERG/I_{Kr}, showing that sparfloxacin displayed the highest potency (IC_{50}=18 μM), while lowest activity was associated to ofloxacin (IC_{50}=1420 μM); sparfloxacin, grepafloxacin, moxifloxacin, and gatifloxacin blocked the hERG channel currents at clinically relevant concentrations, while for levofloxacin, ciprofloxacin, and ofloxacin hERG/I_{Kr} blockade only occurred at much higher (possibly supra-therapeutic) concentrations (Kang *et al*., 2001). Thus, similarly to

macrolides, a reasonable correlation also exists between hERG-blockade potencies and cardiotoxic potential in humans for fluoroquinolones (Frotingham, 2001). Moreover, the existence of significant differences in torsadogenic potential by quinolones has also been demonstrated recently in rabbit hearts under condition of hypokalemia; in this experimental model, moxifluoxacin showed the highest TdP-inducing propensity (69%), followed by ciprofloxacin (40%), ofloxacin (21%), and levofloxacin (20%) (Milberg *et al*., 2007). Based on these considerations, new fluoroquinolone agents have been tested for their 'cardiac liability': DW224a (Kim *et al*., 2004), DW286a (Kim *et al*., 2005), gemifloxacin and balofloxacin (Seop Kim *et al*., 2006) displayed low torsadogenic potentials at doses showing antimicrobial activity.

Intriguingly, analysis of the structure–activity relationships for different fluoroquinolone structures and their cardiotoxic potential shows that, although fluoroquinolone antibiotics carry different substituents at the C_5, C_7, C_8 and N_1 positions (Domagala, 1994), molecules behaving as potent blockers of hERG have substituents at the C_5 position, whereas levofloxacin, ciprofloxacin and ofloxacin all lack C_5 substituents and act as poor hERG inhibitors; thus, the least potent hERG blocker are either unsubstituted (ciprofloxacin) or are substituted in a conformationally-restricted manner with a bridge to N_1 (levofloxacin and ofloxacine) (Kang *et al*., 2001),

3.8.3 Antifungals

Development of systemic antifungal agents has evolved rapidly, mainly because of the increased incidence of mucosal and invasive fungal infections. These drugs are classified into five major classes: allylamines, antimetabolites, azoles, glucan synthesis inhibitors, and polyene macrolides. Others include griseofulvin.

Imidazole and triazole antifungal agents act by blocking ergosterol synthesis via inhibition of a specific cytochrome P450 enzyme; administration of ketoconazole, itraconazole, miconazole and fluconazole also inhibits the metabolism of certain drugs through the hepatic cytochrome P4503A4 and, at least for ketoconazole and fluconazole, 2C19 and 2C9 enzymes, respectively. The combination of azole agents and other QT-prolonging drugs whose metabolism is inhibited (e.g. antihistamines, triciclic antidepressants) can lead to serious consequences: ketoconazole, the first imidazole available in the early 1980s, was associated with TdPs when given together with terfenadine (Monahan *et al*., 1990). Also

triazole antifungal agents, including itraconazole and fluconazole, which were approved for use in the early 1990s, have been associated with TdPs (Pohjola-Sintonen *et al.*, 1993; Wassmann *et al.*, 1999; Tatetsu *et al.*, 2006); although these events have often been described in patients with baseline QT interval prolongation (Khazan and Mathis, 2002), hypokalemia and hypomagnesemia (McMahon and Grayson, 2008), or during association therapy with amitriptyline (Robinson *et al.*, 2000) or terfenadine (Honig *et al.*, 1993; Pohjola-Sintonen *et al.*, 1993), they apparently also occur in the presence of a normal K level, absence of other drug therapy, no cardiac ischemia, and normal findings on baseline ECG (Tholakanahalli *et al.*, 2001). QT interval prolongation and non-sustained, polymorphic ventricular tachycardia has also been described upon use of the newer azole agent voriconazole (Alkan *et al.*, 2004).

A recent metanalysis revealed that, in most reported cases of TdP associated with antifungal drugs, additional risk factors could be easily detected (Justo and Zeltser, 2006); these included: female gender, structural heart disease (ischemic heart disease or cardiomyopathy, resulting in congestive heart failure), hypokalemia, hypocalcemia (Ca serum levels lower than 8.5 mg dl^{-1}), hypomagnesmia, advanced age, impaired antifungals metabolism resulting from liver cirrhosis or renal failure, bradycardia, starvation or malnutrition, hypoalbuminemia, digitalis therapy, HIV infection, history of drug-induced LQTS, family history of LQTS, or a prolonged QT interval in the baseline electrocardiogram before drug initiation. Beyond their pharmacokinetic interactions with other QT-prolonging drugs, less is know about the pharmacodynamic effects of anti-fungals: however, ketoconazole was reported to block I_{Kr} in Xenopus oocytes and cause QT interval prolongation (Dumaine *et al.*, 1998); similarly, miconazole (Kikuchi *et al.*, 2005) and clotrimazole (Tian *et al.*, 2006) have been shown to block activated hERG channels, although the same studies also report a direct inhibitory effects of these drugs on another voltage-gated K channel, namely the $K_v1.5$ which underlies I_{to} during phase 1, which occurs at concentrations only twice larger than those reported to block hERG.

3.8.4 Antiprotozoal Agents

3.8.4.1 Antimalarials

Although quinine is the levorotatory diastereomer of quinidine, the two compounds show differential cardiac toxicity; in fact, while the

antiarrhythmic quinidine significantly lengthens the QT interval and shows propensity to TdPs (Selzer and Wray, 1964), this effect is much less pronounced with quinine, which may lengthen the QT interval by only 10%, mostly as a result of QRS widening (White *et al.*, 1983); however, significant cardiotoxicity has been reported in large prospective studies of quinine when used as an antimalaric drug (Hien *et al.*, 1996; van Hensbroek *et al.*, 1996; Dondorp *et al.*, 2005). Nevertheless, combination of quinine with halofantrine or coadminstration with other QT interval prolonging drugs should be avoided, as suggested by the occurrence of TdPs after a single dose of quinine while taking astemizole (Martin *et al.*, 1997). Chloroquine has been shown to prolong the action potential duration (Harris *et al.*, 1988) and to inhibit I_{Kr} (Sanchez-Chapula *et al.*, 2001). While, in healthy subjects, therapeutic doses of chloroquine cause mild and transient QT_c prolongation (Bustos *et al.*, 1994), in the case of accidental chloroquine poisoning (Demaziere *et al.*, 1995), or after long-term treatment with the drug (Stas *et al.*, 2007), polymorphic ventricular tachycardia, QT interval prolongation, and TdPs may occur.

Halofantrine is a valid alternative drug in cases of chloroquine-resistant Plasmodium falciparum malaria. Cardiotoxicity of halofantrine was discovered in 1993 during a clinical study and was confirmed later by some spontaneous reports of sudden deaths of patients treated with halofantrine (Akhtar *et al.*, 1994; Malvy *et al.*, 2000). QT interval prolongation was noted in several studies of patients on halofantrine therapy, especially using doses higher than those recommended (Karkwang *et al.*, 1994; Lavallee *et al.*, 2001; Monlun *et al.*, 1995), or when mefloquine was previously administered (Wesche *et al.*, 2000). Furthermore, QT_c interval prolongation and episodes of TdP occurred in a mother and her son carrying LQTS mutations after receiving halofantrine (Piippo *et al.*, 2001). *In vitro* experiments revealed that halofantrine blocked hERG channels (Tie *et al.*, 2000), preferentially in their open or inactivated configurations.

Mefloquine has been shown to augment the proarrhythmic effect of halofantrine, but whether mefloquine has QT interval prolonging effect *per se* is controversial; Kang *et al.* (2001) found that mefloquine inhibited K_vLQT1/minK channels underlying I_{Ks} with an IC_{50} of about 1 μM, whereas hERG/I_{Kr} currents were about six-fold less sensitive, suggesting that co-administration of mefloquine and halofantrine may synergistically lead to excessive prolongation of the QT interval by a pharmacodynamic mechanism. However, it has also been suggested that this cardiotoxic potentiation may also result from mefloquine-induced increased circulating concentrations of halofantrine (Lightbown *et al.*, 2001).

Two newer antimalarial drugs seem to show a higher cardiac safety. Lumefantrine, despite a high structural similarity with halofantrine, does not have a significant effect on the QT interval. Co-artemether (artemether–lumefantrine association) did not alter the QT_c interval in 13 healthy subjects in a randomized double-blind crossover study; the same subjects, when challenged with halofrantine, showed a significant mean maximum QTc increase of 28 ms (Bindschedler *et al.*, 2000). Artemether seems to be devoid of serious cardiotoxic potential when used in patients affected by malaria (Touze *et al.*, 2002), even in severe cases (Maude *et al.*, 2009). In large support of these clinical observations, *in vitro* studies have revealed that antimalarial drugs show a wide spectrum of affinities for hERG/I_{Kr}; in particular, halofantrine showed the highest potency ($IC_{50} = 0.04$ μM), followed by chloroquine (2.5 μM), mefloquine (2.6 μM), desbutyl-lumefantrine (5.5 μM), and lumefantrine (8.1 μM) (Traebert *et al.*, 2004). These results, together with the calculated cardiac safety indices, suggests that lumefantrine and desbutyl-lumefantrine have a weaker proarrhythmic potential than their comparator compounds.

3.8.4.2 Other Antiprotozoal Agents

Among antiparasitic compounds, pentamidine is an antiprotozoal agent used for leishmaniasis, trypanosomiasis and Pneumocystis carinii pneumonia. Intravenous (Mitchell *et al.*, 1989) or inhalatory (Cardoso *et al.*, 1997) pentamidine therapy seem to trigger TdPs. The work of Katchman *et al.* (2006) failed to find any direct hERG/I_{Kr}-blocking effect or QT interval prolongation in an isolated perfused rabbit heart model, even at relatively high concentrations. Consistent with these results, Kuryshev *et al.* (2005) showed that prolonged exposure to pentamidine caused a reduction in I_{hERG} not because of a direct channel blockade, but rather as a consequence of a decreased expression of the mature, fully glycosylated form of the hERG protein on the cells surface; this mechanism might explain the untoward cardiac effects of pentamidine (Dennis *et al.*, 2007). Finally, treatment of visceral leishmaniasis with meglumine antimoniate (Ortega-Carnicer *et al.*, 1997) or sodium antimony gluconate (Sundar *et al.*, 1998) has been associated with unexplained sudden ventricular arrhythmias and syncopal episodes, caused by QT interval prolongation leading to TdPs. Investigations of the mechanism of this cardiotoxic action have revealed that these compounds do not cause a reduction in hERG/I_{Kr} currents, but rather they increase in cardiac Ca current density which may prolong the action potential duration (Kuryshev *et al.*, 2006).

3.9 WHY IS hERG/IKr AS A PREFERRED TARGET FOR QT-PROLONGING DRUGS?

Virtually all known drugs delaying ventricular repolarization (prolonging the QT interval, thus predisposing to TdP ventricular arrhythmia) inhibit the functional activity of hERG channels responsible for the "rapid" delayed rectifier K current (I_{Kr}); this is particularly striking given the diversity of chemical structures producing hERG K channel blockade. Therefore, several studies have attempted to reveal the molecular basis of this unique susceptibility of hERG channels to inhibition by chemically and therapeutically diverse drugs.

3.9.1 hERG Channel Structure

hERG K channels, similarly to other voltage-gated K channels, are membrane proteins which assemble as a tetramer of subunits, each with a predicted membrane topology consisting of six α-helical transmembrane segments (S_1–S_6); the N- and C-termini are cytoplasmic, and a cyclic nucleotide-binding domain is located in the C-terminus (Figure 3.3A). A pore-forming region located between the transmembrane segments S_5 and S_6 contributes to the ion conduction pathway and the inner pore gate, and is responsible for the astonishing selectivity of the channel for K over Na ions. The recently solved structure of three bacterial non voltage-gated K^+ channels, KcsA (Doyle *et al.*, 1998), MthK (Jiang *et al.*, 2002a; Jiang *et al.*, 2002b), and KirBac1.1 (Kuo *et al.*, 2003), whose membrane core of each subunit only contains the regions corresponding to the S_5–S_6 domain and the intervening linker, has provided a valuable structural model to explain the molecular mechanisms of ion permeation, selectivity and pore opening/closing behavior.

A highly conserved sequence of amino acids (Thr/Ser-Val-Gly-Tyr/Phe-Gly) represents the K channel selectivity filter that coordinates dehydrated K ions, separated by a water molecule, as they pass through the pore in single file. Below the selectivity filter, the pore widens to form a water-filled vestibule, called the inner cavity, lined by residues from the C-terminal ends of the pore helices and by residues from S6. In the closed state, the cavity is relatively small and the S_6 helices bundle together at the intracellular end of the pore to form a narrow aperture that is sufficiently small to restrict the movement of K ions. However, in the open state, bending of S_6 enables the C-terminal ends of the helices to move apart,

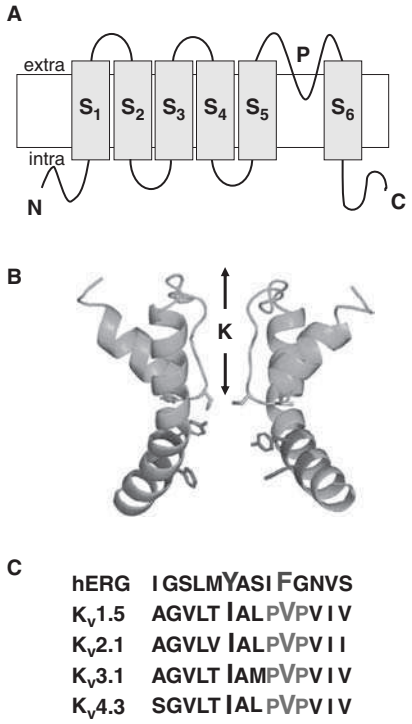

Figure 3.3 hERG subunit structure. A. Topological representation of a single hERG subunit; S_1 to S_6 cylinders indicate transmembrane regions of the channel; P indicates the loop within the pore region. B. Enlarged view of the pore region, which highlights important drug-binding residues, including Tyr652 (blue) and Phe656 (purple) in the S_6 domain, and Thr623 (orange) and Ser624 (cyan) near the selectivity filter. C. Sequence alignment of part of the S_6 domain of hERG and other K_v channels. Tyr652 and Phe 656 of the hERG channel are shown in blue and violet, respectively, and the Pro-Val-Pro motif of other K_V channels is shown in orange. (B and C are from Sanguinetti and Mitcheson, 2005, adapted with permission from Elsevier, Copyright 2005) (see Color Plate 1)

dramatically increasing the diameter of the aperture to allow movement of ions (Figure 3.3B). A conserved Gly function was thought to act as a hinge for activation gating and to confer flexibility for bending; however, Gly is not obligatory and other residues can fulfill this function. Once the activation gate is open, the splaying apart of the cytoplasmic ends of S_6 increases the size and alters the shape of the inner cavity, and this has important consequences for drug binding (Mitcheson, 2008).

In voltage-gated K channel subunits, pore opening is controlled by the voltage-sensing domain (VSD), formed by the S_1–S_4 core region; in particular, a critical gating role has traditionally been assigned to the S_4

segment which contains several positively charged residues spaced by mostly hydrophobic residues, and whose movement through the membrane electric field appears to represent the first gating transition in response to changes in membrane voltage (Bezanilla, 2000; Yellen, 2002). The crystal structure of the first voltage-gated K^+ channel subunits containing six transmembrane segments including a VSD, namely the bacterial K_vAP (Jiang *et al*., 2003;) and the mammalian K_v1.2 (Long *et al*., 2005a; Long *et al*., 2005b) seems to support such a view, although the intimate details of such movement, including the position of the VSD in the closed channel configuration, the extent of VSD dislocation during activation (ranging from 2 to 15–20 Å), the relative role of the hydrophobic membrane interface, and the coupling of such movement to the inner pore gate, still remain highly controversial (Tombola *et al*., 2005).

3.9.2 State-Dependent hERG Blockade

The biophysical properties of drug-induced hERG blockade are consistent with a discrete state-dependent blocking mechanism occurring from the intracellular side of the membrane (Clancy *et al*., 2003). Similarly to other hERG blockers (and to local anesthetics or class I antiarrhtythmics) (Liu *et al*., 2002), methanesulfanonilides such as dofetilide, E-4031, and MK-499 act as open-channel blockers, since they require channel opening for access to this intracellular binding site. In addition, mutations that result in loss of inactivation reduce affinity for methanesulfonanilides, suggesting that inactivation may be required for stabilization of drug binding. Therefore, once they gain access to their binding site during channel opening, drugs become trapped inside the hERG inner cavity by the inactivated channel conformation. Methanesulfanonilides are less effective at inhibiting hERG K channels during strong depolarization (e.g., +60 mV), possibly because, at these positive voltages, extremely rapid inactivation occurs which may reduce the time spent by the channel in the open state, thereby preventing the drug from accessing the binding site. Recovery from hERG blockade by methanesulfonanilides is extremely slow, even at negative holding potentials when most channels are in closed states. Using a mutant hERG channel that has the unusual property of opening in response to hyperpolarization (Sanguinetti and Xu, 1999), it was shown that methanesulfonanilides are trapped in the inner vestibule by closure of the activation gate. Opening of the channel in response to hyperpolarization allowed release of the drug from its receptor.

3.9.3 Molecular Determinants of hERG Blockade

Mitcheson *et al.* (2000), using alanine scanning mutagenesis coupled with homology modelling based on the bacterial KcsA channel, identified amino acids located on the S6 transmembrane domain (G648, Y652, and F656) and pore helix (T623 and V625) of the hERG channel subunit that face the cavity of the channel to be crucial determinants for high-affinity binding by the methanesulfonanilide MK-499 (Figure 3.3C). The crucial role of Y652 and F656 was confirmed by studies using two additional torsadogenic drugs (the gastrointestinal prokinetic drug cisapride and the antihistamine terfenadine), whereas G648, V625, and T623 appeared to be specific for methanesulfonanilides, since mutations at these sites failed to interfere with terfenadine and cisapride ability to block hERG currents. Binding of another methanesulfonanilide drug, dofetilide, also involved Y652, F656, as well as several other residues (Lees-Miller *et al.*, 2000). Evidence supporting the involvement of these aromatic residues in S6 in drug binding has also been obtained for thioridazine (Milnes *et al.*, 2006), droperidol (Schwoerer *et al.*, 2007), ziprasidone (Su *et al.*, 2006), doxepin (Duncan *et al.*, 2007), maprotiline (Kiesecker *et al.*, 2006), and chlorimipramine (Jo *et al.*, 2008); despite slight quantitative changes in the dependence of drug efficacy on membrane voltage, all these drugs also appear to preferentially block hERG currents in the open/inactivated state.

Noticeably, alignment of hERG with other voltage-dependent K_V channels reveals the presence of isoleucine or valine residues in the corresponding positions of Y652 and F656 residues of hERG channels, respectively (Figure 3.3C) (Sanguinetti and Mitcheson, 2005); this led to the suggestion that aromatic residues in S_6 may be consensus determinants of high-affinity hERG channel blockade via cation-π and/or π-stacking interactions with the basic tertiary nitrogen and aromatic groups of the blocking drug. Since aromatic residues are capable of both hydrophobic and electrostatic interactions, Y652 and F656 were systematically mutated to different residues, in order to determine how the physicochemical properties of the amino acid side group affected channel block by cisapride, terfenadine, and MK-499. The results obtained revealed that the potency for block by all three drugs was well correlated with measures of hydrophobicity, especially the two-dimensional approximation of the van der Waals hydrophobic surface area of the side chain of residue 656; thus, the side chain of this residue may be mostly involved in π-stacking interactions with aromatic portions of the blocking molecule.

For residue 652, an aromatic side group was essential for high affinity block, suggesting the importance of a cation-π interaction between Y652 and the basic tertiary nitrogen of these drugs (Fernandez *et al*., 2004).

Differential sensitivity of drug-induced hERG inhibition by mutations of either Y652 and F656 residues seems evident; as an example, while Y652 appears not to be important as a molecular determinant of blockade by erythromycin (Duncan *et al*., 2006), the F656C mutation in the distal S6 of hERG completely abrogated block; binding to this site by the macrolide antibiotic appears strongly influenced by temperature, an effect explained by the temperature-dependent access of erythromycin to its intracellular binding site (Guo *et al*., 2005). On the other hand, mutagenesis of the S_6 helix residue F656 failed to eliminate or reduce the moxifloxacin-mediated block whereas mutation of Y652 reduced it by approximately 66% (Alexandrou *et al*., 2006). In the case of azole antifungals, which appear to interfere with hERG function both by a direct blocking mechanism and by disrupting intracellular maturation of the hERG protein, mutations in the drug-binding sites (F656C or Y652A) significantly attenuated the hERG current blockade by ketoconazole (Ridley *et al*., 2006) and miconazole (Kikuchi *et al*., 2005), but did not affect the ketoconazole-induced disruption of trafficking (Takemasa *et al*., 2008), suggesting that the molecular determinants for these two effects are distinct. Also hERG current blockade by halofrantine was reduced by mutations of Y652 and F656 aromatic residues in S_6 (Sanchez-Chapula, 2004).

While the physicochemical implications of the two aromatic residues in S_6 appear rather clear, the role of T623, S624, and V625 in the S_5–S_6 linker, which are not unique to hERG channels, is much less understood; it seems likely that, together with the aromatic residues in S_6, they might create an energetically favorable environment in which compounds can establish several contacts with the channels (Mitcheson, 2008). This issue was also approached by a combined patch clamp electrophysiology and comparative molecular similarity analysis (CoMSiA) approach by Pearlstein *et al*. (2003), using a series of sertindole analogs in which structural features were systematically omitted or changed. The results obtained were summarized as follows: (i) The hydrophobic features optimally consist of an aromatic group that is capable of engaging in π-stacking with a F656 side chain. Optionally, a second aromatic or hydrophobic group present in some inhibitors may contact a different F656 side chain (one of the first evidences for subunit cooperation in drug binding, but see below). (ii) The basic nitrogen is proposed to undergo a π-cation

interaction with Y652. The variation in basicity of this feature across the studied inhibitors is suggestive of a π-cation versus salt bridge interaction, which would preferentially involve a tertiary nitrogen. (iii) The tail region of the molecules occupies the pore between Tyr652 and the selectivity filter. This region is structurally and conformationally diverse, and extends maximally to the selectivity loop. These modeling studies generate a 'drain plug' picture of hERG channel inhibition, with the drain plug shape complementary to the cylindrical architecture of the pore, with key interaction sites on the drain plug 'handle' [aromatic feature(s)] and along the length of the plug (basic nitrogen and polar group). The depth of penetration can vary, depending on the length of the tail region, maximally extending to the intracellular end of the selectivity filter. Qualitatively similar conclusions were also reached by Choe *et al.* (2006).

Altogether, these results suggest that a basic nitrogen and two or three aromatic components at appropriate distances from the amine confer increased hERG activity. Based on these considerations, Murphy *et al.* (2007) synthesized a novel series of fluoroquinolone antibacterials by decreasing the basicity of the amine with the electron-withdrawing nitrile group: some of these compounds seem to be promising as they showed reduced hERG activity, low potential for human genotoxicity, good pharmacokinetics in rats, and activity against resistant strains of *S. aureus* and *S. pneumoniae*. However, it should be mentioned that, in this work, displacement of dofetilide binding was used as an indication of hERG activity; although remote, the possibility remains that these novel compounds might affect hERG function upon binding in a non-competitive manner with methanesulfonanilides.

Another important characteristic that appears to contribute to the pharmacological promiscuity of hERG channels and that can be derived from sequence comparison between hERG and other voltage-gated K channels, is the lack of the S_6 proline motif (P-x-P) that restricts inner cavity size in the other K_V channels, probably by causing a kink in the inner (S_6) helices (Figure 3.3C); this suggests that the vestibule of the hERG channel is larger than the well-studied Shaker K^+ channel, allowing molecules of larger size to gain access to the channel cytoplasmic cavity.

hERG channels are composed of four identical subunits that are assembled into a four-fold symmetric tetramer structure and, to date, structure function analyses have compared only wild type or mutant homotetrameric channels. However, the blocking drugs are asymmetric in their structure and large enough to span multiple subunits. Thus, aromatic residues (Y652 and F656) from different subunits could contribute unequally to the binding during drug–channel interaction. To

identify the contributing residues and their positions in a tetrameric channel, Myokai *et al.* (2008) assessed cisapride blockade and binding by using tandem dimers of hERG incorporating wild type and/or mutant subunits in which one or both of Y652 and F656 had been mutated to alanine residues. These experiments revealed that both Y652 residues on adjacent subunits are required for high-affinity drug binding; in contrast, the role of F656 in drug blocking affinity seems to be less crucial. These results provided the first direct experimental evidence of the importance of the particular arrangement of the aromatic residues for high affinity binding: drug affinity was higher on dimers containing only wild type or double mutant subunits, than the affinity measured on dimers incorporating a single mutation for each subunit. The data are interpreted as suggestive of additive interactions between Y652 and F656 on the same subunit, but cooperative interactions between these residues on adjacent subunits (Hancox and James, 2008). The findings of Myokai *et al.* (2008) are also broadly consistent with a recent simulation study involving cisapride docking to the hERG K channel tetramer (using a template based on KvAP), which suggested T-shaped π–π stacking interactions between cisapride and diagonally opposite Y652s and a parallel displaced interaction with an F656 (Farid *et al.*, 2006).

3.10 CONCLUSIONS

Since the first drug-induced syncopal episodes and sudden deaths in the 1960s (Selzer and Wray, 1964), a huge number of clinical studies report that QT prolongation and/or TdPs, possibly leading to sudden death, are associated with the use of many drugs (as listed at www.torsade.org), having widely diverse pharmacological actions. Because of the enormous clinical relevance of these serious adverse efects, and of the widespread use of these potentially fatal drugs, many efforts have been addressed to identify the cardiotoxic potential very early during the drug development process, using both *in vitro* and *in vivo* tests. Moreover, recently developed *in silico* methods to predict hERG channel blockade may provide cost-effective screening tools for use in conjunction with other *in vitro* and *in vivo* assays that predict the cardiac liability of new chemical entities (Recanatini *et al.*, 2008). In the near future, *in silico* models are likely to facilitate the prediction of QT-prolongation liability and identify strategies for improving drug safety. Several examples in which binding to hERG has been successfully removed during the lead optimization

phase of drug discovery without affecting target biological activity have been recently reported; these include phosphodiesterase-4 (PDE4) inhibitors, neuropeptide-Y (NPY) antagonists, KDR kinase inhibitors, and β-tryptase inhibitors (Li *et al.*, 2006).

It seems astonishing that only about 25 years ago we were largely unaware of the molecular basis of most cardiac ion currents, of the genetic diseases affecting them, and of how their detailed functional coordination allowed heart contraction to occur; building on the many contributions achieved in these years, a much better knowledge of drug-induced arrhythmogenesis is available, which has already translated into improved patient care and the availability of safer drugs for clinical use.

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4

Cardiovascular Toxicity of Antitumor Drugs: Dimensions of the Problem in Children

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4.1 INTRODUCTION

Advances in chemotherapy have greatly improved the survival of children diagnosed with cancer. Before the 1970s, 5-year survival of these children was less than 50%; today it is 80% [1,2]. The spectrum of cancers that can now be successfully treated ranges widely and includes a variety of hematologic malignancies and solid tumors. Despite this success, many antitumor drugs are limited by marked toxicity. The cardiotoxic effects of these drugs are of particular concern; cardiac injury related to chemotherapy is often progressive and irreversible. In the US alone, the population of long-term survivors of pediatric cancers is estimated to be more than 360 000, and this number is increasing [1,2]. As such, clinicians should employ cardioprotective strategies during therapy and monitor cardiac function before, during, and after administration of cardiotoxic antitumor drugs.

Cardiotoxicity of Non-Cardiovascular Drugs Edited by Giorgio Minotti

Several classes of antitumor drugs have cardiotoxic effects in children. Drugs such as anthracyclines, alkylating agents, and metabolites are well recognized for their cardiotoxicity and are common components of pediatric cancer treatment regimens. The mechanisms by which these agents damage cardiac tissue differ by drug type and are associated with a range of cardiac sequelae, including cardiomyopathy, arrhythmia, ischemia, and hypertension. In the era of multi-agent cancer treatment regimens, damage to cardiac tissue is not only sequential but also synergistic, as illustrated by the marked acute cardiotoxicity of anthracyclines used in conjunction with Herceptin. The trade-offs between treatment efficacy and cardiotoxicity must be considered before any chemotherapy regimen is initiated.

Several potential cardioprotective strategies have been successful to varying degrees. The iron-chelator dexrazoxane has shown the greatest success in reducing the cardiotoxic effects of doxorubicin in women with breast cancer and in children with acute lymphoblastic leukemia. Other potentially cardioprotective agents include intravenous immunoglobulin, beta-blockers, angiotensin-converting enzyme inhibitors, and calcium channel blockers. A metabolic cocktail, including levocarnitine, co-enzyme Q_{10}, thiamine, and riboflavin has also been suggested.

Vigilant cardiac monitoring is also vital. During treatment, cardiac biomarkers, including troponin T, brain natriuretic peptide, and various growth factors, hold the greatest promise for the sensitive and specific identification of acute cardiac cell damage and death, the early injury that sets the stage for long-term cardiac declines. Currently, echocardiography remains the reference standard for cardiac monitoring during the years after anthracycline treatment.

Knowing these risks, treatment strategies, and diagnostic options allows clinicians to individualize treatment, and to monitor cardiac function both during and after therapy. Understanding risk factors will also improve long-term planning for childhood cancer survivors, where underlying cardiac vulnerability can be taken into account when anticipating cardiac stresses from, events such as, pregnancy or exercise programs.

4.1.1 Survivorship and Incidence

The incidence and severity of cardiac damage in children receiving antitumor drugs varies with age, sex, type of drug, dosage, and the presence of comorbidities. The damage occurring in childhood may appear at or near the time of treatment, but it also often develops several years after

treatment. A retrospective study of the Childhood Cancer Survivor Study by Oeffinger *et al*. examined the late-effects of childhood cancer in 10 397 adults diagnosed with cancer between 1970 and 1986. Compared to 3034 age-matched controls, survivors had a 15-fold higher rate of congestive heart failure and a 10-fold higher rate of cardiovascular disease [3]. The number of survivors suffering from any severe or life-threatening chronic health condition was also higher than in controls, and this risk was greater in women. A similar report by Mertens *et al*. analyzing the 5-year mortality rates of 20 227 survivors in the same study cohort showed that their cardiac mortality was 8-fold higher than that of healthy controls. As expected, treatment with anthracyclines, bleomycin, or chest radiation was strongly correlated with increases in cardiac mortality [4]. Extended follow-up has found that these increases persist throughout survivorship [5].

These longitudinal studies of childhood cancer survivors illustrate the late-effects of drug-induced cardiac damage acquired at the time of treatment. The extended survivorship of pediatric cancer patients shows that defining successful cancer treatment in this population means balancing the efficacy of treatment against its late effects, such as cardiotoxicity.

4.2 DRUGS WITH KNOWN CARDIOTOXICITY

4.2.1 Anthracyclines

Anthracyclines, a class of highly effective antitumor agents, are the first-line treatment for a wide spectrum of hematologic malignancies and solid tumors. The first anthracyclines were isolated from Streptomyces bacteria in the early 1950s and were immediately recognized for their highly active cytotoxicity [6]. Anthracyclines, such as doxorubicin, daunorubicin, epirubicin, and idarubicin, have been fundamental in improving 5-year survival for pediatric cancer patients and are a mainstay in many chemotherapy regimens. Despite this success, anthracyclines are limited by their well recognized cardiotoxicity.

Anthracycline cardiotoxicity can be categorized as acute, early-onset, or late-onset [10]. In acute cases, symptoms occur within hours or days of administration, often presenting as arrhythmias and disturbances in intracardiac conduction, such as nonspecific ST-segment and T-wave abnormalities [11–14]. In rare cases, pericarditis and acute left

ventricular (LV) failure can occur. Clinically important declines in cardiac function that occur within 1 year of treatment are considered early-onset, and any declines occurring after 1 year of treatment are considered late-onset.

Early- and late-onset anthracycline cardiotoxicity is characterized by progressive LV dysfunction that in some cases may progress to heart failure (HF). The incidence of clinical cardiotoxicity ranges from 1% to 16% and increases over time [7–10,15–20]. A study by Lipshultz *et al.*, evaluating the cardiac health of 115 children who received doxorubicin during treatment for acute lymphoblastic leukemia (ALL), found that 65% of children had subclinical changes in cardiac function, including increased left ventricular afterload, decreased contractility, or both, at a median of 6.4 years after treatment [7]. In these children, the most significant predictor of abnormal left ventricular afterload (measured as end-systolic wall stress) was the cumulative dose of doxorubicin ($P < 0.002$). Other longitudinal studies have reported similar effects of doxorubicin on the late cardiac health of children treated for cancer [8,9]. These cardiotoxic effects are often persistent, pervasive, and progress over time.

Although anthracycline-related cardiac damage is often defined as a decrease in LV fractional shortening or ejection fraction (LVEF), patients who present with only mild changes in LV systolic function may still have hemodynamically important cardiac dysfunction, and the severity of their cardiac symptoms may not be immediately apparent [7,8]. Despite preserved left ventricular systolic function (LVEF $> 50\%$), patients may still experience HF in which symptoms are related more to filling pressures than to the LVEF. In contrast to the HF associated with reduced LVEF, the 1- and 5-year survival rates for the diastolic form of HF in cancer patients have remained unchanged [21,22]. The better survival rates for patients with HF associated with reduced LVEF underscores the fact that there is no proven therapy for HF with preserved or normal LVEF of the kind seen in anthracycline-treated pediatric cancer patients.

The effect of anthracycline-related cardiotoxicity is clearest on pathologic examination. In patients treated with anthracyclines, cardiac tissue shows a marked loss of myocytes that is accompanied by vascular and mitochondrial degeneration and interstitial fibrosis [7,8,15,35–39]. The initial loss of myocytes causes the LV wall to thin and is detectable by echocardiography. As the child grows, the remaining cardiac cells compensate by becoming hypertrophic to maintain an LV mass that is closer to normal for body surface area. This compensatory effect may only by histology of individual cardiomyocyte hypertrophy. The dilated cardiomyopathy that

develops during the first year after treatment can progress to a restrictive type characterized by abnormal diastolic function and elevated LV filling pressures [7,8]. The progressive nature of anthracycline cardiotoxicity can also be accelerated by underlying heart disease, such as congenital defects or those acquired later in life, from vascular disease or hypertension [40,41]. Additive factors, such as radiation, may worsen atherosclerosis and increase cardiac injury.

The mechanism of anthracycline cardiotoxicity continues to be explored but appears to be in part related to free radicals. Anthracyclines enter cells through passive diffusion and accumulate in concentrations 10- to 500-times higher than their extracellular concentrations. Here, anthracyclines bind to iron and form complexes that generate free radicals capable of lipid peroxidation and DNA damage [23–28]. Within the cardiomyocyte, anthracyclines shows a high affinity for cardiolipin, a lipid in the inner mitochondrial membrane. This affinity leads to acute concentrations of anthracyclines within the mitochondria, which impairs energy metabolism by damaging mtDNA and membrane integrity in these vitally active cells [29–31].

Numerous additional mechanisms of anthracycline damage have also been implicated in treatment-related cardiomyopathy (Figure 4.1). These mechanisms include the formation of toxic metabolites, reduced gene expression, inhibition of protein synthesis, release of vasoactive amines, disturbances in creatine kinase activity, apoptosis, changes in intracellular calcium homeostasis, induction of nitric oxide synthetase, and increases in certain immune functions [32–34].

The occurrence and severity of anthracycline-related cardiotoxicity depends on several drug- and patient-related factors. The strongest predictor of acute cardiac injury is the total cumulative dose of anthracycline received [7–9,16,17]. Children receiving doses greater than 400 mg m^{-2} are at the greatest risk of cardiac damage [16,17]. At cumulative doses of 400 mg m^{-2}, the incidence of doxorubicin-related HF approaches 3%; at doses of 550 mg m^{-2}, the incidence is 7%; and at doses of 700 mg m^{-2}, the incidence is 18% [16,17]. The incidence of non-HF cardiac injury is higher.

The agents comprising the anthracycline class of antitumor drugs are cardiotoxic in varying degrees and should not be assumed to have similar effects. Anthracyclines at any dose can result in cardiac damage, and the long-term risk of HF is unclear [8]. Children who received cumulative doses of anthracyclines less than 300 mg m^{-2} can have normal echocardiographic values up to 8 years after anthracycline therapy, but over the long-term, even children who received a single dose of doxorubicin may

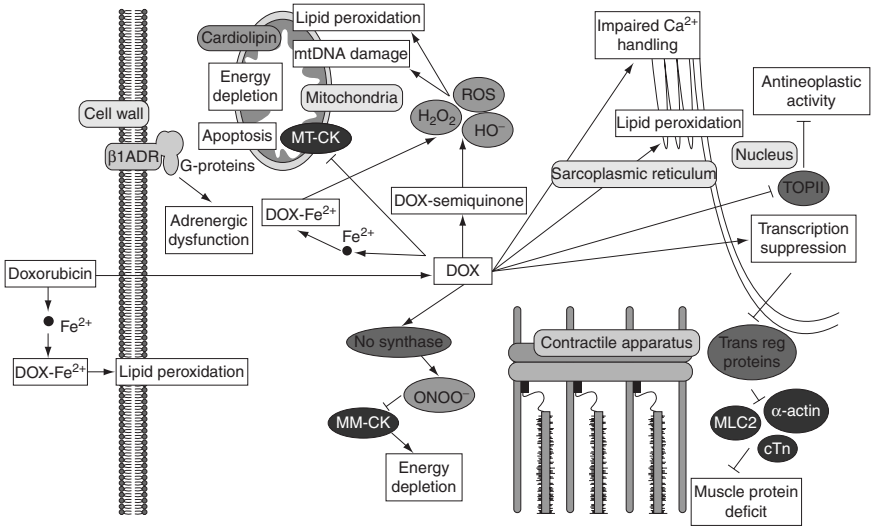

Figure 4.1 Mechanism of anthracycline toxicity in the cardiac myocyte. Anthracyclines enter cardiomyocytes by passive diffusion and spur the generation of free radicals, leading to cell damage. Anthracyclines also directly and indirectly inhibit gene transcription, mitochondrial functioning, and energy production in the cell. β1ADR: beta 1 adrenergic receptor; Ca: calcium; cTn: cardiac troponin; DOX: doxorubicin; Fe: iron; MLC2: myosin light chain; MM-CK: muscular creatine kinase; MT-CK: mitochondrial creatine kinase; ROS: reactive oxygen species; TopII: topoisomerase II. (Lipshultz S. E., Rusconi P., Scully R. E., Chapter 18: Assessment of cardiotoxicity during anti-cancer therapy. in *NT-proBNP as a Biomarker in Cardiovascular Diseases*, eds: Januzzi J. L., Bayes-Genis A., 2008, Chapter 18, Prous Science, Barcelona. (Reproduced with permission from Prous Science. Copyright 2008)

exhibit echocardiographic abnormalities [8,9]. There is no safe dose of anthracyclines.

The age of patients receiving anthracyclines is also a key predictor of cardiotoxic risk. In general, the younger the patient, the greater the susceptibility to cardiac damage, with infants under the age of 1 year being the most vulnerable. Likewise, the synergistic and additive effects of concomitant therapies are critical in assessing anthracycline cardiotoxicity. These factors include the use of multi-drug regimens with other cardiotoxic chemotherapeutic agents, as well as the cumulative dose of mediastinal radiation. Treatment protocols using more than 30 Gy of radiation clearly increase the risk of cardiac injury and may amplify anthracycline-induced damage [42,43].

The incidence of acute and late-onset cardiotoxicity may also depend on the rate and schedule of anthracycline delivery [44,45]. The cardiotoxic

effects of anthracyclines are also greater in females, Black children, and patients with trisomy 21 [7–10,35,46–48]. The combination and sequence of administration of antitumor drugs can also amplify cardiotoxicity, as can previous anthracycline therapy. Preexisting heart disease can also increase an agent's toxicity when conditions such as hypertension and structural or valvular heart disease are exacerbated. Different patients may also have drug hypersensitivities. Children with co-morbidities, such as diabetes, obesity, renal dysfunction, pulmonary disease, endocrinopathies, electrolyte and metabolic abnormalities, or infection may also be more vulnerable to cardiotoxic antitumor drugs.

Despite the identification of risk factors and their recognized effects on children receiving chemotherapy, many questions remain regarding the safe dosing of anthracyclines. Optimal treatment is still unclear for patients with preexisting heart disease or those who have received prior anthracycline therapy, a factor that limits the cumulative secondary dose. Optimal treatment for patients with diabetes, obesity, and other comorbidities is also uncertain. Likewise, the administration of multiple cardiotoxic drugs, such as cyclophosphamide, paclitaxel, and trastuzumab, all need to be considered when planning anthracycline dosing.

4.2.2 Mitoxantrone

Mitoxantrone is commonly used to treat acute leukemias, breast cancer, and lymphomas. It is an anthracycline analog and was developed to maintain or improve the antitumor activity of anthracyclines while at the same time reducing cardiotoxic side effects. Some studies have shown that mitoxantrone-related cardiotoxicity is lower than that of other anthracyclines, but mitoxantrone is associated with the development of HF, as well as with the deterioration of cardiac function, although to a lesser extent than that seen with doxorubicin [49–51].

In a systematic review, van Dalen *et al*. estimated that the cumulative incidence of mitoxantrone-related symptomatic cardiotoxicity varies between 0 and 6.7% and that asymptomatic cardiac damage varies between 0 and 80% [52]. These estimates are limited, however, given variations in methodologies among the studies reviewed. Some evidence shows that mitoxantrone is less cardiotoxic than doxorubicin and other anthracyclines, but it is still cardiotoxic. Reports of asymptomatic cardiac damage in a large portion of children receiving mitoxantrone should

also underscore the need for long-term monitoring of these patients to detect and treat any decreases in heart function.

4.2.3 5-Fluorouracil

The pyrimidine analog, 5-fluorouracil (5-FU), is an antimetabolite recognized for its antitumor properties. This agent is a key component of various cancer treatment protocols and effectively treats several types of cancer. Despite this success, the incidence of cardiotoxicity associated with 5-FU is 1.2 to 7.6% [53–55a,b]. Although this cardiac damage is rarely life-threatening (<1%), morbidities such as arrhythmia, ischemia, and HF can occur [53–57]. Additionally, 5-FU cardiotoxicity is dose-dependent but typically reverses when administration is stopped [54].

Children at risk of 5-FU cardiotoxicity must be monitored closely. Although the mechanism by which 5-FU damages cardiac tissue is unclear, symptoms implicating myocardial ischemia are often present [53]. Patients presenting with chest pain or electrical disturbances, such as elevation or depression of the ST segment and an inverted T wave, are compatible with 5-FU toxicity [53,58]. Severe dysfunction is rare, but impaired contractility and arrhythmias may also indicate cardiac injury and hence the need to stop administration [54]. As is the case with other cardiotoxic antitumor drugs, in patients with preexisting cardiovascular disease, combination chemotherapy and mediastinal radiation may worsen cardiotoxic effects.

The frequency of ischemia-related symptoms in 5-FU cardiotoxicity suggests changes in the coagulation system [53–59]. These changes are thought to result from vasospasms in the coronary arteries, direct toxicity of the myocardium, and the upregulation of coagulation factors; however, direct mechanisms are still unclear [60,61]. In mice, radiolabeled 5-FU concentrates in the myocardium, where it readily enters cells and is converted to several active metabolites [61,62]. These 5-FU metabolites exert their anticancer activity by inhibiting thymidylate synthase, as well as by being misincorporated into RNA and DNA. The inhibition of thymidylate synthase prevents the synthesis of thymidine monophosphate and, thus, stops an essential step in the only pathway providing this essential nucleotide to the cell. Any inhibition in thymidylate synthase will have cytotoxic effects [61–64]. As with other cardiotoxic antitumor agents, children receiving 5-FU treatment should be monitored closely for signs of cardiac distress both during and after treatment.

4.2.4 Cyclophosphamide

The alkylating agent cyclophosphamide is an essential component of many pediatric chemotherapeutic regimens as well as pretransplant protocols [65–69]. In low doses, cyclophosphamide is well tolerated. At high doses, or when used with other cardiotoxic chemotherapeutic agents, it can cause cardiac damage, resulting in arrhythmias, myopericarditis, ischemia, and HF [65–69]. The risk of HF increases with dosage; Gottdiener *et al*. showed that 28% of 32 cancer patients receiving high doses of cyclophosphamide (180 mg kg^{-1} over 4 days) experienced congestive heart failure [66,67]. Other studies of cyclophosphamide cardiotoxicity in non-cancer patients report similar cardiotoxicity.

4.3 ADDITIVE CARDIOTOXICITY RISK FACTORS

4.3.1 Multidrug Regimens

Cardiac complications of specific antitumor agents are a serious concern when treating children, and these complications are compounded in multi-drug regimens. Treatment protocols may involve several antitumor drugs, each capable of injuring cardiac tissue with its own unique cardiotoxic effects. Even agents without known cardiotoxicity can potentially worsen the effects of other antitumor drugs, substantially increasing the risk and severity of cardiovascular disease.

As new agents are introduced, clinicians should remain aware of the risk of additive cardiac injury and the potential for synergistic effects. The additive risk of multidrug chemotherapy is best exemplified by a clinical trial of trastuzumab in conjunction with doxorubicin [74]. Trastuzumab is a highly effective monoclonal antibody that binds to the extracellular domain of human epidermal growth factor receptor-2, a membrane-bound receptor tyrosine kinase that is overexpressed in some breast cancers [70–73]. During an early clinical trial comparing trastuzumab plus chemotherapy with chemotherapy alone in 469 patients with metastatic breast cancer, 27% of patients receiving both trastuzumab and doxorubicin experienced symptomatic or asymptomatic cardiotoxicity. These cardiac effects were evident in women receiving only trastuzumab, but only in 4% of patients, which highlights the almost 4-fold increase in cardiac injury these drugs exert when used together [74]. The ability of

trastuzumab to hypersensitize the heart to anthracycline toxicity illustrates the complexity of drug interactions and the need for careful cardiac monitoring both during and after treatment, particularly when novel agents or drug combinations are used.

4.3.2 Radiation

Mediastinal radiation in conjunction with chemotherapy is associated with an increased risk of cardiovascular disease. This risk includes progressive development of coronary artery disease, arrhythmias, valvular disease, and restrictive cardiomyopathy [75–77]. The risk of developing atherosclerosis is of particular concern where radiotherapy can damage endothelial cells, leading to ischemia and myocyte death [76]. Cardiac mortality in children receiving radiation treatment is greater than that in the general population and in survivors of childhood cancer not treated with radiation [75]. Adams *et al*. reported that 14 years after mediastinal radiotherapy (median, 40.0 Gy; range, 27.0 to 51.7 Gy), 20 of 48 long-term survivors of pediatric Hodgkin's lymphoma had marked valvular disease, 36 had conduction defects or arrhythmias, and 16 had grossly abnormal peak myocardial oxygen consumption during exercise testing (<20 mL kg^{-1} m^{-2}) [77]. Although radiation therapy is highly effective in treating childhood cancer, especially when used with antitumor drugs, its use should be balanced with the understanding of its additive effect on cardiac damage.

4.4 CARDIOPROTECTIVE STRATEGIES

Despite the clear cardiotoxicity of certain antitumor drugs, their effectiveness in treating cancer ensures that they will remain widely used. To reduce the risk of cardiac damage caused by these drugs, several preventive strategies have been developed. These strategies include closely monitoring heart function before, during, and after treatment, as well as variations in drug administration and combining cardioprotective agents with antitumor drugs. However to date no cardiac screening strategy during childhood cancer therapy has been shown if used to individualize therapy based on screening results to optimize the overall outcome of reducing cardiotoxicity and maximizing oncologic efficacy. It has been hoped that these

strategies can markedly reduce and sometimes prevent cardiotoxicity while maintaining the efficacy of antitumor drugs. Given the irreversible and progressive nature of cardiac damage caused by chemotherapy in many children, using cardioprotective strategies should be a priority.

4.4.1 Continuous Infusion

Reducing peak delivery dose by administering the drug as a continuous infusion may prevent cardiac injury. In adults, this strategy has shown some promise. Analysis of different doxorubicin dosing schedules revealed that infusion times longer than 6 hours were associated with a reduced risk of clinical HF relative to infusion times of less than 1 hour (relative risk [RR] = 0.27; 95% CI 0.09 to 0.81; combined $n = 557$) [45]. However, the risk of subclinical cardiac dysfunction did not differ significantly between groups. In children, the benefit of continuous infusion has yet to be seen. Neither 6- nor 48-hour continuous infusion schedules protected children with ALL receiving daunorubicin at an average cumulative dose of 180 mg m^{-2} or doxorubicin to 360 mg m^{-2}, respectively [78,79]. Although some studies found slight decreases in the rate of asymptomatic cardiac dysfunction, as measured by left ventricular shortening fraction, in children with ALL receiving either bolus or 48-hour continuous infusions of daunorubicin, the difference was not statistically significant [80,81].

4.4.2 Dexrazoxane

In children receiving anthracyclines, one of the most promising cardioprotectants is dexrazoxane. The cardioprotectant activity of dexrazoxane comes from its ability to chelate iron and thus prevent the production of free radicals when iron reacts with anthracycline. In adults, dexrazoxane has well recognized cardioprotective properties and is recommended by the American Society of Clinical Oncology for use in patients with metastatic breast cancer receiving a cumulative dose of anthracycline above 300 mg m^{-2} [82]. Although dexrazoxane is not widely used in children, it clearly reduces cardiac injury from anthracyclines.

In 2004, Lipshultz *et al*. reported the cardioprotective efficacy of dexrazoxane in children receiving doxorubicin for ALL [83]. Half the children were randomly assigned to receive dexrazoxane with doxorubicin, and the other half received doxorubicin alone. Serial biomarker assessments

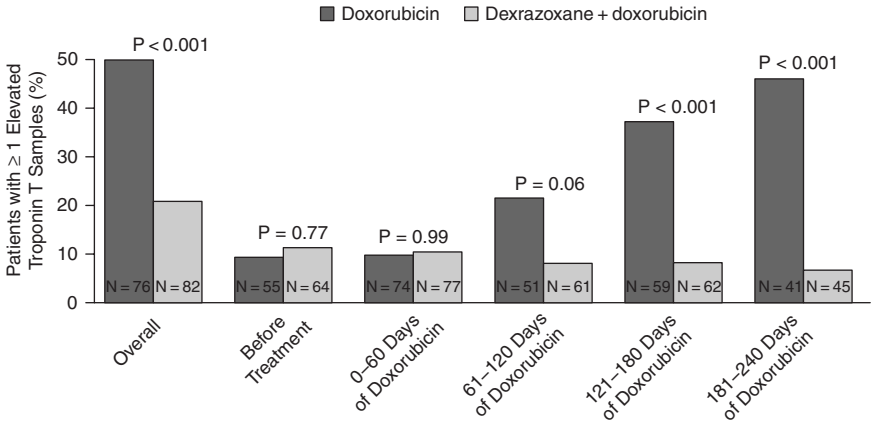

Figure 4.2 Percentage of patients with at least one elevated cardiac troponin T. level overall, before and during treatment with doxorubicin. An elevated level of troponin T was defined as one that exceeded 0.01 ng mL^{-1}. The number of patients in whom troponin T was measured at least once during the specified intervals is shown in each bar. (Lipshultz S. E., Rifai N., Dalton V. M. *et al*. The effect of dexrazoxane on myocardial injury in doxorubicin-treated children with acute lymphoblastic leukemia. *New Engl. J. Med*. 2004, 351, 145–153. (Reproduced with permission from Massachusetts Medical Society. Copyright 2004. All rights reserved)

indicated that dexrazoxane reduced cardiac injury (Figure 4.2). Serum levels of cardiac troponin T (cTnT) in children receiving dexrazoxane plus doxorubicin were lower than those receiving doxorubicin alone (21% vs. 50%, $P < 0.001$; elevated >0.01 ng mL^{-1}). Any elevation in the cardiomyocyte structural protein cardiac troponin T in the serum indicates myocyte damage. In this study and others, dexrazoxane did not reduce the efficacy of doxorubicin treatment, nor was it associated with increased secondary malignancies among survivors of childhood ALL [83,131].

The efficacy of dexrazoxane as a cardioprotectant in children shows promise, but further study is needed. Whether dexrazoxane reduces the antineoplastic efficacy of anthracyclines is a primary concern and has prevented the acceptance of dexrazoxane as a standard component of pediatric anthracycline regimens. Although most studies have found that dexrazoxane does not reduce oncologic efficacy, the American Society of Clinical Oncology does not yet recommend using dexrazoxane outside of its approved use in women with metastatic breast cancer who would benefit from cumulative anthracycline doses greater than 300 mg m^{-2} [84,85].

Table 4.1 Cardioprotective strategies used with anthracyclines [136]. (Reproduced with permission from Informa Healthcare, Copyright 2007)

Class	Examples
Antihistamines	chlorpheniramine
	ketotifen
	disodiumcromoglycate
Antioxidants	*N*-acetyl cysteine
	alpha tocopherol
	carvedilol
	coenzyme Q10
	Resveratrol
Chelating agents	Dexrazoxane
Cytokines	Erythropoietin
	granulocyte stimulating factor
	Thrombopoietin
Energy regulators	Adenosine
	Carnitine
Enzyme inhibitors	Cox-2 inhibitors
	Digoxin
	Amrinone
Exercise	
Hormones	Estrogen
Inhibitors of mediator release	Cromolyn
Ion regulators	calcium channel blockers
	α and β adrenergic antagonists
Membrane stabilizers	steroids
	taurine
Metabolic agents	probucol
	lovastatin
Miscellaneous agents	bismuth, zinc, cadmium
Uptake inhibitors	tetracyclines

The use of traditional cardiac medications with antineoplastic therapies may reduce or prevent cardiac damage. Agents such as ACE inhibitors, beta-blockers, and others have shown some promise in this regard (Table 4.1). These agents are useful in treating patients who already have drug-induced cardiac damage, but their efficacy during treatment is still unclear, and conclusions are limited by the small sample sizes in which they were evaluated.

4.4.3 Additional Agents with Cardioprotective Potential

In order to further minimize the deleterious effects of antitumor drugs, a wide spectrum of additional cardioprotective agents have been evaluated

(Table 4.1). Although preliminary evidence shows some agents may prove beneficial, results are limited by small sample size and uncontrolled experiment design [138,139]. Despite this uncertainty, the use of dietary supplements and homeopathic remedies in treating cancer, heart disease, and other illnesses is widespread amongst patient populations. In many cases, the benefits and risks of such complementary treatments are unknown and require further assessment to determine their suggested use. Additionally, while the use of widely used drugs such as digoxin, beta-blockers, and calcium channel inhibitors have proven beneficial in treating certain patient populations, their use in preventing cardiotoxicity in children receiving antitumor drugs requires further investigation.

The beta-blocker carvedilol, which is used to treat hypertension, angina, and congestive heart failure, has some cardioprotectant properties. In a short-term follow-up of 6 months, Kalay *et al.* showed that among 50 adults receiving doxorubicin or epirubicin for various malignancies, those randomly assigned to receive carvedilol daily ($n = 25$) during treatment showed no significant decrease in LVEF = 70.5% vs. 69.7%, P = 0.3. Alternatively, the control group not receiving carvedilol ($n = 25$) had significantly decreased post-treatment LVEF = 68.9% vs. 52.3%, $P = 0.001$ [86,87]. In addition to their use in post-treatment care, traditional therapeutics, such as beta-blockers, appear to delay deterioration into symptomatic HF.

Further investigations into cardioprotective strategies have involved attempts to alleviate the macronutrient deficiencies of cardiomyopathy. Drug-related cardiac injury may be reduced by metabolic cocktails containing levocarnitine, coenzyme Q10, thiamine, and riboflavin. The cardiomyopathy seen in autosomal recessive disorders involving L-carnitine membrane transport is reversible with L-carnitine supplementation. In cancer treatment, L-carnitine has been cardioprotective in rats, where it reduced the 120-day mortality from doxorubicin cardiomyopathy from 53 to 8% [133]. Although more research is needed, one small study of 38 children treated with anthracyclines showed that those with ventricular dysfunction more than 6 months after doxorubicin treatment had serum carnitine levels that were markedly lower than those of controls [134].

Similarly, the use of coenzyme Q10 as supplemental therapy may prevent cardiac damage. Coenzyme Q10 acts through the electron transport chain during oxidative phosphorylation, where it is thought to improve ATP production. A study of anthracycline-induced cardiotoxicity in children with ALL or non-Hodgkin's lymphoma compared 10 children who received coenzyme Q10 with 10 who did not. After treatment, LV fractional shortening was significantly decreased in both

groups, although the decline was greater in the group that did not receive coenzyme Q10 [135]. The addition of thiamine and riboflavin, both of which improve mitochondrial function, may improve a multi-agent metabolic cocktail for use in children receiving antitumor drugs. Supplemental therapy is potentially viable and is a promising area of research [139].

4.5 CARDIOVASCULAR MONITORING

To manage children at risk of drug-induced cardiomyopathy, clinicians must closely monitor cardiac function before, during, and after treatment. Electrocardiography, echocardiography, and measurements of serum biomarkers can detect early cardiac injury and in so doing may help guide treatment. Several groups have proposed screening guidelines for patients at risk, but there is no standard protocol for monitoring these patients. Similarly, echocardiographic interpretation is substantially limited by differences in measurement and variability in methodologies. This need for risk–benefit data to guide protocol development is highlighted by van Dalen *et al.*, who reported that 10 of 12 European pediatric oncology protocols recommended modifying doses at the onset of abnormal cardiac function, but none required long-term follow-up to assess treatment-related cardiac damage [105]. The long-term cardiotoxic effect of cancer treatment is well recognized, but longitudinal monitoring is needed to assess potential endpoints for progressive cardiac risk [97,105].

4.5.1 Echocardiography

Current recommendations focus on echocardiography as the primary means to evaluate cardiac health in children before, during, and after exposure to cardiotoxic antitumor agents. The American Heart Association recommends both pediatric and adult patients receive routine echocardiograms at baseline as well as regular re-evaluations during treatment [137]. Although guidelines for adjusting therapy based on echocardiographic results are incomplete, serial monitoring of patients during therapy might allow dose modification at the first signs of cardiac abnormality [105]. No cardiac biomarker-directed strategy to tailor chemotherapy based on biomarker levels has been tested to determine if the overall therapeutic outcome is improved that is based on maximizing oncologic efficacy

while minimizing toxicities and late effects. Due to the progressive nature of chemotherapy-related cardiac injury, pediatric cancer survivors who received cardiotoxic drug regimens should receive regular echocardiograph evaluation following the end of treatment. Although specific endpoints for cardiac risk are unavailable, the recommended frequency for evaluating adult survivors is 5 years while patients with abnormal cardiac findings should receive follow-up on a yearly basis [136,137].

4.5.2 Cardiac Markers

Serum biomarkers provide an alternative and increasingly valuable means of monitoring children at risk for cardiac damage. Biomarkers can complement echocardiography in assessing children receiving cardiotoxic agents. Serial measurements of biomarkers for inflammation, neurohormones, myocyte injury, and myocyte stress can create a composite picture of the pathogenesis of cardiac injury [106].

4.5.2.1 Troponins

The presence of cardiac troponin proteins in the serum are some of the most sensitive serum markers of myocardial injury. These structural proteins are found in striated muscle cells, where they regulate the calcium-mediated interaction between actin and myosin. When cardiac cells are damaged, troponins in the cytoplasm are immediately released into the serum and become readily detectable [107–114]. The majority of these troponins are bound to actin and are released slowly. This characteristic, along with the slow clearance of troponins from the body, permits the detection of not only acute damage but also of ongoing injury.

In cancer treatment, the most widely studied troponins are cardiac troponins T and I (cTnT and cTnI). Both cTnT and cTnI are protein isomers specific to cardiomyocytes and are easily detected with commercial assays [107–114]. The link between elevated cTnT and cardiac injury has been described in adults with myocardial ischemia. The presence of cTnT or cTnI in the serum indicates myocyte damage; even the presence of cTnT at its lowest measurable levels ($\geq$0.01 ng mL^{-1}) can be associated with acute cardiac injury [108]. Lipshultz *et al*. confirmed this association in children with ALL receiving doxorubicin in which

elevations in cTnT correlated with cumulative dose, LV dilation, and wall thinning [83,107,108]. These elevations in cTnT predicted left ventricular dilation ($r = 0.80$ when variables were treated as continuous, $P = 0.003$) and wall thinning ($r = 0.61$, $P = 0.044$) 9 months after exposure to doxorubicin [107].

As is the case with cTnT, elevations in cTnI correlate strongly with adverse cardiac events. Cardinale *et al*. showed that in adults being treated for Hodgkin's disease, persistent elevations in cTnI (> 0.08 ng mL^{-1}) were associated with an increase in cardiac events [115,132]. Patients with elevated serum cTnI levels immediately and 1 month after treatment had a higher risk for cardiac events than did patients in whom cTnI was increased only after the initial dose (84% versus 37% $P < 0.001$) [115]. As a result of their limited expression and the high sensitivity of commercially available assays, further use of these attractive biomarkers in monitoring cardiac health is warranted.

4.5.2.2 Natriuretic Peptides

Pro-brain natriuretic peptide (proBNP) is an independent marker of myocyte stress. In contrast to troponins, which indicate the presence of dead cardiac tissue, elevations in proBNP and its metabolic products, BNP and N-terminal proBNP, correlate with early indicators of stress, such as load-induced wall stretch, and factors such as fibrosis, ventricular mass, ischemia, and inflammation [127,128]. These correlations suggest that proBNP can indicate cardiac distress early, before irreversible damage occurs. Under normal conditions, natriuretic proteins circulate in the blood at predictable levels. Comparing a patient's natriuretic serum levels to that of age-matched healthy controls can be an inexpensive, rapid diagnostic test for cardiac damage.

The use of BNP, as well as other natriuretic proteins, such as atrial natriuretic peptide (ANP) and proANP, in diagnosing cardiac distress is not new. Elevations in these proteins have long been associated with heart disease and LV dysfunction. Brain natriuretic peptide levels have been shown to be highly sensitive and specific in diagnosing HF in patients presenting with dyspnea in the emergency room and can also predict cardiac mortality in patients with coronary heart disease [116,117]. This high specificity of BNP and NT-proBNP to heart damage is independent of other risk factors, including exercise capacity, LV mass, systolic and diastolic dysfunction, and other biomarkers (e.g., cTnT and CRP) [117]. This independence and the availability of commercial assays

make natriuretic proteins an attractive additional component in evaluating patients with suspected HF.

In children, the use of natriuretic biomarkers to identify cardiac dysfunction during or after treatment with antitumor drugs is promising. Children who survived a median of 5 years after cancer diagnosis and who had received anthracyclines had mean serum NT-proANP levels that were significantly higher than those in healthy age-matched controls, although these levels did not increase with chemotherapy [118]. Serum levels of BNP also correlate with echocardiographic findings of clinical and subclinical heart failure in children who received anthracyclines, and these changes were identified before radionuclide angiography diagnosed any abnormality [119,121]. Furthermore, chronically elevated levels of BNP indicate a poor prognosis in children at risk for drug-related cardiomyopathy and may indicate increased deterioration of systolic function [120,121].

The value of natriuretic markers was further assessed by the National Cancer Institute's Dana-Farber Cancer Institute Acute Lymphoblastic Leukemia Consortium. Between 1995 and 2000, serial serum samples and echocardiograms were collected from children with ALL. These patients were randomly assigned to receive doxorubicin alone ($n = 74$) or doxorubicin preceded by the cardioprotectant dexrazoxane ($n = 80$) [121]. In both groups, children with elevated NT-proBNP levels (above 100 pg mL^{-1} for children less than 1 year old; above 150 pg mL^{-1} for older children) 6 months after the start of doxorubicin were 2.39 times as likely to have elevated cTnT levels (above 0.01 ng mL^{-1}; $P = 0.019$). The increase in NT-proBNP also correlated with abnormal fractional shortening, both during and after treatment. NT-proBNP elevation was also associated with decreased LV mass in asymptomatic children 4 years after receiving an average dose of 240 mg m^{-2} of doxorubicin [121]. During treatment, elevated serum NT-proBNP levels were signs of worsening abnormal LV fractional shortening and elevated cTnT levels among children with ALL receiving doxorubicin-based multi-agent chemotherapy [122]. Although increases in natriuretic proteins are positively associated with cardiac injury, they do not correlate with anthracycline dose [123].

With mounting evidence for the use of BNP in assessing heart damage, several studies have reported results of BNP-driven cardiac treatments. A multicenter study in France reported findings for 220 patients with HF whose treatment was directed by plasma BNP levels [124]. Patients whose treatment was directed to maintaining plasma BNP levels below 100 pg mL^{-1} had more adjustments to their drug regimens, including

higher mean doses of ACE inhibitors and beta-blockers, as well as a lower rate of death and shorter hospital stays for HF than did patients receiving standard care.

A similar study in New Zealand randomly assigned patients presenting with systolic dysfunction and symptomatic heart failure to receive treatment based on either plasma NT-proBNP levels or standard medical evaluation. During a median follow-up of 9.5 months, patients treated based on NT-proBNP levels had fewer total cardiovascular events (death, hospital admission, and heart failure exacerbation) than did patients in the control group, and their first cardiac event occurred later [125,126].

Despite increasing evidence that NT-proBNP is a marker of cardiac damage, it must be validated further in clinical trials before its use in assessing children with cancer is widely accepted. The strong correlation between natriuretic peptides and heart stress means that they may offer a relatively inexpensive and rapid means of monitoring heart function. Further, using biomarker analysis to monitor the progression of pediatric heart failure can provide an objective assessment of the effects of treatments, especially in patients who cannot articulate their symptoms. Research should clarify how incremental changes in marker levels over time correlate with the degree of cardiac damage. These biomarkers are potentially quite valuable in improving the management of children receiving cardiotoxic drugs.

4.5.2.3 Highly Sensitive C-Reactive Protein

Inflammation in patients with HF makes markers of generalized inflammation, such as high-sensitivity C-reactive protein (hsCRP), an appealing tool in patient evaluation. In adults, levels of inflammatory markers like hsCRP correlate positively with HF symptom severity [129,130]. However, these elevations may be partly caused by advancing atherosclerosis, which induces the release of inflammatory factors [128]. In children, who are typically free of atherosclerosis, increased levels of hsCRP may indicate cardiac damage. The strongest evidence for an association between hsCRP and cardiac damage comes from a prospective study of 156 childhood cancer survivors who had received anthracycline and radiation therapy. All survivors had significantly higher hsCRP and proBNP levels than did their healthy siblings [40]. Inflammatory biomarkers may potentially be used to evaluate cardiac conditions in pediatric oncology, but further study is needed.

4.6 CARDIAC FOLLOW-UP IN SURVIVORS OF CHILDHOOD CANCER

There is no specific treatment for chemotherapeutic-related cardiomyopathy in children. Given the success of managing cardiomyopathy in non-cancer settings, children presenting with cardiac injury should receive standard treatments for congestive heart failure and be followed regularly. Guidelines from the American College of Cardiology, the American Heart Association, the Heart Failure Society of America, and the International Society for Heart and Lung Transplantation recommend ACE inhibitors, beta-blockers, diuretics, and other standard medications to treat HF [88–90]. These guidelines recognize the need for improved outcomes in symptomatic patients but also the benefits in reducing disease progression in asymptomatic patients. The goal of treating chemotherapy-related cardiomyopathy includes reducing afterload as well as improving systolic function. For patients with end-stage heart disease, cardiac transplantation remains an option.

4.6.1 ACE Inhibitors

In non-cancer settings, the use of angiotensin converting enzyme (ACE) inhibitors for reducing afterload in patients with LV dysfunction is well recognized. ACE inhibitors, such as enalapril and captopril, markedly reduce the incidence of HF and improve long-term survival in patients with heart disease [91–94]. Anthracyclines can thin the LV wall and subsequently increase afterload and wall stress, so treatments that reduce afterload may be beneficial in these children. Despite their ability to reduce afterload, ACE inhibitors may not be efficacious in treating children with drug-related cardiomyopathy. For example, although enalapril can reduce wall stress in anthracycline-treated childhood cancer survivors, it may not halt deterioration of heart function [95]. Other studies of enalapril in childhood cancer survivors with anthracycline-related injury have had similar results; cardiac performance improved initially but was eclipsed by a return to pre-enalapril levels 3 to 5 years after treatment [96,97]. This inability of ACE inhibitors to provide the long-term results seen in non-cancer patients may be explained by the restrictive nature of the cardiomyopathy typically seen in children treated with anthracyclines. Restrictive cardiomyopathy does not usually respond to ACE inhibitors, which may explain the eventual relapse seen in these patients [97].

4.6.2 Beta-Blockers

The success of beta-adrenergic receptor antagonists in managing heart disease indicates their potential for treating chemotherapy-related cardiac damage. In non-cancer patients, beta-blockers substantially improve heart function, especially for patients already receiving maximum dosages of diuretics and ACE inhibitors. Similar benefits have been shown in adults with anthracycline-related cardiomyopathy, where beta-blockers increased mean LVEF from a baseline of 28% to 41% [98]. A retrospective study of children with dilated cardiomyopathy found similar effects among those receiving the beta-blocker carvedilol; mean LVEF increased from 25% at baseline to 42% after a mean follow-up of 27 months ($P < 0.001$) [99]. Other studies have confirmed that beta-blockers improve children's heart function, in both the short and medium term, though further evaluation is still needed [100,101].

4.6.3 Calcium Channel Blockers

Calcium channel blockers have had mixed results in treating chemotherapy-induced cardiomyopathy. The results of animal studies have been inconclusive, but a double-blind pilot study found that the calcium channel blocker prenylamine can reduce anthracycline-induced cardiotoxicity [102]. Interest in calcium channel blockers as cardioprotectants led to their use in a randomized study of adults with acute myeloid leukemia who were receiving doxorubicin. Patients receiving the calcium channel blocker verapamil had slightly less cardiac dysfunction than did placebo controls; however, both groups had evidence of cardiomyopathy, and the differences between groups was not statistically significant [103]. Interest in calcium channel blockers continues, but their efficacy is not yet proven.

4.6.4 Growth Hormone

The common finding of growth hormone deficiency in anthracycline-treated childhood cancer survivors suggests that replacement therapy may be beneficial. Growth hormone therapy initially improves cardiac health, but dysfunction still occurs. In a study monitoring cardiac function in anthracycline-treated childhood survivors, several years of growth hormone therapy was associated with improvements in LV wall thickness

and mass. However, cardiomyopathy still progressed, and improvements were lost when therapy was discontinued [104].

4.7 CONCLUSION

Many children and adolescents with cancer are treated with cardiotoxic chemotherapeutic agents. Although these agents have greatly improved long-term survival, many survivors experience cardiovascular complications that impair quality of life. Attempts to reduce these cardiac complications limit treatment options, which may compromise the efficacy of chemotherapy. Anthracyclines are well known for their cardiotoxicity, as are alkylating agents such as cyclophosphamide and the metabolite 5-fluorouracil. New antitumor agents such as trastuzumab have also been associated with an increased risk of cardiac injury. Children are especially vulnerable to cardiac stress because their cardiac growth must keep pace with their rapid somatic growth.

Children with or at risk of cardiac injury may present with arrhythmias, echocardiographic abnormalities, myocarditis, ischemia, and HF. The effects of treatment-induced cardiomyopathy can occur at the time of treatment, or shortly after, or even several years after the initial dose. Given that chemotherapy-induced cardiac damage is often irreversible, treatment strategies aimed at preventing or reducing cardiac injury are important. Such strategies include the use of cardioprotective agents, such as dexrazoxane, screening patients for additive risk factors, and close serial monitoring for signs and symptoms of cardiac damage during chemotherapy and in long-term follow-up. Drug-related cardiomyopathy may also be prevented or reduced by changing the dosing, delivery, and schedule of potentially cardiotoxic agents.

Future research should focus on the continued refinement of chemotherapy protocols to minimize side effects while maintaining acceptable treatment response rates. The use of cardioprotectants, as well as the development of new drugs with fewer cardiotoxic effects, is also of great importance.

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5

Cardiovascular Toxicity of Antitumor Drugs: Dimension of the Problem in Adult Settings

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5.1 INTRODUCTION

Cardiotoxicity (CT) is a rare but potentially catastrophic complication of chemotherapy and anti-cancer drugs. The incidence is dependent on *pharmacology* (drug, route and dosing schedule, cumulative load, concomitantly administered drugs), *patient characteristics* (age, presence of underlying cardiovascular or renal disease and cardiac risk factors) and associated *mediastinal radiation*.

To standardize the reporting of drug adverse events, most studies use either the grading system proposed by the World Health Organization in 1981 [1] or the National Cancer Institute Common Terminology Criteria for Adverse Events [2] to define symptomatic CT. The latter will be preferentially used in this report.

Factors that confound the understanding of the true incidence of CT are:

- A historically enduring reliance on a body of sporadic case reports that are often poorly documented with minimally substantiated conclusions.

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- The lack of definition and reporting uniformity across large studies that eliminates the potential for study comparisons.
- The absence of denominators in many reports.
- Methodological inconsistencies.
- Exclusion of patients with known cardiac disease from studies.
- Equating acute and chronic toxicity as identical entities.
- Combining grade 1–2 asymptomatic abnormalities in imaging (electrocardiogram (ECG]), echocardiogram (ECHO) gated nuclear angiogram (MUGA)) and grade 3–4 symptomatic disease and/or death.
- Inconsistent threshold for reporting adverse events (often only $\geq$5% incidence).
- Basing the presence of heart failure solely on reductions in left ventricular ejection fraction (LVEF) with definitions that range from reductions as little as 10% to $\geq$20% or only reductions that are below the lower limit of normal for the laboratory.
- Reporting absolute versus percentage reductions of LVEF, that is, 10 points versus 10%.

In addition, chemotherapy-related adverse events are frequently unreported by physicians [3].

CT may involve all parts of the heart from the pericardium to the endocardium. Clinical manifestations include ECG abnormalities, pericardial inflammation, ischemia, left ventricular and valvular dysfunction and alterations of atherosclerotic risk factors. In this chapter we will attempt to quantitate the incidence and risk of CT for each of the commonly used drugs in the current era of cancer chemotherapy. Although radiation is a risk factor for CT and may augment the impact of concomitant chemotherapy, the effects of radiation will not be specifically addressed. Table 5.1 offers an overview of chemotherapy-related CT by drug class.

5.2 ANTHRACYCLINES AND RELATED DRUGS

The anthracyclines (doxorubicin [Adriamycin], daunorubicin [Cerubidine], idarubicin [Zavedose], epirubicin [Ellence]) and mitoxanthrone (Novantrone) have been the anchor drugs of cancer chemotherapy for more than 60 years and continue to be critical components in the

Table 5.1 Summary of chemotherapeutic toxicity

Chemotherapeutic agent by class	Cardiac toxicity
Anthracyclines	Acute CT: arrhythmias, myocarditis, pericarditis, sudden cardiac death
Doxorubicin	
Mitoxantrone	Sub acute and late CT: asymptomatic and symptomatic reductions in LVEF
Daunorubicin	
Idarubicin	
Epirubicin	
Liposomal preparations	
Monoclonal antibodies	
Rituximab	Infusion-related hypotension, atrial & ventricular arrhythmias, heart block, chest pain, and acute myocardial infarction
Cetuximab	Possible hypomagnesemia-induced arrhythmia, atrial fibrillation
Alemtuzimab	First dose infusion-related hypotension
Trastuzumab	Asymtpomatic and symptomatic decrease in LVEF
Laptinib	Asymptomatic decrease in LVEF
Bevacizumab	Hypertension, venous and arterial thromboembolism
Antimetabolites	
Gemcitabine	Rare radiation recall
Cytarabine	Pericarditis, asymptomatic bradycardia
Fluorouracil	Chest pain with or without ST segment elevation, arrhythmia, heart failure, sudden cardiac death
Capecitabine	Chest pain due to coronary vasospasm with ST elevations +/− arrhythmias and +/− HF
Histone deacetylase inhibitors (HDAC)	QT prolongation, supraventricular and ventricular premature depolarizations, supraventricular and ventricular tachycardia, decreased LVEF, sudden death
Alkylating Agents	
Cyclophosphamide (high doses)	Myo-pericarditis
Ifosfamide (high doses)	Asymptomatic decrease in LVEF, myo-pericarditis
Microtubule-targeting agents	
Vinca alkaloids	Myocardial infarction or ischemia
Vinflunine	Angina
Taxanes	
Paclitaxel	Asymptomatic sinus bradycardia, premature ventricular depolarizations, ventricular tachycardia and atrio-ventricular block
Docetaxel	None

(*continued overleaf*)

Table 5.1 (*continued*)

Epilithones	
Ixabepilone	Symptomatic palpitation, atrial flutter, myocardial infarction
Immunomodulating agents	
Lenalidomide	Venous thromboembolism
Pomalidomide	Venous thromboembolism
Tyrosine kinase inhibitors (TKI)	
Imatinib	Heart failure in patients with risk factors or pre-existing CV disease
Sunitinib	Hypertension, myocardial infarction, heart failure, cardiovascular death
Sorafenib	Hypertension
Epidermal Growth Factor TKI	
Erlotinib	None reported
Gefitinib	None reported
Retinoids	
Bexarotene	Hypertriglyceridemia, hypercholesterolemia
Proteosome inhibitors	
Bortezomib	Heart failure, QT prol ongation, angina, atrioventricular block, atrial fibrillation
Platinum agents	
Cisplatin	Acute: small and large vessel vasospasm Long-term: hypercholesterolemia, hypertension, increased incidence of CV events
Oxaliplatin	Chest pain
Folate antagonists	
Methotrexate	Sinus bradycardia, ventricular tachycardia, chest pain
Pemtrexed	None reported
Cytokines	
Interferon	Arrhythmia, dilated cardiomyopathy, myocardial ischemia, myocardial infarction
Interleukins	Infusion related hypotension, myocardial ischemia, arrhythmias
Radioimmunotherapy	
Tositumomab	None reported
Ibritumomab tiuxetan	Hypertension
Gemtuzumab ozogamicin	Non-specific arrhythmias, hypotension, hypertension
Arsenic trioxide	QT prolongation, atrioventricular block
Tamoxifen	Stroke, venous thromboembolism

modern treatment of breast cancer and lymphomas. The CT associated with their use has been the most extensively studied non-hematologic complication of chemotherapy and is the most widely recognized cardiac complication of cancer therapy. Anthracycline CT may present at three distinct times defined from the onset of treatment initiation. *Acute CT* is a broadly defined syndrome that occurs during or immediately after treatment initiation, with an incidence that varies over a wide range proportional to the inclusion (30–40%) or exclusion of sinus tachycardia (0.5–10%). The manifestations can be either electrophysiological with transient ECG changes (nonspecific ST and T wave changes, low voltage, QT prolongation), arrhythmias (sinus tachycardia, atrial /ventricular premature depolarizations or tachycardia, atrioventricular block) and overt myo-pericarditis [4–6]. Isolated cases of sudden death have been reported that may be due to sustained ventricular arrhythmias, hypersensitivity or hypotension [7,8]. The incidence of arrhythmia is low during infusion and increases in the 24 h after infusion. Pre-existing ECG abnormalities do not impact the incidence or predict the occurrence of CT [9].

This manifestation of CT is not dose related and withdrawal of the anthracycline usually results in recovery. The development of acute CT does not increase the risk or impact the incidence of late CT. The mechanism is most likely due to drug-induced myocardial damage and/or an associated catecholamine or histamine surge.

Most instances of acute anthracycline cardiotoxicty consist of minor electrocardiographic changes.

Sub-acute CT presents in the first year in approximately 3% of patients and *Late CT* occurs after the first year. Both have been characterized by various degrees of heart failure (HF), historically defined by a symptomatic decrease in LVEF. It has become increasingly clear that this decrease in systolic function may be preceded by a decrease in diastolic function [10] and/or may exist without clinical manifestations, that is, there is an asymptomatic latent period. Late CT can occur decades after treatment completion with the risk increasing over time. Several factors increase the risk and include extremes of age (young and elderly), female sex, cumulative anthracycline dose, mediastinal radiation, pre-existing cardiovascular disease and risk factors.

The incidence of sub acute anthracycline cardiotoxicity is 3%.

The development of CT is independent, for the most part, of the type of the underlying cancer, related to treatment protocol [11] and clinically indistinguishable from other types of nonischemic cardiomyopathies.

Symptomatic HF The classic relationship for the risk of developing CT was described by Von Hoff who showed an association with the total cumulative dose of anthracycline. CT incidence is 0.14% up to a cumulative dose of 399 mg m^{-2} and increases to 3% above 400 mg m^{-2}, 7% up to 550 mg m^{-2}, 18% at 700 mg m^{-2} and 50% at 1 g m^{-2} or higher [12] with individual variation. We have subsequently learned that the real world risk, although generally proportional to the total accumulated dose, can actually occur in a less linear fashion and the incidence may be more time dependent [13] so that there probably is no safe dose of medication that is 100% protective.

Zambetti *et al*. reported long-term cardiac effects in 1000 patients after adjuvant chemotherapy for breast cancer. In women who were free of recurrence for a median of 11 years, the incidence of systolic dysfunction was 8% in doxorubicin-treated patients and 2% in cyclophosphamide-methotrexate-fluorouracil-treated patients [14].

The incidence of CT in the modern adjuvant treatment of breast cancer with doxorubicin doses between 240 and 360 mg m^{-2} has been estimated at 1.6–2.1% and may be up to 2.5 times higher in patients over the age of 65 years [15].

Asymptomatic HF It is increasingly recognized that asymptomatic abnormalities in noninvasive studies can be found in greater frequency and at a lower cumulative anthracycline dose than previously reported. This sub-clinical cardiomyopathy in patients receiving lower doses of doxorubicin has been studied by Hequet *et al*. [16] and even at doses less than 300 mg m^{-2}, asymptomatic decreases in fractional shortening, a measure of systolic function, was found in 27.6% of 141 lymphoma patients.

In spite of an abundant literature, the cause of CT remains speculative. Hypotheses include the development of free radicals and/or reduction in free radical scavengers, inhibition of ionic pumps through toxic metabolites, iron complexes that damage cellular membranes and DNA, inflammatory cytokines and most recently, p53-induced inhibition of mTor signaling and loss of myocardial mass [17].

> Late asymptomatic cardiomyopathy can occur at any anthracycline dose.

Because of the success in treating cancer with millions of adult survivors who have been treated with an anthracycline, there exists a potential epidemic of anthracycline-induced heart disease for the future. Recently, a predictive model to estimate cardiotoxic risk has been published that could be helpful in up-front decision making and assessing the risks of CT after treatment completion [18]. A case for ongoing monitoring and need for a systematic study of late survivors has been made [19,20].

> The first manifestation of anthracycline cardiotoxicty may be diastolic dysfunction.

Although less studied than doxorubicin, the incidence of CT with daunorubicin and idarubicin is similar with equivalent dosing regimens.

5.2.1 Doxorubicin Analogs

5.2.1.1 Epirubicin

Epirubicin is a semi-synthetic derivative of doxorubicin designed for faster elimination, decreasing overall myocardial exposure time to reduce CT. This change in pharmacodynamics allows higher dosing, with a shift in the Von Hoff curves to the right with a cumulative risk of CT at 950 mg m^{-2}, equal to a total doxorubicin dose of 450 mg m^{-2} [21].

In a study of 469 patients with metastatic breast cancer the overall incidence of CT was 7.2% with a 4% incidence at 900 mg m^{-2} that rose dramatically to 15% at 1000 mg m^{-2} [22].

In a study of120 patients who received high dose epirubicin (850–1000 mg m^{-2}), the risk increased over time post treatment completion from 11% at 1 year to 14% at 2 years and 20% at 5 years. This corresponded

to a 1.9% incidence at a total cumulative dose of 800 mg m^{-2} and 4.3% at 900 mg m^{-2} [23].

A recent analysis of 1097 breast cancer patients confirmed that there is a risk of CT with epirubicin that is higher than originally recognized and re-emphasized its dependence on cumulative dosing as well as increasing age, pretreatment predisposition to heart disease, and mediastinal radiation. They found that the hazard ratio of CT was constant as the CT rate increased by 40% per each increase in epirubicin cumulative dose of 100 mg m^{-2} [24].

The need for high dose epirubicin for clinical efficacy has negated the theoretical advantage for CT. This led the Cochrane Group to conclude that there was no established benefit for epirubicin compared to standard doxorubicin [25].

> Epirubicin at equivalent doses to other anthracyclines has an equal incidence of cardiotoxicity.

5.2.1.2 Liposomal Encapsulation

In a similar effort to preserve or increase antitumor efficacy while reducing CT, pegylation or liposmal encapsulation of anthracyclines was developed. If liposomes cannot escape the vasculature due to tight capillary junctions (e.g., the heart and GI track), then they cannot enter these tissues, while tumors that characteristically have more porous junctions should receive the full power of the chemotherapy. There are three liposomal preparations available (Doxil® or Caelix®, Lipodox®, DaunoXome®). In two head to head comparisons of pegylated liposomal doxorubicin compared to doxorubicin, a decrease in CT was noted without any decrease in clinical efficacy. Batist *et al.* studied 297 patients and found a 6% versus 21% incidence of CT and O'brien *et al.* found a 3.9% versus 18.8% incidence of CT between patients receiving encapsulated versus standard doxorubicin. In the latter trial only two patients in the conventional doxorubicin group had overt HF [26, 27].

In summary, encapsulated doxorubicin/daunorubicin probably have a decreased incidence of CT compared to conventional anthracycline administration.

Liposomal encapsulation may decrease cardiotoxicity compared to standard anthracycline preparations.

5.2.1.3 Mitoxantrone

Mitoxantrone is an anthracenedione designed to yield broad spectrum antitumor activity similar to the anthracyclines. The risk of symptomatic HF has been estimated to be 2.6% for patients receiving up to a cumulative dose of 140 mg m^{-2}. The presence or history of cardiovascular disease and/or mediastinal radiation, or concurrent use of other cardiotoxic drugs may increase the risk of CT. The incidence of CT (overall decrease in LVEF 4.9–6.25%) is not different from the anthracyclines [28–29].

5.2.1.4 Third Generation Anthracyclines

The focus of drug development has been to increase tumor selectivity to minimize CT. A recent paper by Sessa *et al.* [30] reviewed the development of newer forms of doxorubicin tumor-targeted formulations that are currently undergoing clinical trials. Little information is available concerning their CT potential.

- Liposomal encapsulation mitoxantrone and annamycin Prodrugs (PK1[doxorubicin-HPNA-copolymer conjugate], PK2 [*N*-galactosamine linked doxorubicin-HPMA-coplymer conjugate], L-377,202 [doxorubicin prodrug activated by Prostatic Specific Antigen] and CPI-004NA (DTS-201) [*N*-succinyl-alanyl-leucyl-doxorubicin]). DTS-201 has been found to deliver at least eight-fold higher doses of doxorubicin with minimal or no CT in preclinical small and large animal studies [31–32].
- Dissacharide anthracycline analogs in which the amino sugar group is displaced to the second sugar (sabarubicin).
- Morpholinyl anthracycline derivatives (nemorubicin).

Because the anthracyclines continue to be the cornerstone for the treatment of many solid and hematologic malignancies, the search continues

for drugs with equal or enhanced efficacy and less CT than the existing first and second generation formulations.

5.3 MONOCLONAL ANTIBODIES

Monoclonal antibodies bind to a cancer-specific antigen leading to an immune reaction against the targeted cancer cell.

5.3.1 Rituximab

Rituximab (Rituxan), a chimeric monoclonal antibody that binds to the lymphocyte surface antigen CD20 was the first monoclonal antibody approved by the FDA for cancer treatment. Rituximab is indicated for the treatment of non-Hodgkin's B-cell lymphoma in combination with CHOP chemotherapy. Rituximab has also been studied with virtually no CT in idiopathic thrombocytopenic purpura [33], rheumatoid arthritis [34] and other connective tissue diseases [35].

Infusion is associated with some combination of fever, chills, nausea, vomiting, urticaria, hypotension, and bronchospasm in more than 80% of patients. This occurs most often during the first treatment and is a result of cytokine release due to the monoclonal antibody or the treated lymphocytes. Symptoms can be attenuated by preparatory regimens and adjustment of the infusion rate. Moderate to severe reactions occur in about 15% of patients [36,37].

A large series of lymphoma patients have been studied with no increase in CT when rituximab is added to standard therapy. In a study of 824 patients aged 18–60 years assigned to six cycles of therapy with or without rituximab, there was no difference in CT between the rituximab plus chemotherapy versus the chemotherapy group (1 vs. 1%) [38].

In a study of 399 patients aged >60 years receiving up to eight cycles of chemotherapy, there was also no difference in CT with or without rituximab. There were more grade I infusion related cardiac episodes in the rituximab group and an equal number of grade 3–4 adverse cardiac events. The overall incidence of CHF was identical in both groups [39].

In cancer patients, there are isolated and poorly documented case reports of atrial and ventricular arrhythmias, heart block, HF, chest

pain and acute myocardial infarction related to infusion [40,41]. With incidences that are less than 0.5%, these are most likely dependent on the baseline characteristics of patients who are unable to cope with the hemodynamic burden of the infusion.

> Rituximab-related cardiac events are the result of an increased infusion-related hemodynamic burden that mainly occurs in patients with pre-existing cardiac disease.

5.3.2 Cetuximab

Cetuximab (Erbitux) is a chimeric monoclonal antibody that binds to extracellular epidermal growth factor receptors (EGFR) inhibiting ligand–EGFR interaction as well as ligand-independent EGFR activity. Its utility, as an adjunctive agent, lies in malignancies that overexpress EGFR, such as metastatic colorectal cancer (CRC), non-small cell lung cancer (NSCLC) and head and neck cancers.

The most commonly observed toxicities with cetuximab-based regimens are dermatological (acneiform rash), hematologic, and infusion reactions. Aside from causing hypomagnesemia that may increase the chance of arrhythmia, major CT has not been reported [42–46].

CT reporting has been limited to a single report of atrial fibrillation with no documentation of causality. In most studies, patients with underlying cardiac disease were excluded so the true incidence of CT may be underestimated in the literature [47].

5.3.3 Alemtuzumab

Alemtuzumab (Campath) is a humanized monoclonal antibody that binds to the CD52 antigen on T and B lymphocytes promoting cell lysis. Efficacy has been demonstrated for the treatment of fludarabine-refractory chronic lymphocytic leukemia and various T cell lymphomas, including mycosis fungoides and the Sezary syndrome.

Infusion-related grade 1–2 rigors, fever, nausea, vomiting and rash are most common. Dyspnea and hypotension result from the release of cytokines. These symptoms generally occur with the first dose and can

be overcome with pretreatment (diphenhidramine and acetaminophen) and careful adherence to a strict initiation protocol that starts with low dose infusions with gradual dose escalation during the first 1–2 weeks of therapy. Infusion-related events may also be significantly reduced by subcutaneous administration.

The incidence of CT is controversial and based on a single study from MD Anderson Cancer Center published in 2004 by Lenihan and his group. They reported adverse cardiac events in four of eight patients with mycosis fungoides/Sezary syndrome who had no prior history of cardiac disease. Those patients developed HF or atrial arrhythmias that 'mostly improved after alemtuzumab discontinuation'. All three patients who developed HF had prior exposure to doxorubicin [48].

Prior studies showed an incidence of grade 1–2 hypotension from 17–50% and sporadic case reports describe arrhythmias, myocardial infarction and HF in both T-cell and B-cell malignancies [49–52].

The Lenihan study prompted a review of 30 patients with advanced mycosis fungoides/Sezary syndrome who had participated in the European trials of alemtuzumab. The population consisted of seven patients who were considered to have pre-existing cardiac risk (prior MI, hypertension, cardiomyopathy, angina, HF/mitral insufficiency), five patients who had been previously treated with doxorubicin and a second cohort of eight patients. No clinical CT occurred during or after alemtuzumab therapy in these patients [53].

In another Sezary syndrome study, 14 patients received subcutaneous alemtuzumab without any CT [54],.and in a randomized phase III trial comparing alemtuzumab with chlorambucil as first-line therapy, hypotension occurred in 14% of patients during alemtuzumab infusion (1.4% grade 3–4). Cardiac events were rare, but more frequent in patients treated with alemtuzumab (one each: atrial fibrillation, sinus bradycardia with hypotension) compared to no CT in the chlorambucil arm [55]. An additional report confirms the low incidence of alemtuzumab-induced CT in patients treated for chronic lymphocytic leukemia [56].

Of interest is the use of alemtuzumab as successful salvage therapy for intractable heart transplant rejection [57].

In summary, it is difficult to reconcile the incidence of serious CT described by the Lenihan group. Subsequent studies have not reproduced their findings. The incidence of first dose infusion related hypotension is real and isolated cases of non-life threatening arrhythmias, cardiac ischemia and reversible LV dysfunction may occur.

5.3.4 Trastuzumab

The human epidermal growth factor receptor 2 (HER2) is a transmembrane tyrosine kinase receptor, that is involved in many cellular processes, including the regulation of cell growth and cellular survival in normal healthy tissue. In 20–25% of new cases of breast cancer, either the HER2 gene is amplified or the HER2 protein is overexpressed and these patients have a relatively poor prognosis [58].

Trastuzumab (Herceptin) is a humanized monoclonal antibody designed to target HER2 on the surface of HER2-overexpressing tumor cells. Trastuzumab is approved for the treatment of HER2-positive breast cancer in both the metastatic and adjuvant settings.

Trastuzumab is generally well tolerated and is not associated with the side-effects common to cytotoxic chemotherapy, but has been associated with an increased incidence of CT. This can manifest as either asymptomatic decreases in LVEF or as overt HF.

The association between trastuzumab and cardiac dysfunction was not described in the clinical development process because HER2 is not overexpressed in the heart, and no CT was observed in preclinical or early clinical studies.

Unanticipated trastuzumab-associated cardiac dysfunction was first identified in the metastatic breast cancer (MBC) trials. In the phase III trial by Slamon *et al.*, any CT and grade 3–4 CT was reported in 27% and 16% of patients, respectively, who received prior anthracycline (up to 360 mg m^{-2} of doxorubicin) /cyclophosphamide (AC) plus trastuzumab (concurrent therapy) and 13% of patients who received trastuzumab plus paclitaxel [59]. This relatively high incidence of CT may have resulted, at least in part, from the lack of prior knowledge about the relationship between trastuzumab and cardiac effects. This meant that for the phase III trial, patients with preexisting cardiac disease were not excluded from participating.

A Cardiac Review and Evaluation Committee (CREC) retrospectively reviewed data from >1200 patients from 7 phase II/III trials using a broad definition that included at least one end point: wall motion abnormality, decline in LVEF, signs and/or symptoms of HF. They concluded that patients treated with trastuzumab were at increased risk of cardiac dysfunction: the incidence was highest in patients receiving concurrent trastuzumab and AC (27%) with lower risk in patients receiving trastuzumab plus paclitaxel (13%) or trastuzumab alone (3 to 7%). Most of the latter patients also received prior, but not concurrent, AC therapy. Most of the

patients (79%) who had developed cardiac dysfunction in these trials experienced an improvement after receiving standard treatment for heart failure [60].

Following the identification of the risk of CT associated with trastuzumab, subsequent prospective trials have not given trastuzumab and anthracyclines concurrently, and have included cardiac-specific eligibility criteria excluding most patients with underlying cardiac disease or baseline LVEF below 50%. Studies have also combined trastuzumab with other non-anthracycline chemotherapeutic agents. Based on these facts, the incidence of cardiac dysfunction has decreased in comparison to the incidence initially reported in the early MBC trials. The incidence varies based on a distinction between asymptomatic decreases in LVEF (with definitions that vary by trial design) and symptomatic heart failure; all are dependent on the other chemotherapy administered with limitations due to small patient numbers in each study.

5.3.4.1 Metastatic Breast Cancer Studies

- In a study that included 572 patients, there was a 2.7% incidence of grade 3 CT with trastuzumab (1% decrease LVEF and 1% symptomatic HF) versus 0% among patients not receiving trastuzumab [61].
- In a study that included 162 patients, the incidence of absolute decreases in LVEF of $\geq$15% was 17% in the 86 patients who received docetaxel/trastuzumab (TH) compared with 8% in the 76 patients who received docetaxel monotherapy. Symptomatic HF was reported in 2.3% of patients who received combination therapy. The majority of patients who experienced decreases in LVEF of $\geq$15% had received previous treatment with adjuvant anthracycline therapy [62].
- In a study with 131 patients in each arm comparing TC (paclitxel) with TCH, the incidence of HF was 2.1% in the TCH arm and 0.0% in the TC arm [63].
- In a study of 38 patients treated with either TC or TCH, the absolute decline in LVEF of >15% was 5.5% and 6.7% for TH and TCH, respectively. One patient had symptomatic HF (0.8) in the TC arm and there was no Grade 3–4 CT in the TCH arm [64].
- In a study of first-line trastuzumab plus vinorelbine in 54 patients with MBC, 44 patients completed a 16 week evaluation of cardiac function. In total, 13% had an asymptomatic decline in LVEF and 2% had symptomatic HF [65].

- No cardiac dysfunction was reported in a study combining trastuzumab and gemcitabine [66].
- In the Capecitabine, Herceptin, And Taxotere (CHAT) trial, symptomatic HF was reported in 2 of 225 patients who received trastuzumab (0.9%), both of whom had received adjuvant anthracycline therapy [67].
- In a retrospective analysis, 173 MBC patients who had received trastuzumab were evaluated for cardiac safety. 28% experienced a cardiac event: 1.7% had an asymptomatic decrease in the LVEF of 20 percentage points, 15.6% and 10.9% experienced grade 2–3 CT, respectively. CT was related to lower baseline LVEF (HR = 0.9444; $P = 0.001$) [68].
- The incidence of CT of trastuzumab concurrently given with up to 6 cycles of cyclophosphamide plus low (60 mg m^{-2}) or high (90 mg m^{-2}) dose epirubicin in women with MBC was studied in a Phase I–II trial. The control group was HER-2 negative and did not receive trastuzumab. CT was defined as a >10 point decrease in LVEF to <50%. The incidence of CT was 6% versus zero between the treatment and control groups and asymptomatic decreases in LVEF >10% but above 50% were higher in the high compared to the low dose epirubicin group [69].

These studies suggest that in patients with MBC, CT is more often manifested as an asymptomatic decline in LVEF (1–28%) and that symptomatic HF occurs less frequently (0.9–3.2%). Prior anthracycline therapy and a low baseline LVEF may help to identify the higher risk patients. In the majority of patients, cardiac dysfunction is reversible with the potential of post-treatment recovery of cardiac function.

As a result of the MBC trial experience, subsequent trastuzumab trials in the adjuvant setting were subject to strict screening and monitoring procedures. Before enrolment into an adjuvant trial, patients had a thorough cardiac assessment to exclude high risk patients and all protocols included a schedule of regular cardiac monitoring to detect any changes in LVEF. Direct cross-trial comparisons are difficult because of disparate cardiac endpoints and event criteria. However, trastuzumab therapy given after anthracycline-based chemotherapy in the adjuvant setting was associated with an increased disease-free survival benefit with a cardiac event rate that remained below the 4% safety cut-off set by independent data monitoring committees.

5.3.4.2 Adjuvant Studies

- **NSABP B-31:** 814 patients post-AC therapy were assigned to one of two treatment arms: paclitaxel alone or with trastuzumab. The three year cumulative incidence of cardiac events (HF or cardiac death) was 0.8% and 4.1%, respectively. 14% of patients discontinued trastuzumab because of asymptomatic decreases in LVEF. HF was more frequent in older patients and patients with marginal post-AC LVEF [70].
- **NCCTG N9831:** 1944 patients post-AC therapy were assigned to one of three arms: weekly T, T then H, or TH, then H. The three year cumulative incidence of cardiac events (HF or cardiac death) was 0.3%, 2.8%, and 3.3%, respectively. Cardiac function improved in most HF cases following trastuzumab discontinuation and/or cardiac medication. Factors associated with increased risk of a cardiac event with trastuzumab were older age ($P < 0.003$), prior/current antihypertensive agents ($P = 0.005$), and lower pretreatment LVEF ($P = 0.033$). The incidence of asymptomatic LVEF decreases requiring holding trastuzumab was 8–10% [71].
- **BCIRG 006:** 3222 patients were treated in three arms: 1072 with AC then T, 1076 with AC then TH and 1074 with TCH. The cumulative 3 year CT (HF) was 0.4%, 1.9%, and 0.4%, respectively [72]. The recent approval of TCH in the adjuvant setting coupled with the low incidence of cardiac dysfunction associated with TCH makes this a particularly suitable regimen for patients with baseline reductions in LVEF and cardiac risk factors.
- **FinHer:** Compared docetaxel with vinorelbine, administered with or without trastuzumab, as adjuvant treatment for early breast cancer. In this study, trastuzumab was administered before other cardiotoxic therapies and concomitantly with potentially synergistic chemotherapy for only 9 weeks to test the hypothesis that such a schedule would limit CT and maintain efficacy. None of the women who were treated with trastuzumab had cardiac failure, and, unexpectedly, these women had slightly better maintenance of LVEF than did those who did not receive the antibody [73].
- **HERA:** 3796 patients were assigned to trastuzumab or observation. The incidence of trastuzumab discontinuation due to cardiac events was 4.3%, with an incidence of asymptomatic decline in LVEF of 3.04% versus 0.53%, symptomatic HF of 2.15% versus 0.12%, and severe HF of 0.60% versus 0% in the trastuzumab and observation-only arms, respectively. Patients with trastuzumab-associated cardiac dysfunction received higher cumulative doses of anthracyclines, had a lower baseline LVEF, and a higher body mass index [74].

- Similar data regarding the incidence of CT have been reported when trastuzumab is combined with liposomal doxorubicin as a substitute for doxorubicin. The results of four major trials were summarized by Rayson *et al*. The aggregate overall incidence of symptomatic or protocol-defined CT was 2–5% with asymptomatic decreases in LVEF no more than 11% [75].
- In 70 women with HER 2 overexpressing breast cancer who received AC followed by TH, there was a 7% incidence of asymptomatic decreases in LVEF and a 1.4% incidence of HF [76].

Summaries of these studies are presented in Tables 5.2 and 5.3. The large amount of data gathered from the adjuvant trials complements MBC trial data, establishing the clinical efficacy of trastuzumab therapy in patients with HER2-positive breast cancer. This is associated with a small, but manageable risk of cardiac dysfunction manifested by asymptomatic declines in LVEF (3–14%) and/or symptomatic HF (1.9–4.1%). The risk and incidence varies according to the treatment protocol and definition of CT.

Table 5.2 Metastatic breast cancer trials.

Trial design and reference	Number of patients studied	Incidence of cardiotoxicity
Trastuzumab [61]	572	2.7% incidence of grade 3 CT with trastuzumab(1% decrease LVEF and 1% symptomatic HF) versus 0% among patients not receiving trastuzumab
TH vs. docetaxel monotherapy [62]	162	Absolute decreases in LVEF of $\geq$15% was 17% in the 86 patients who received docetaxel/trastuzumab (TH) compared with 8% in the 76 patients who received docetaxel monotherapy. Symptomatic HF was reported in 2.3% of patients who received combination therapy. The majority of patients who experienced decreases in LVEF of $\geq$15% had received previous treatment with adjuvant anthracycline therapy

(*continued overleaf*)

Table 5.2 (*continued*)

Trial design and reference	Number of patients studied	Incidence of cardiotoxicity
TC (paclitaxel) vs. TCH [63]	131 in each arm	HF occurred 2.1% in the TCH arm and 0.0% in the TC arm
TC or TCH [64]	38	Absolute decline in LVEF of >15% was 5.5% and 6.7% for TH and TCH respectively. One patient has symptomatic HF (0.8) in the TC arm and there was no Grade 3–4 CT in the TCH arm
First-line trastuzumab plus vinorelbine [65]	44 of the 54 enrolled completed a 16 week evaluation of cardiac function	13% had an asymptomatic decline in LVEF and 2% had symptomatic HF
Trastuzumab and gemcitabine combination [66]		None reported
Capecitabine, Herceptin, And Taxotere (CHAT) [67]		Symptomatic HF was reported in 2 out of 225 patients who received trastuzumab (0.9%), both of whom had received adjuvant anthracycline therapy
Retrospective analysis of trastuzumab therapy [68]	173	28% experienced a cardiac event: 1.7% had an asymptomatic decrease in the LVEF of 20 percentage points, 15.6% and 10.9% experienced grade 2/3 CT respectively. CT was related to lower baseline LVEF (HR = 0.9444; $P = 0.001$)
Phase II trial of trastuzumab given with up to 6 cycles of cyclophosphamide plus low (60 mg m^{-2}) or high (90 mg m^{-2}) dose epirubicin in women with MBC.The control group was HER^{-2} negative and did not receive trastuzumab [69]		CT was defined as a >10 point decrease in LVEF to <50%. The incidence of CT was 6% versus zero between the treatment and control groups and asymptomatic decreases in LVEF >10% but above 50% were higher in the high compared to the low dose epirubicin group

Table 5.3 Adjuvant breast cancer studies

Trial design	Number of patients studied	Incidence of cardiotoxicity
NSABP B-31 [70] Paclitaxel alone or with trastuzumab	814 post-AC therapy	The three year cumulative incidence of cardiac events (HF or cardiac death) was 0.8% and 4.1% respectively. 14% of patients discontinued trastuzumab because of asymptomatic decreases in LVEF. HF was more frequent in older patients and patients with marginal post-AC LVEF.
NCCTG N9831 [71] Weekly T, T then H, or TH, then H	1944 post-AC therapy	The three year cumulative incidence of cardiac events (HF or cardiac death: was 0.3%, 2.8%, and 3.3%, respectively. Cardiac function improved in most HF cases following trastuzumab discontinuation and cardiac medication. Factors associated with increased risk of a cardiac event with trastuzumab were older age ($P < 0.003$), prior/current antihypertensive agents ($P = 0.005$), and lower registration LVEF ($P = 0.033$). The incidence of asymptomatic LVEF decreases requiring holding trastuzumab was 8% to 10%.
BCIRG 006 [72] AC then T, AC then TH, or TCH	3222	The cumulative 3 year CT (HF) was 0.4%, 1.9%, and 0.4%, respectively.
FinHer [73] Docetaxel with vinorelbine + trastuzumab		None of the women treated with trastuzumab had HF. In this study, trastuzumab was administered before other cardiotoxic therapies and concomitantly with potentially synergistic chemotherapy for only nine weeks to test the hypothesis that such a schedule would limit CT and maintain efficacy.

(*continued overleaf*)

Table 5.3 (*continued*)

Trial design	Number of patients studied	Incidence of cardiotoxicity
HERA [74] Trastuzumab or observation	3796	The incidence of trastuzumab discontinuation due to cardiac events was 4.3%, with an incidence of asymptomatic decline in LVEF of 3.04% versus 0.53%, symptomatic HF of 2.15% versus 0.12%, and severe HF of 0.60% versus 0% in the trastuzumab and observation-only arms, respectively. Patients with trastuzumab-associated cardiac dysfunction received higher cumulative doses of anthracyclines, had a lower baseline LVEF, and a higher body mass index.
Rayson *et al.* [75] Trastuzumab combined with liposomal doxorubicin as a substitute for doxorubicin		The aggregate overall incidence of symptomatic or protocol defined CT was 2–5% with asymptomatic decreases in LVEF no more than 11%. Results of 4 major trials were summarized.
AC followed by TH [76]	70 women with HER 2 overexpressing breast cancer	7% incidence of asymptomatic decreases in LVEF and a 1.4% incidence of HF.

The consistently identified risk factors for development of CT are related to the age of the patient (elderly), pretreatment with anthracyclines, a lower LVEF pretreatment and possibly the co-association of hypertension.

> The incidence of reversible asymptomatic and symptomatic cardiomyopathy with trastuzumab use is <16% and 4%, respectively.

5.3.5 Lapatinib

Lapatinib (Tycarb) is a selective oral dual tyrosine kinase receptor inhibitor targeting EGFR1 and EGFR2. Clinical efficacy has been demonstrated for MBC and a variety of solid tumors when used as monotherapy, or

combined with capacitabine, FOLFOX (oxaloplatin, leucovorin, 5-fluorouracil), FOLFIRI (irinotecan) and trastuzumab. Similar to trastuzumab, it is well tolerated with low hematologic toxicity and, to date, a low incidence of cardiac side-effects. In a study of 138 patients with locally advanced or MBC who were treated with lapatinib (either 1500 mg once daily or 500 mg twice daily), three patients withdrew secondary to CT, four additional patients (3%) had asymptomatic >20% relative LVEF reductions and more than the defined lower limit of normal from baseline. The decrease was recognized 3 to 11 weeks after starting lapatinib. All had received prior treatment with an anthracycline, none developed symptoms and all returned to baseline with either interruption or discontinuation of lapatinib and/or HF treatment [77].

In a recent review by Perez *et al*., 44 trials conducted (2001 to 2006) were analyzed, including 3689 patients treated with lapatinib alone or as part of combination chemotherapy. Overall, the incidence of CT was 1.6% (60 patients), 83% of these were asymptomatic with only 7 symptomatic patients. Of the group, 20% had prior anthracycline therapy, 23% had prior trastuzumab therapy and 57% were anthracycline and trastuzumab naïve. The mean onset of CT was at 13± 9 weeks (95% CI: 2–54 weeks) and the mean decrease in LVEF was 18.8 ± 5.2% (95% CI: 11–32%) with a mean nadir of 43%. Of 35 analyzable patients, there were 19 and 16 patients with full and partial recovery, respectively. In a comparator group of 1301 patients treated without lapatinib, the incidence of CT was 0.7% [78].

In a phase I study in patients with advanced squamous carcinoma of the head and neck, there was no symptomatic CT in doses up to 1500 mg d^{-1} [79].

The overall rate of lapatinib induced CT is less than 2%, with the majority of patients manifesting asymptomatic decreases in LVEF. An explanation for the difference in CT between lapatinib and trastuzumab is currently speculative. The real difference may only be related to the pretreatment use of anthracyclines and, as further studies and longer intervals from original treatment become available, there may be no clinically significant difference in CT between the two agents.

Lapatinib produces less cardiotoxicity than trastuzumab.

5.3.6 Bevacizumab

Bevacizumab (Avastin) is a recombinant humanized monoclonal antibody with activity against vascular endothelial growth factor (VEGF)

that disrupts tumor angiogenesis. It has been studied in various malignancies including CRC, NSCLC, RCC, and breast cancer when used alone or as part of combination chemotherapy (pactitaxel, capatitabine, lapatanib, docetaxel, nab-pactitaxel, AC,TAC).

Because of the physiologic role of VEGF in regulating arterial tone and promoting arterial vasodilatation, it is not surprising that the most common adverse effect of bevacizumab therapy is hypertension as has been recognized consistently from the first phase I trial [80]: hypertension may be newly diagnosed or existing hypertension may be exacerbated.

The publication from the pivotal phase III trial of bevacizumab, fluorouracil, and leucovorin in metastatic CRC reported a 22.4% incidence of any grade of hypertension in the bevacizumab treatment arm, of which 11% of patients had grade 3 hypertension [81].

Bevacizumab-induced hypertension is reversible, may occur early or late in treatment and may be dose related. In a meta-analysis of 1850 patients in 10 trials treated with bevacizumab, the incidence of hypertension ranged between 2.7 and 32% and between 17.6 and 36% between low and high-dose treated patients with a RR of 3.0 for low dose (95% CI: 2.2 to 4.2;) and 7.5 for high dose (95% CI: 4.2 to 13.4) [82]. Overall, the incidence of hypertension may be >60% [83] and grade 3–4 hypertension has been reported from 8–19% [84] with initial or subsequent doses.

Early studies in patients with metastatic CRC and NSCLC treated with bevacizumab suggested that these patients are also at an increased risk for venous thromboembolic (VTE) and/or arterial thromboembolic events (ATE). Arterial events can occur in any arterial bed but are reported most commonly in the coronary and cerebral circulations, manifested by transient ischemic attacks (TIA), cerebrovascular accidents (CVA), angina, acute coronary syndromes (ACS) and myocardial infarctions (MI).

The mechanism for increased thrombosis is multifactoral and includes the decreased production of endothelial nitric oxide with a decrease in vasodilatation, increase in platelet aggregation and enhanced thrombin formation. Current analyses suggest that the risk for bevacizumab ATE is increased approximately two-fold. In the above noted Hurwitz trial [84], there was a 19.4% incidence of thrombotic phenomenon compared to 16.2% in the non-bevacizumab group.

In a pooled analysis from five randomized controlled trials that included a total of 1745 patients with metastatic CRC, breast cancer, or NSCLC, published by Scappiticci *et al.*, the risk of arterial or venous thromboembolism was assessed combining bevacizumab with chemotherapy compared to chemotherapy alone. Among patients treated

with both, 3.8% experienced ATE events, compared with 1.7% of patients on chemotherapy alone. Death from ATE was 0.62% compared to 0.26%, respectively. There was no statistically significant difference in the incidence of venous thromboembolism (VTE). Risk factors for blood clots in both arteries and veins included previous blood clots and age >65 years [85].

A similar incidence of ATE was found in a study of 1401 patients who were randomly assigned in a 2 × 2 factorial design to oxaliplatin-based chemotherapy with or without bevacizumab. The incidence of grade 3–4 ATE was 1% and 2%, respectively, in the bevacizumab treatment groups [86].

The risk of ATE is not related to dose or duration of bevacizumab therapy. In a preliminary report of the BRITE study of 1953 patients, the incidence of ATE in the first year was 2.1% and 0.7% beyond 12 months [87]. The incidence of hypertension and ATE is increased in the elderly and in patients who have had prior arterial embolic events [88,89].

Although there was no statistical difference for VTE in the Scappiticci pooled analysis, an increased risk of venous thrombosis in addition to the risk associated with malignancy has been reported with incidences that vary from 3% to 19.4% across phase II and phase III trials. In a recent meta-analysis, a total of 7956 patients with a variety of advanced solid tumors from 15 randomized controlled trials were identified. Among those patients receiving bevacizumab, the summary incidences of all-grade and high-grade VTE were 11.9% (95% CI: 6.8–19.9%) and 6.3% (95% CI: 4.8–8.3%), respectively, with an RR of 1.33 (95% CI: 1.13–1.56; $P < 0.001$) compared with controls.

Tumor type and the bevacizumab dose may influence the risk of thromboembolism. Patients with metastatic CRC were found to have a 19.1% incidence of all grade VTE (95% CI: 16.1–22.6%), NSCLC 14.9% (95% CI: 8.2–25.5%), breast cancer 7.3% (95% CI: 4.6–11.5%), and renal cell carcinoma 3.0% (95% CI: 1.6–5.5%) [90].

An additional consideration is a presumed interaction in patients who received prior anthracycline-containing chemotherapy, resulting in an increased incidence of left ventricular dysfunction with rates ranging from 6 to 35% [91–93].

The use of bevacizumab is associated with an increased incidence of hypertension and arterial and venous thrombosis.

5.3.7 New Anti-VEGF Drugs

At present, there are at least four specific anti-VEGF agents in phase I development:

- an engineered VEGF that binds VEGF-A 100- to 1000- fold more tightly than monoclonal antibodies (VEGF-Trap);
- an antisense oligonucleotide (VEGF-AS);
- two peptides (Aplidin and Dehydrodidemnin B).

For these, the incidence of treatment-associated CT remains to be elucidated although a 31.6% incidence of hypertension was found in patients with macular degeneration treated with VEGF Trap [94].

In summary, bevacizumab has CT that includes dose-related hypertension and an increased incidence of ATE and VTE above that which would be expected on the basis of cancer-related thrombosis. This is variable depending on the patient's age and history of prior thromboembolism and tumor type.

5.4 ANTIMETABOLITES

Antimetabolites are chemically similar to substances required in normal biochemical pathways. These 'decoys' interfere with normal function preferentially in cancer cells, leading to abnormalities in cell division and replication.

5.4.1 Gemcitabine

Gemcitabine (Gemzar) is an antimetabolite that is effective as monotherapy or in combination with other drugs in a variety of solid tumors. Toxicity related to the cardiovascular system is rare. When used as monotherapy, no CT has been reported [95,96].

Non-cardiogenic pulmonary edema has been described in up to 7.1% of patients [97] and mild peripheral edema has been reported in up to 20% of patients with <1% of these developing severe edema necessitating drug discontinuation [98]. The mechanism for edema is unknown, but not considered to be cardiac. Preclinical and phase I data from 1997

described incidences of ventricular tachycardia in 1.4, 0.7, 0.2 and 0% and decreases in LVEF in 0.9, 0, 0.7 and 0.2% for grades 1–4, respectively, along with a single case of pericarditis [99]. There have been three case reports describing atrial fibrillation within 18–24 h of gemcitabine infusion, with recurrence following second and/or third subsequent infusions. One patient had paroxysmal atrial fibrillation prior to treatment and two of the patients had advanced lung cancer [100–102].

Similarly, ACS and AMI have also been described in sporadic case reports: all in patients with known CAD and underlying heart disease [103–104].

Gemcitabine has been implicated in radiation recall and Vogl *et al.* reported four cases of hemodynamically significant pericardial effusions in patients with refractory lymphoma who received gemcitabine after prior mediastinal radiation without subcarinal blocking [105].

If there is indeed a causal relationship to CT, the incidence is some percentage significantly lower than 1%. Vigilance for CT should be present in the older patient, especially those with underlying cardiac disease and/or prior atrial fibrillation and in patients with prior radiation therapy.

5.4.2 Cytarabine (Ara C, Cytosine Arabinoside)

Cytarabine (Cytosar) is an antimetabolite that is the backbone of treatment of acute myelogenous leukemia. CT is rare, with sporadic case reports of pericarditis [106–109] and arrhythmias. The latter have been dominated by asymptomatic sinus bradycardia and idioventricular rhythms [110–112].

5.4.3 Fluorouracil (5-FU)

Fluorouracil (Efudex) has been used for more than five decades as the first line therapy for GI, head and neck cancers and breast cancer. Initially, the mode of delivery was as an intravenous bolus. Treatment has evolved to include continuous infusion to reduce toxicity and improve clinical response rates.

5-FU pharmacokinetics are characterized by a wide inter- and intrapatient variability, leading to unpredictable blood levels significantly impacting efficacy and toxicity. Clinical efficacy is well established with

cardiac side-effects being less common than GI and hematological adverse events.

The antitumor activity of the drug is mediated through 5-fluoronucleotides that interfere with normal DNA and RNA function. Both genetic and non-genetic factors have been implicated in toxicity risk. More than 80% of a given dose is rapidly metabolized by dihrodropyrimidine dehydrogenase (DPD) [113]. A potential role of DPD in determining 5-FU toxicity has been suggested with a speculated relationship between lymphocyte DPD activity, 5-FU clearance and neurotoxicity [114]. Other patient characteristics including age and sex (females>males) have also been suggested to influence clearance.

CT has been manifested by chest pain without ECG changes and with ST elevation suggestive of acute STEMI, arrhythmias, HF and sudden death. Literature incidence ranges from less than 1% to 1.8% with an associated mortality rate of 2.2–13%. In a recent study of 683 patients who received various regimens of 5-FU, there was a 0.8% incidence of grade 3–4 CT.

Labianco reported a 1.6% incidence of CT manifested mainly by angina in 1083 patients treated with 5-FU. Multivariate analysis showed a relationship to known prior heart disease rather than age or concomitant chemotherapy [116].

Keefe *et al*. reported their experience at MSKCC over a 16 month period from 1990 to1991 in a series of 910 patients treated with 5-fluorouracil. Five patients (0.55%) developed life-threatening chest pain associated with ST elevation and ventricular arrhythmias thought to be secondary to coronary vasospasm. The events occurred on the third or fourth day of a planned 5-day infusion and after the fourth intravenous bolus in a patient receiving bolus therapy [117].

Soft confirmation of the low incidence of CT is reflected in multiple large studies and 2 meta-analyses, that included 4496 patients, published from 1994 to 2002. CT is not included among the reported common toxicities (diarrhea, mucositis, neutropenia, neutropenic hand-foot syndrome) [118–121].

A classic study by deGrament published in 1997 included 448 patients randomly assigned to monthly bolus 5-FU compared to a bimonthly dose intense regimen. A 1% (2/216) incidence of CT was seen in the monthly group versus 3.7% (8/217) in the higher dose bimonthly group with no grade 3–4 CT [122]. Recent studies that reflect a realistic picture of the incidence of CT include an incidence of 0.6% with one of 162 patients developing angina [123] and an incidence of 1.2% that was reported in16 of 1350 patients with a variety of cardiac adverse events. These included ten patients with angina accompanied by electrocardiographic changes,

two patients with asymptomatic ECG changes, three patients with symptoms without ECG changes and one patient with heart failure. All clinical signs and ECG changes were observed early after initiation of therapy and all but one patient fully recovered. One patient had an actual myocardial infarction and died 2 months later. All manifestations appeared in patients with no prior history of heart disease [124].

CT manifested by acute nonischemic HF has been described by case report with an incidence that is less than 1%. In one case, myocardial biopsy showed proliferation of the sarcoplasmic reticulum with marked vacuolization [125,126].

Four cases of cardiac failure associated with the combined administration of 5- FU and cisplatin have also been reported [127]. This suggests a possible synergistic effect of cisplatin on fluorouracil CT and should, at least, raise the awareness of clinicians using this combination chemotherapy.

There is a large body of literature that attempts to predict 5-FU CT. These include patient profiles (hereditary DPD deficiency, underlying ischemia, older age, female sex, elevated creatinine), delivery method (infusion, bolus versus continuous, cycle >1) and screening studies (for SNPs, DPD deficiency and individual response to the drug by giving test doses, measuring blood levels) [128]. Currently, no reliable markers to predict 5-FU toxicity have been validated to permit their use as a standard of care. CT is not satisfactorily explained by age, sex, hepatic or renal function, method of administration, comorbidity or comedication [129].

In summary, the incidence of CT manifested by angina with or without ECG changes is in the range of 1–2%. Rarely, patients develop manifestations of HF suggesting a direct toxic effect of 5-FU on the myocardium without vasospasm, or vasospasm at the microvascular level, that presents like myocarditis.

> Chest pain with and without electrocardiographic changes occurs in 2% of patients treated with fluorouracil.

5.4.4 Capecitabine

Capecitabine (Xeloda), an oral fluoropyrimidine, is a 5-FU prodrug drug used in the treatment of MBC and CRC. Capecitabine is metabolized to

5-FU via a complex enzymatic pathway. There are more than 50 case reports to date of capecitabine-induced chest pain. The most frequent description implicates coronary spasm suggested by ST segment elevation with or without arrhythmias [130], global left ventricular dysfunction or overt HF [131,132].

A meta-analysis of 53 patients from the literature found 38 (71%) cases with angina, 6 (11.3%) with arrhythmias and 6 (11.3%) with myocardial infarction. Rechallenge in 16 patients led to symptoms in 10 [133].

Typically, ECG changes and symptoms are transient and respond to drug withdrawal, nitrates and/or calcium channel blockers. Coronary arteriography [134] or CT angiography [135] have typically demonstrated no obstructive epicardial lesions in the coronary arteries.

Chest pain without ECG changes has also been infrequently seen occurring either at rest or with activity only during treatment weeks and responds to either cessation of therapy or the addition of oral calcium channel blockers [136].

The overall incidence of chest pain is probably similar to that reported with fluorouracil.

> The spectrum and incidence of chest pain with and without electrocardiographic changes associated with capacitabine is similar to fluorouracil.

5.4.5 Histone Deacetylase Inhibitors (HDAC)

Epigenetic modification is an important mechanism in tumor cell biology. Drugs that target epigenetic silencing mechanisms are under development. Depsipeptide is one such drug. A phase I preclinical trial of 15 patients with metastatic neuroendocrine tumors was stopped prematurely due to serious CT that included one case of sudden death, 2 cases of grade 2 ventricular tachycardia and 3 cases of grade 2 QT prolongation [137]. Toxicity of depsipeptide may be mediated through HDAC inhibition.

To date, more than 500 patients have been treated with depsipeptide. In one eloquently documented study, more than 50% of patients had non-specific ST-T wave changes, 65% and 38% had isolated supraventricular and ventricular premature depolorizations, respectively, and

unsustained supraventricular tachycardia/ventricular tachycardia in 38%/14%. There have been reports of decreases in LVEF or liberation of cardiac biomarkers (mb-CPK or troponin) [138].

The drug is arrhythmogenic and the issue of associated potential QT prolongation modulates the severity of drug-induced arrhythmias. The true incidence of QT prolongation remains speculative, but probably real and less than seen in pre-clinical studies. This warrants ECG monitoring and QT measurement with treatment.

5.5 ALKYLATING AGENTS

Alkylating agents add an alkyl group to DNA that makes it unable to uncoil and separate and therefore divide.

5.5.1 Cyclophosphamide

Cyclophosphamide (Cytoxan) is the alkylating agent that is the mainstay of chemotherapy for breast and many solid tumors, most hematologic malignancies and as a preconditioning agent prior to bone marrow transplantation. Highly differing rates of CT have been described with a lower incidence reported today than in the past. CT has been associated with high dose chemotherapy and the first fatal case of drug-induced HF was described in 1971 [139].

Prior to 1986, the development of CT manifested by peri-myocarditis was thought to be related to high dose treatment (120–180 mg/kg/day) over a standard 7-day delivery regimen with an incidence of 22% and a fatality rate of 11%. Gottdiener *et al.* [140] described their experience in 32 patients with hematologic malignancies treated with 180 mg/kg/day for 4 days and found a 28% incidence of HF and a 33% incidence of pericardial effusion. In their series, 6 patients (19%) died and 6 patients (19%) had pericardial tamponade. This series elucidated high dose cyclophosphamide-induced CT, its severity, high fatality rate, as well as its reversibility.

In 1986, Goldberg and associates studied 84 consecutive patients with hematological malignancies and found an overall incidence of CT of 17% that occurred within the first 10 days of treatment. When dosing was related to body surface area, a cutoff point of 1.55 g/m^2/day was evident.

Below this level 1/32 (3%) developed CT and above this level 13/52 (25%) developed CT with a 12% mortality rate [141].

More recently, with the advent of multifractionated schedules of administration, the incidence of overt HF has declined and the recognition of subclinical myocardial dysfunction has been recognized. Zver and his group studied 23 consecutive patients with multiple myeloma. They measured biomarkers of myocardial damage and performed serial echocardiograms during high dose cyclophosphamide therapy. They found evidence of consistent neurohormonal activation with elevation of BNP and ET-1 levels after treatment compared to baseline with echocardiographic evidence of diastolic dysfunction [142].

Cardiac biopsy has shown changes of toxic endothelial and myocardial damage with subsequent interstitial hemorrhage and edema. A recent mouse study showed a consistent suppression of cyctoplasmic thioredoxin reductase activity and non-protein free thiol levels [143].

Unlike the chronic CT associated with anthracyclines that is related to cumulative dosing, the CT associated with cyclophasphamide is related to the magnitude of single dosing, is more often reversible without permanent structural myocardial damage and lacks the latency for development with all cases occurring within a week to 10 days of treatment.

> The cardiotoxicity of cyclophosphamide is not related to cumulative dose.

5.5.2 Ifosfamide

Ifosfamide (Mitoxana) is an oxazaphosphorine nitrogen mustard compound that is structurally similar to cyclophosphamide with indications and side-effects that are virtually identical [144].

Ifosfamide has little CT at standard doses but high-dose therapy has caused asymptomatic decreases in LVEF, myocarditis with HF, pericarditis, and arrhythmias. The total dose that causes myocardial injury has usually has been >1000 mg m^{-2} [145,146].

In one series of 52 patients who received combination chemotherapy and autologous bone marrow transplantation, 17% (9 patients) developed clinical HF [147].

5.6 MICROTUBULE-TARGETING DRUGS

The microtubule-targeting drugs have the ability to disrupt microtubule function by interacting with tubulin. Drugs are divided into those that inhibit tubulin polymerization (vinca alkaloids) or promote tubulin polymerization (taxanes) [148].

5.6.1 Vinca Alkaloids

These are microtubule-targeting drugs derived from the pink periwinkle plant. There are four drugs in this class that vary by minor structural differences with different spectrums of clinical efficacy: vincristine (Oncovin), vinblastine (Velban), vindesine (Eldisine) and the semisynthetic vinorelbine (Navelbine).

The vinca alkaloids are also part of the backbone of regimens for hematologic and solid malignancies with a rare incidence of CT that includes repolarization changes, autonomic neuropathy (postural blood pressure and heart rate fluctuations) and HF. The most commonly reported CT is the development of myocardial ischemia and infarction [149,150].

The mechanism for CT is speculative and includes drug-induced vasoconstriction and hypertension, a direct effect on cellular microtubules with impairment of myocardial metabolism and coronary spasm [151].

Recent literature focuses on vinorelbine chemotherapy. In a meta-analysis of 19 trials 'comparing' 2441 patients with a variety of hematologic malignancies treated with vinorelbine to 2050 controls, the overall incidence of CT was 1.19% (95% CI: 0.75–1.67) for patients receiving vinorelbone. Approximately 60% of the CT was related to asymptomatic or symptomatic decrease in LVEF with the remainder divided between chest pain and acute myocardial infarction. CT incidence appears to be related to the population being treated so that studies with lower and higher incidences either excluded or included patients with pre-existing cardiac disease, respectively [152]. For comparison, in a study where monotherapy vinorelbine was used to treat women with breast cancer, there was no CT [153] or 3% grade 1–2 decrease in LVEF [154]. In a meta-analysis of 13 trials involving 492 women, the incidence of CT was 1.4% with 82% manifested as changes in LVEF and 28% as ischemia or infarction [155].

5.6.2 New Drugs

5.6.2.1 Vinflunine

Vinflunine (Javlor) is the first fluorinated microtubule inhibitor belonging to the vinca alkaloids family. In an analysis of 880 patients in phase II/III clinical trials with a dose of 320 mg m^{-2} in a variety of advanced solid tumors, the incidence of nonspecific chest pain was 5.2% with 4.1% of instances being grade 1–2 [156].

In summary, it is impossible to know the true incidence of CT with the vinca alkaloids. Myocardial ischemia is a potential side-effect with vigilance recommended for its recognition, especially in patients with known coronary artery disease prior to treatment.

> Chest pain occurs in approximately 1.5% of patients receiving vinca alkaloids.

5.6.3 Taxanes

The taxanes (paclitaxel [*Taxol*], docetaxel [*Taxotere*]) are derived from the bark of the Yew tree and have demonstrated clinical efficacy in a variety of solid tumors since the early 1990s. Their anti-tumor activity comes from an inhibition of microtubular function. Paclitaxel, but not docetaxel is formulated in a Cremophor EL vehicle that enhances drug solubility. As single agents they have negligible cardiac effects [157,158].

Paclitaxel infusion has been associated with asymptomatic sinus bradycardia in up to 29% of patients with a 5% incidence of other cardiac arrhythmias (premature ventricular depolarizations, ventricular tachycardia and atrio-ventricular block) [159]. There is speculation that these arrhythmias are a result of histamine release triggered by Cremophor EL. There is no direct myocardial effect and no HF incidence.

However, when paclitaxel was initially combined with doxorubicin, the incidence of CT manifested by left ventricular dysfunction increased by 20% compared to doxorubicin alone. This was due to either a pharmacokinetic interaction of the two drugs leading to a 30% increase in doxorubicin drug levels or an increase in a toxic secondary metabolite of

doxorubicin, doxorubicinol, or a combination of both. Subsequent research implicated the dosing schedule as the culprit and current alterations of total dose, sequence and timing has minimized (<5%) the CT incidence with combination therapy [160]. There is no pharmacokinetic interaction or increase in CT when docetaxel is combined with doxorubicin [161].

When paclitaxel is added to modern adjuvant chemotherapy regimens for breast cancer, there is no increase in CT. Giannini *et al*. found a 0.3% incidence of severe CT compared to 0.5% incidence when doxorubicin followed by cyclophosphamide, methotrexate, and fluorouracil (CMF) was given without or with paclitaxel, respectively [162].

Because of changes in pharmacokinetics in the elderly, there has been concern about administering taxanes to patients >65 years. A recent detailed review of studies in the elderly reinforces their efficacy given as weekly infusions with CT incidence that is not different from that encountered in younger populations [163].

A nanoparticle albumin-bound form of paclitaxel (*Abraxane*) has emerged with less myelosupression than standard paclitaxel. A 3% incidence of grade 3–4 CT has been reported, manifested by chest pain, tachyarrhythmias, cardiac arrest, hypotension and fluid retention [164]. In a study of 1000 patients with ovarian cancer, an overall incidence of CT was 14% (76% of these were asymptomatic) [165]. Hypersensitivity manifested by profound hypotension and pulmonary edema and drug-induced thrombosis are also rare [166].

Two new taxane formulations are in early clinical trials: larotaxel (XRP9981) and ortataxel with no reported CT [167,168].

5.6.4 Epilithones

The epothilones are nontaxane microtubule-stabilizing agents. Like taxanes, epothilones have anti-microtubule effects. Five epothilone analogs are in phase I and II clinical trials and have shown clinical activity in patients whose tumors are refractory to prior chemotherapy [169,170].

The epothilone ixabepilone (Ixempra) was approved by the Food and Drug Administration. In pre-approval studies there was a <1% incidence of mild cardiac abnormalities ranging from symptomatic palpitation to atrial flutter. Myocardial infarction has also been reported [171]. In a review of 13 trials in a variety of solid tumors that include more than 1600 patients, no grade 3–4 CT was noted [172].

5.7 IMMUNOMODULATING AGENTS

Immunomodulating compounds are novel small molecule, orally available compounds that impact the immune system and other biologically important targets through multiple mechanisms of action, including angiogenesis inhibition, modulation of the levels of key pro-inflammatory and regulatory cytokines and immune cell co-stimulation.

5.7.1 Thalidomide

The immunomodulatory drug thalidomide inhibits angiogenesis and induces apoptosis of established neovasculature in experimental models. Thalidomide is an oral cancer drug that has been used to treat multiple myeloma and some NHL. The known toxicities of thalidomide include peripheral neuropathy, constipation, fatigue, and sedation. Therapy for myeloma has revealed a new and previously unrecognized toxicity of thalidomide: deep venous thrombosis with a 2% incidence when used as a single agent [173].

When thalidomide is administered with other cytotoxic agents, the incidence of DVT increases substantially. With a single course of VAD, VTE risk increased seven-fold to 14%. In a group of 100 patients who received induction chemotherapy including four cycles of continuous infusion of combinations of dexamethasone, vincristine, doxorubicin, cyclophosphamide, etoposide, and cisplatin, VTE developed in 14 of 50 patients (28%) randomly assigned to receive thalidomide but in only 2 of 50 patients (4%) not given the agent ($P = 0.002$). All episodes of DVT occurred during the first three cycles of induction. Administration of thalidomide was resumed safely in 75% of patients after receiving anticoagulation therapy [174]. A similar increased incidence of VTE has been seen when thalidomide is combined with doxorubicin, fluorouracil or gemcitabine [165–178].

Other manifestations of CT have been reported. The incidence of sinus bradycardia is $<5\%$ [179,180], but this number may be exaggerated because of thalidomide-induced hypothyroidism. Up to 20% of patients develop grade 1–2 edema [181].

Dyspnea is a rare but disabling complaint with thalidomide use that occurs in 11–44% of patients. This often occurs without a clear objective etiology and with symptoms that are out of proportion to the clinical findings: there is no overt HF, pneumonia or interstitial lung disease.

Myelodysplasia may be a predisposing risk factor [182,183]. Reversible pulmonary hypertension has also been implicated [184]. In most of these case reports, withdrawal of thalidomide has reduced or eliminated the dyspnea.

5.7.2 Lenalidomide

Lenalidomide (Revlimid) is structurally similar to thalidomide and was developed to enhance its efficacy while mitigating its neurotoxicity. It is used for the treatment of multiple myeloma and CLL. A unique toxicity is a potentially life threatening tumor flare reaction and the major CT, similar to thalidomide, is the development of VTE. The overall incidence of VTE is increased in patients with multiple myeloma and with concurrent steroid use. Overall, the incidence of grade 3–4 VTE in myeloma patients is 13% when lenalidomide and dexamethasone are used, compared to 4% with dexamethasone alone.

The relationship of VTE to steroid use is dose-related: an incidence of 6.3% was noted with low dose versus 18% with high dose dexamethasone [186]. In a study with patients with relapsed and refractory multiple myeloma, there was a 2% incidence of VTE and events only occurred with the addition of dexamethasone to the treatment regimen [187].

Review of data for the treatment of CLL shows no incidence of VTE [185].

5.7.3 Pomalidomide

Pomalidomide (Actimid) is structurally similar to thalidomide with similar immunomodulating effects and is under investigation. Early results show a similar incidence of VTE [188].

5.8 TRANSMEMBRANE RECEPTOR INHIBITORS: SMALL MOLECULE EGFR/TYROSINE KINASE INHIBITORS (TKI)

Transmembrane receptors are involved in a complex set of essential biologic processes. Dysregulation is associated with altered tumor

development, growth, metastasis and survival. Inhibition of tumor specific receptors produces anti-tumor effects.

5.8.1 Imatinib

Imatinib mesylate (Gleevec) is a TKI that targets BCR-ABL, platelet-derived growth factor receptor, and stem cell receptor c-Kit . Imatinib is used for the treatment of chronic myeloid leukemia, Philadelphia chromosome-positive acute lymphoblastic leukemia , gastrointestinal stromal tumors (GIST), and other diseases.

Treatment with imatinib is generally well tolerated and data from the early large clinical trials failed to show significant CT [189].

Fluid retention and edema occur in up to 66% of patients (4–5% grade 3–4) and dyspnea in up to 16% of patients (4–5% grade 3–4) [190].

CT in both experimental and clinical settings was studied by Kerkila *et al*. They reported data from a mouse model and 10 patients with HF, suggesting imatinib induced myocardial injury. The mechanism of cardiomyocyte toxicity was suggested to be a result of c-Abl inhibition inducing mitochondrial changes. Two of the 10 patients had a myocardial biopsy that showed non-specific prominent membrane whorls in the myocytes, pleomorphic mitochondria, glycogen accumulation, and cytosolic lipid droplets and vacuoles. This report did not assess incidence, just possible causality [191].

With an increased awareness of the possibility of CT with imatinib therapy, multiple letters and papers were generated. Breccia *et al*. reported their real-world experience of 285 CML patients who received imatinib. Retrospectively, they were only able to identify three patients (1%) who had CT and referenced 4 other letters where the incidence of CT was 0.2–0.5% [192].

Park *et al*. reported 2 cases of overt HF in a group of 16 patients treated with imatinib. Both were >60 years of age and both had underlying hypertension and diabetes [193]. In another study, data were collected and analyzed from 1276 patients to determine the incidence of HF from imatinib therapy. After a median follow-up of 47 months, they identified 22 patients (1.7%) who might have had HF. Diabetes, hypertension, CAD, arrhythmia and history of HF were present in 19/22 patients and 59% had previously received cardiotoxic drugs [194,195]. Recently, Ribeiro *et al*. evaluated the CT in 103 consecutive patients with CML and compared them to a matched group of 57 patients not treated with imatinib. There was no statistical difference when comparing symptoms

and signs, biomarkers of HF (BNP levels) and echocardiographic measurements. The treated group had only an increased incidence of edema compared to the controls [196].

In summary, in patients with risk factors and/or pre-existing CV disease, the incidence of CT manifested by HF with imatinib is in the range of 1–2%. Noncardiac edema is common and asymptomatic increases in BNP levels may also be detected with unknown clinical significance. In most reported studies, rechallenge with a lower dose of imitamib has been tolerated after resolution of the acute HF.

Imatanib use is associated with edema and fluid retention.

5.8.2 Sunitinib

Sunitinib malate (Sutent) is a multitarget receptor TKI with activity against VEGF receptor, 1–3, platelet-derived growth factor receptor α and β, c-KIT, FLT3 kinase, colony-stimulating factor 1 receptor and RET kinase [197]. Sunitinib is approved for the treatment of advanced RCC and imatinib-resistant GISTs or patients with GISTs who do not tolerate imatinib.

The most common cardiac side-effect is hypertension (defined as $\geq$ 150/100 mm Hg) with an incidence according to the package insert of 30% [198] and reported as high as 47% by Chu *et al*. [199].

The first indication of LV dysfunction and HF was seen when sunitinib was compared to interferon in patients with metastatic RCC. Sunitinib was associated with decreases in LVEF that were uniformly twice as frequent compared with interferon [199].

The incidence of MI, HF, or cardiovascular death has been observed to be as high as 11% in sunitinib-treated patients with imatinib-resistant GIST [200].

In a recent study by Telli *et al*., in a more 'real-world' population of patients, 15% developed symptomatic decreases in LVEF [201] while a retrospective review from MD Anderson showed a 2.7% (6/224) incidence of Grade 3–4 CT in a population with underlying hypertension and a mean age of 65 years [202].

The cause of CT is not fully understood. An animal model suggested a potential correlation between mitochondrial dysfunction, cardiomyocyte apoptosis and underlying hypertension [203].

5.8.3 Sorafenib

Sorafenib (Nexavar) is another oral multikinase inhibitor (Raf-1, A-Raf, and B-Raf) as well as VEGFR2/3, FLT3, c-Kit, and PDGFRs. Sorafenib is approved for the treatment of metastatic RCC as a second-line agent and for hepatocellular carcinomas.

Similar to other VEGFR inhibitors, hypertension is the most frequent treatment-related serious adverse effect. Hypertension occurred in 17% of sorafenib-treated patients compared to placebo in a study of 903 patients with advanced clear cell RCC. There was no ischemia or HF reported [204].

In a meta-analysis of nine studies that included 4599 patients with various solid tumors the overall incidence of hypertension was 23.4% (95% CI: 16.0–32.9%) and grade 3–4 incidence of 5.7% (95% CI:2.5–12.6%) [205].

Cardiac ischemia has been reported. One paper described, without detail, an increased CT manifested by chest pain when sorafenib therapy immediately followed sunitinib therapy [206].

An observational study of sorafenib and sunitinib reported the incidence of cardiac events to be higher than reported in clinical trials. The use of TKIs led to mild asymptomatic to severe symptomatic cardiac events in 33.8% of patients and the incidence may be higher in sunitinib compared to sorafenib-treated patients (5 versus 14%) [207].

In summary, both sunitinib and sorafenib are associated with CT manifested by hypertension and varying degrees of LV dysfunction. The reported incidence of the latter is blurred by varying definitions of CT, the lack of distinction in most studies between asymptomatic decreases in LVEF and overt HF and the high incidence at baseline of cardiac risk factors and underlying cardiovascular disease in treated patients. Similar to the CT defined by trastuzumab, it appears that the CT associated with sunitinib and sorafenib is not associated with permanent myocardial damage and is largely reversible when the offending drugs are discontinued. Rechallenge with lower doses of medication has been successful in treatment continuation.

Sunitinib and sorafinib are associated with hypertension and a reversible cardiomyopathy.

5.8.4 New Drugs

There are at least 16 new drugs in this class that are in phase I/II clinical trials. Data from one, AMG 706, shows a similar incidence of hypertension compared to available, approved oral preparations. In a study of 71 patients there was a 42% incidence of any hypertension and 20% incidence of grade 3 hypertension [208].

In summary, chemotherapeutic drugs that target VEGF and TK are associated with an increased risk of hypertension, arterial embolic events that are more common in patients over the age of 65 years and/or who have pretreatment cardiac risk factors or prior arterial disease. The relationship to age is highlighted by the 2.2% incidence of thrombosis/embolism described in a recent study of young women with carcinoma of the cervix (median age 46 years with a range of 29–62 years) treated with bevacizumab [209].

5.9 EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) TYROSINE KINASE INHIBITORS

The EGFR has a major role in tumorigenesis, inhibitors of the receptor have been approved for the treatment of tumors that overexpress EGFR.

5.9.1 Erlotinib

Erlotinib (Tarceva) is a specific EGFR tyrosine kinase inhibitor. It is indicated for advanced non-small cell lung cancer and advanced pancreatic cancer.

The most commonly reported adverse events are skin rash (75% with 8% grade 3–4) and diarrhea (54% with 6% grade 3–4). Less than 1% of patients develop dyspnea, cough and fever associated with interstitial lung disease that becomes treatment limiting. No CT has been reported [210–212].

5.9.2 Gefitinib

Gefitinib (Iressa) like erlotinib inhibits the EGFR tyrosine kinase and shares a common chemical structure, a similar spectrum of clinical efficacy and a similar incidence of non-cardiac adverse effects. No CT has been reported [213–217].

5.10 RETINOIDS

Retinoids are a class of compounds structurally related to vitamin A that have anti-cancer effects.

5.10.1 Bexarotene

Bexarotene (Targretin) is a synthetic retinoid that selectively binds to retinoid X receptors. It is approved for the treatment of recurring or refractory cutaneous T-cell lymphoma (CTCL) and currently is being studied in other malignancies.

Treatment may be limited by reversible drug-related adverse effects. In phase II and III clinical trials, 98% of the patients reported at least one adverse event. Non-cardiac side-effects include headache, hypothyroidism (up to 30%), asthenia, leucopenia, and pruritus [218,219]. Major CT is manifested by a mixed hyperlipidemia, primarily hypertriglyceridemia in over 80% and hypercholesterolemia in over 30% of patients. Hyperlipedemia is dose related and begins 1 to 2 weeks after initiating therapy. Triglyceride levels left untreated can lead to acute pancreatitis. Prophylactic and therapeutic use of lipid lowering agents can temper the degree of lipid elevation [220].

> Bexarotene causes marked hypertriglyceridemia in over 80% of patients.

5.11 PROTEOSOME INHIBITORS

Intracellular protein degradation occurs predominantly through the proteasome, which is the final common effector for proteolysis.

5.11.1 Bortezomib

Bortezomib (Velcade) inhibits proteasome 26S activity impairing the degradation of inhibitory κB (IκB) and further inhibiting nuclear factor

kappa B (NF-κB). It is indicated for the treatment of refractory multiple myeloma.

The efficacy and safety of bortezomib at multiple dosages have been studied in two phase II studies (SUMMIT/CREST [221]) and in one phase III study (APEX [222]). In these studies, patients with relapsed or refractory multiple myeloma received single-agent bortezomib. The major toxicities were hematologic and neurologic. In the APEX study, there was a 2% incidence of HF in a group of more than 600 patients with a mean age of 62 years, 77% who had prior anthracycline exposure. Later studies showed no CT [223,224].

Controversy regarding incidence and causality of bortezomib-induced CT developed based on a small number of patients/case reports repeatedly cited in the literature:

- Hachihanefioglu reported on a single case with HF during bortezomib therapy following VAD for multiple myeloma. Although the development of HF was documented, the causality was not clear [225].
- Voortman reported the development of reversible HF during treatment that may have been due to bortezomib in one of the first 16 treated patients. Subsequently, all patients had LVEF determinations prior to and at the conclusion of therapy. Of the 15 patients who had both studies there was no treatment related change in LVEF [226].
- Suvannasankha reported HF in one of 29 patients that his group attributed to bortezomib [227].
- Orciuolo *et al*. in a letter described eight patients out of a group of 69 (11.6%) with CT. Of these, 3 had HF, 1 had angina, 2 had atrioventricular block and 2 had atrial fibrillation. All were $\geq$60 years of age. All CT occurred after at least 4 cycles of bortezomib with cumulative doses of more than 20 mg m^{-2} and two of three patients with HF had prior anthracycline exposure [228].
- A single case described by Berenson with pretreatment QT prolongation who also received arsenic trioxide, has been referenced as bortezomib-induced CT [229].

In summary, the preponderance of literature supports the lack of CT caused by bortezomib. The reviewed case reports do not support causality between bortezomib treatment and CT, and in spite of the theoretical negative effect of proteasome inhibition on ventricular remodeling and apoptosis, vascular plague disruption and

anti-angiogenesis, there is little evidence to support a relationship with bortezomib and CT.

5.11.2 New Drugs

Second-generation agents have entered phase I trials: NPI-0052 (salinosporamide A) and carfilzomib (formerly PR-171). Unlike bortezomib, that binds the proteasome in a slowly reversible manner, these bind irreversibly. No CT has been reported [230].

5.12 PLATINUM-BASED CHEMOTHERAPY

The platinum drugs target DNA and are essential components of many established chemotherapy regimens, including lung, ovarian, colorectal and head and neck cancer. Their effective use, however, is limited by side-effects, the need for IV hydration and acquired or intrinsic resistance. There are three platinum-based agents: cisplatin, introduced in the 1970s, followed by second-generation analogs carboplatin and oxaliplatin. They share some structural similarity with minor differences that alter their pharmacokinetics, tissue distribution, efficacy and side-effects profiles. They all are administered intravenously.

5.12.1 Cisplatin

Cisplatin-based chemotherapy has been associated with acute treatment-related and long-term CT.

Acute CT: There are sporadic case reports describing heightened vascular reactivity and/or arterial thrombosis with the acute administration of cisplatin. Manifestations range from small vessel vasospasm (Raynaud's) [231] to large vessel spasm and/or occlusion with resultant angina, MI, TIA or CVA. Most of the reports predate 2000, and none include a denominator to define a true incidence and a direct link to cisplatin use is often lacking. The incidence of stroke has been reported as 1 in 2000 [232,233].

Mechanisms postulated include direct endothelial damage leading to vasospasm, increased thrombogenicity with increased platelet aggregation, an increase in von Willebrand factor (VWF) and hypomagnesemia. There may be a higher incidence in patients with pre-existing high

levels of VWF [234]. The incidence of arterial thrombosis may be 1–3% and VTE as high as 10–15% when cisplatin is combined with a vinca alkaloid and bleomycin [235].

Nuver *et al*. reviewed their experience in 65 patients with testicular cancer who received combination chemotherapy that included cisplatin, etoposide and bleomycin and reported a 3% (2/65) with acute MI and 7.7% (5/65) with VTE [236].

In a review of 932 patients with NSCLC who received cisplatin with another single agent (vinca alkaloid or etoposide), no CT was reported [237] and a meta-analysis of 5 trials with cisplatin/vinorelbine that were conducted after 1995 and included 4584 patients showed no CT [238].

There are also reports of electrophysiologic toxicity (sinus bradycardia, atrial and ventricular arrhythmias, heart block) implying that atrial arrhythmias with hemodynamic consequences are commonly associated with cisplatin treatment [240–243]. Although these studies are all referenced as cisplatin-induced atrial arrhythmias, it is a stretch to deduce that cisplatin is the culprit. Confounding issues include concomitant anthracycline administration, advanced stage lung cancer with associated hypoxia and possible pericardial involvement and advanced age. In a recent study, heart rhythm monitoring was performed before and after cisplatin infusion in 37 patients. Holter analysis demonstrated asymptomatic and unsustained supraventricular or ventricular arrhythmia in 66.7% (20 patients) [244].

The discrepancy between early reports of frequent CT and later trials with a low incidence of CT is most likely related to changes in drug delivery: we currently are more vigilant in monitoring and management of magnesium and potassium levels and hydration.

Carboplatin and oxaliplatin have similar CT profiles to cisplatin. Eighty one patients with recurrent ovarian carcinoma, 6 months after treatment with platinum- based chemotherapy (PBCT) were randomized to receive carboplatin (arm A) or paclitaxel + carboplatin (arm B). No CT was reported in either arm [245]. A 1% incidence of chest pain is reported in the approval summary for oxaliplatin [246].

Short-term cardiotoxicity of platinum containing chemotherapy consists of small and large vessel arterial spasm.

Long-term: Cardiovascular morbidity is increasingly recognized as a long-term complication of cisplatin-containing chemotherapy. Survivors of platinum-based chemotherapy (PBCT) have an excess of cardiac risk factors

(hypertension, dyslipidemia, obesity, and insulin resistance [metabolic syndrome]) and an increased risk of development of premature atherosclerosis that appears to become evident 10 or more years after treatment completion.

Huddart *et al*. assessed the cardiovascular morbidity and risk factors in long-term survivors of testicular cancer. Data were available for 992 patients after a median follow-up of 10.2 years. Sixty eight events were reported. After adjustment for age, a relative risk of 2.59 (95%CI: 1.15–5.84; $P = 0.22$) was seen after PBCT and this increased risk was not due to an increase in cardiac risk factors [247].

Raghaven *et al*. found that 41% (7/17 patients) had higher than desirable levels of total serum cholesterol and low-density lipoprotein cholesterol. Two had normal levels before treatment, four had preexisting hypercholesterolemia that increased further, and one patient had an elevated pretreatment level that was unchanged. Absolute increases in serum cholesterol were noted in 14 of 17 patients [248].

In another study, Meinardi *et al*. studied 87 patients treated with cisplatin-containing chemotherapy before 1987 who were in remission for at least 10 years and whose ages were ≤50 years at the time of analysis. Patients were evaluated for the occurrence of cardiovascular events. Sixty two of 87 patients were additionally evaluated for cardiac damage and cardiovascular risk factors. Their cardiovascular risk profile was compared with that of 40 patients of comparable age and follow-up duration treated with orchidectomy only for stage I disease: 79% had hypercholesterolemia, 39% had hypertension and 25% experienced Raynaud's phenomenon. Major vascular events were found in 6.9% of patients (6/87) who were 30 to 42 years old at the time of study and 9 to 16 years post chemotherapy: two with MI, three with angina pectoris with proven myocardial ischemia and one CVA. An increased observed-to-expected ratio of 7.1 (95% CI: 1.9 to 18.3) for coronary artery disease, as compared with the general male Dutch population, was found [249].

In a recent study, long-term survivors of testicular cancer who received PBCT demonstrated objective evidence of endothelial injury and dysfunction (impairment of brachial artery flow-mediated dilatation and elevated levels of soluble intercellular adhesion molecule-1) as potential mechanisms for increased cardiovascular risk [250].

Late cardiotoxicity of platinum-containing chemotherapy includes an increase in cardiovascular risk factors and premature atherosclerosis.

5.12.2 New Platinum Drugs

5.12.2.1 Satraplatin

Satraplatin is an oral platinum preparation now in clinical trials. It has been studied primarily in prostate cancer but clinical efficacy has been demonstrated in NSCLC, head and neck cancer and ovarian cancer. To date no CT has been reported [251,252].

Picoplatin, an intravenous platinum compound is also under development for a variety of solid tumors with no reported CT [253–255].

5.13 FOLATE ANTAGONISTS

Folate antagonists inhibit dihydrofolate reductase that disrupts DNA replication and cell division.

5.13.1 Methotrexate

Methotrexate is a folate antagonist used in the treatment of many hematologic and solid malignancies. High dose methotrexate is generally well tolerated from a cardiac standpoint. There are sporadic case reports of arrhythmias that include sinus bradycardia with pauses and ventricular tachycardia as well as atypical chest pain with incidence rates that are not calculable [256,257].

5.13.2 Pemtrexed

Pemetrexed (Alimta) is an antifolate drug used to treat malignant mesothelioma, NSCLC and pancreatic cancer. In multiple large trials, across a variety of solid tumors, there has been no reported CT [258–260].

5.14 CYTOKINES

Cytokines are signaling polypeptides that are critical for the immune response that have anticancer effects when used alone or in combination chemotherapy.

5.14.1 Interferon

The interferons are a family of glycoproteins that include Interferon alpha (from leukocytes), beta (from fibroblasts) and gamma (from T lymphocytes). Interferon was the first cytokine to show activity in patients with metastatic RCC. All three Interferons cause a flu-like syndrome whose hemodynamic burden may increase the myocardial oxygen demand beyond the limits of coronary blood flow and/or ventricular function in patients with underlying cardiac disease. Sonnenblick and Rosin reviewed the literature and found 15 reports on 44 patients with interferon-induced CT. In their review, CT was not related to type of interferon nor the daily or cumulative dose. CT was manifested by arrhythmia, dilated cardiomyopathy and ischemia including MI. In 44 patients, there were arrhythmias in 25 (supraventricular tachycardia and ventricular ectopy), ischemia/infarction in 9, atrioventricular block in 2, cardiomyopathy in 6 and sudden death in 2 [261]. Clinical trial data implicate up to a 20% incidence of arrhythmia [262]. Similar to other stress cardiomyopathies, left ventricular dysfunction is transient and is generally reversible with cessation of medication [263] and /or treatment. Because of the relationship to the febrile illness, symptoms usually occur in the first 2–8 h of treatment.

With the modern awareness of the potential CT and the major risk factor being underlying cardiac disease, screening, especially for coronary artery disease is the standard prior to interferon use. Patients with unrevascularized coronary artery disease virtually never receive this drug and therefore the currently reported incidence of CT is extremely low and occurs in patients with pretreatment unrecognized cardiac disease.

5.14.2 Interleukins

Since the 1980s, interluken-2 has been utilized as a cancer therapy, with improved survival in RCC and metastatic melanoma. It is a glycoprotein produced by activated lymphocytes that induces T-cell proliferation. Toxicity is secondary to a capillary leak syndrome with resultant tachycardia, decreased peripheral vascular resistance (PVR), hypotension and increased cardiac output – a picture similar to septic shock that is probably related to the release of tumor necrosis factor. Early studies showed grade 3–4 CT that included a 3% incidence of ischemia and an 81% incidence of hypotension. The decrease in PVR may last for days

following infusion. Postulates regarding mechanism include direct CT and demand ischemia [264, 265]. Arrhythmias and conduction delay have also been reported in 14–21% of patients [266].

In studies when high-risk cardiac patients are excluded, either no CT or grade 1–2 toxicity occurs in <3% of patients with interluken [267,268]. Infusion-related hypotension is common (up to 53%) and may be associated with a pot pourri of hemodynamically inconsequential cardiac adverse events including a 6% incidence of arrhythmia (atrial fibrillation or supraventricular tachycardia 5.2%, unsustained VT 1%), and a 1.6% incidence of CPK/MB elevation with no other evidence of MI. CPK elevations are asymptomatic and associated with minor ST-T wave changes, mild diffuse hypokinesia when ECHOS are done and no obstructive coronary artery disease when coronary angiography is performed. All patients responded to a change in treatment regimen [269].

> Cytokines (interferons and interlukens) cause a febrile reaction that increases cardiac demand that may lead to ischemia and/or ventricular dysfunction in susceptible patients.

5.14.3 New Therapies

A rich menu of immunomodulatory therapies targeting T lymphocytes are in various stages of small clinical trials. Effectors include monoclonal antibodies (ipilimumab, tremelimumab and ticlimumab), T cell transfer and vaccine therapy. To date, autoimmune side-effects but no CT has been reported [270–274].

5.15 RADIOIMMUNOTHERAPY

The combination of radiotherapy and chemotherapy to increase therapeutic efficacy was first shown to possess high levels of clinical activity in patients with relapsed or refractory B-cell lymphomas [275]. Several radiolabeled antibodies that target different B-cell associated CD20 antigens are currently available and play an important therapeutic role in the management of B-cell lymphomas.

5.15.1 Tositumomab

Tositumomab (Bexxar) is an IgG2a murine anti-CD20 monoclonal antibody that, when combined with a radionuclide vehicle such as iodine 131, irradiates lymphoma cells. The dosing methodology and maximum tolerated total-body dose is established and no CT has been reported [276].

5.15.2 Ibritumomab Tiuxetan

The radioimmunoconjugate ^{90}Y ibritumomab tiuxetan (Zevalin) is currently approved for treatment of patients with relapsed or refractory, low-grade or follicular B-cell NHL. In a study of 414 patients, a 2.9% incidence of grades 1–3 hypertension was the only CT reported [277].

5.15.3 Gemtuzumab Ozogamicin

Gemtuzumab Ozogamacin (Mylotarg) is a humanized anti-CD33 monoclonal antibody linked to a derivative of calicheamicin, a potent cytotoxic antibiotic that is released inside the myeloblast by hydrolysis causing DNA breaks and subsequent cell death. It is approved for relapsed CD33 positive AML. Myelosuppression and thrombocytopenia are the most common hematological toxicities Aside from infusion related reactions due to cytokine release (fever, chills) non-specific arrhythmias in 10% of patients and changes in blood pressure (4–8% incidence of hypotension and 9% incidence of hypertension), no major CT has been reported [278–281].

5.16 MISCELLANEOUS DRUGS

5.16.1 Arsenic Trioxide

Arsenic trioxide has efficacy in the treatment of acute promyelocytic leukemia, pancreatic carcinoma and metastatic melanoma. Its use is associated with grade 1–2 fluid accumulation/edema: pleural effusion in

up to 23% and peripheral edema in up to 83% of patients and sporadic reports of pericardial effusion [282,283].

QTc prolongation may occur in up to 63% of treated patients with <1% incidence of torsade de pointes and case reports of sudden death. QT prolongation is due to a dose-dependent inhibition of the potassium ion channel by arsenic. There is less QT prolongation with oral versus intravenous dosing [284–286]. Other less frequent electrophysiologic adverse events include high-grade atrioventricular block that occasionally requires pacemaker implantation [287].

5.16.2 Etoposide

Etoposide is a semisynthetic topoisomerase II inhibitor that is a derivative of podophyllotoxin. The major side-effects are bone marrow suppression and peripheral neuropathy. Sporadic case reports suggesting that etoposide combined with either cisplatin, methotrexate or bleomycin can cause chest pain and/or acute MI have appeared in the literature. The latter may be due to coronary vasospasm, direct myocardial injury, hypersensitivity or an immune response and because of the nature of combination therapy, it is impossible to tease out which drug is the real culprit [288].

5.16.3 Bendamustine

Bendamustine (Treanda) is an alkylating agent, with a unique chemical structure that has recently been 'rediscovered' in the treatment of refractory NHL. To date, no CT has been reported [289,290].

5.17 BONE MARROW TRANSPLANTATION (BMT)

Allogeneic (Allo) and autologous (Auto) BMT have increased survival in hematologic malignancies. Cardiac complications may occur during pretransplant conditioning (including high-dose chemotherapy), early transplant (the first 100 days) and late transplant (beyond 100 days).

Conditioning and the First 100 Days:
Cardiac complications due to conditioning regimens are well recognized, and these include peri-myocarditis disease (pericarditis/HF) and

arrhythmias. The aggregate reported incidence has been as high as 26%. In a series reported by Sakata-Yanagimoto *et al*. from Japan, the incidence of severe cardiac complications was 4.9%. They found that the cumulative dose of anthracyclines correlated independently with the development of grade 3–4 cardiac complications [291].

In a retrospective study from the University of Minnesota the records of 2821 patients who received a BMT were evaluated. Excluding patients with septic shock, pneumonia and/or multi-organ failure, 26 patients (0.9%) were identified: 2 had a major or fatal cardiac event during the first 100 post-transplant days presenting as rapidly progressive HF [292]. Hertenstein *et al*. found a 4.7% incidence of CT in a series of 170 patients undergoing Allo (150 patients) or Auto (20 patients) BMT with a 1.8% incidence of life-threatening CT [293].

A higher frequency of CT following BMT was reported by Bearman *et al*. with a relationship between CT and pre-transplant cardiac history. They identified 14 patients who had an LVEF $<50\%$ prior to transplant. Of these, 10 began the preparative regimen and two (20%) experienced grade 3–4 CT versus 5 of 116 patients (4%) with LVEF $\geq$ 50% ($P = 0.096$). The incidence of grade 3–4 CT among patients with any history of cardiac dysfunction or with an abnormal cardiac examination prior to transplant was 9% (2 of 22) versus 5% among the remaining patients (5 of 104) ($P = 0.605$) [294].

Chung *et al*. investigated the incidence of subacute CT (grade 1–2) unrelated to sepsis in 32 patients with normal baseline LV function following Auto BMT. Serial echocardiograms, BNP and troponin measurements were obtained pre- and post-conditioning and serially after transplant. A $>10\%$ decline in LVEF with global systolic dysfunction developed in 31% of patients within a mean of 17 days (14–28 days) of transplantation. This was generally reversible by a mean of 16 days with a tail of up to 6 weeks. BNP and troponin levels were not different between those with or without CT [295].

In a study of Auto BMT for breast cancer, a 5.1% incidence of CT (manifested as HF) was found in 443 women following STAMP-1 (cyclophosphamide, cisplatin, BCNU) preconditioning [296].

The following conclusions are applicable to clinical practice:

- The incidence of asymptomatic CT manifested by decreases in LVEF is more common than appreciated clinically and may occur in up to 1/3 of patients.
- The incidence of symptomatic HF is in the 5% range.
- The risk is increased in patients with prior anthracycline treatment.

- The risk is higher in patients with pre-transplant reductions in LVEF.
- There is no difference in early CT between Auto and Allo BMT.

> In the first 100 days following bone marrow transplantation asymptomatic decreases in left ventricular function are common and symptomatic heart failure occurs in approximately 5% of patients.

Late: Beyond Day 100:
Late CT following BMT may be classified into four categories: HF and LV dysfunction, emergence of atherosclerotic risk factors (RF), arterial events and graft versus host disease (GVHD).

5.17.1 Heart Failure and LV Dysfunction

Armenian *et al*. studied 60 patients with HF that developed >1 year post BMT. The median time to symptom recognition was 3.0 years with a 68:32 split between Auto: Allo BMT. The mean LVEF was 39% with a range of 15–53%. The development of HF was associated with more cycles of preconditioning high-dose chemotherapy (8.6 vs. 4.9, $P = 0.01$), a larger BMI at conditioning (28.4 vs. 26.2, $P = 0.01$), a greater lifetime anthracycline exposure (285.3 vs. 175.6 mg m^{-2}) and two or more pre-transplant comorbidities (renal insufficiency, hypertension, diabetes, chronic lung disease) [297]. HF incidence was more strongly related to prior anthracycline dose and comorbidity and probably not related to transplant type (Allo vs. Auto).

5.17.2 Atherosclerotic Risk Factors and Arterial Events

The prevalence of *de novo* hypertension, diabetes and dyslipidemia increases over time following BMT, irrespective of GVHD status. Long-term BMT survivors are nearly four times more likely to report diabetes, and two times more likely to report hypertension compared to sibling controls. Compared to Auto recipients, Allo recipients with hypertension were found to have a 2.5-fold increased risk of a cardiovascular event, while those with diabetes had a 2.3-fold increased risk [298,299].

Majhail *et al.* conducted a cross-sectional study to estimate the prevalence of metabolic syndrome among 86 adults who had Allo BMT as compared with 258 age- and gender-matched United States population controls selected from the 2005–2006 National Health and Nutrition Examination Survey database. The median age at study enrollment was 50 years (range, 21–71 years), and patients were at a median of 3 years (range, 1–21 years) from transplantation. The prevalence of metabolic syndrome was 49% (95% CI: 38–60%) that was a 2.2-fold (95% CI: 1.3–3.6, $P = 0.002$) increase compared with controls. The prevalence rates of hypertension and hypertriglyceridemia were significantly higher among transplanted patients than among controls without increased rates of abdominal obesity, elevated blood glucose and low high-density lipoprotein cholesterol levels [300].

Postulated mechanisms for this increased incidence of RF include an increase in circulating catecholamines, the damage of tumor lysis and cytokines (TNF, interleukin) and myocardial depressant substance.

Late vascular events involve the coronary, cerebrovascular and peripheral arterial circulations. A 6% cumulative incidence of arterial events at 15 years has been reported associated with an older age at the time of transplantation and the presence of pre-established cardiovascular RF [301].

In a retrospective mutlicenter European Group of Blood and Marrow Transplantation (EBMT) analysis, that included 548 long-term survivors (>1 year) treated in 10 EBMT transplant centers, 3.6% (20 out of 548 patients) had a cardiovascular event in at least one arterial territory. The median age at occurrence was 54 years (range, 41–70 years). The cumulative incidence of a first arterial event 15 years after hematopoietic stem cell transplantation was 6% (95% CI: 3%–10%). The cumulative incidence for patients with a high global cardiovascular risk score, defined as having ≥50% of the risk factors (hypertension, diabetes, dyslipidemia, increased body-mass index, physical inactivity, smoking) was 17%, as compared to 4% in those with a low risk score. In multivariate analysis age >30 years at last follow-up, and a high global cardiovascular risk score were associated with 6.4-fold and 9.8-fold increases in the risk of an arterial event, respectively [302].

5.17.3 Graft versus Host Disease

GVHD affecting the heart has been sporadically described with manifestations that include heart block, peri-myocarditis, CAD and bradycardia. It is an infrequent but recognized post-BMT complication [303].

Late cardiotoxicity after bone marrow transplantation (>100 days) consists of heart failure and left ventricular dysfunction, an increase in cardiovascular risk factors and arterial events and graft versus host disease.

5.18 TAMOXIFEN AND AROMATASE INHIBITORS

Tamoxifen (Nolvadex) is a selective estrogen receptor modulator that has proven efficacy in the treatment of hormone positive breast cancer and prophylactically to reduce the risk of estrogen receptor-positive tumors in women at increased risk for breast cancer. Tamoxifen treatment has also been associated with a protective effect against atherosclerotic cardiovascular events because of its favorable effect on cholesterol: reducing LDL and raising HDL levels [304,305].

There is no evidence that the administration of tamoxifen increases the incidence of acute arterial events compared to controls. However, there is a statistically nonsignificant 0.05% increased risk of stroke but a definite increase in VTE and pulmonary embolism incidence [306,307].

The aromatase inhibitors (AI) consisting of anastrozole (Arimidex), letrozole (Femara), and exemestane (Aromasin) target estrogen sensitive tumors in postmenopausal women by inhibiting the production of estrogen via the aromatase enzyme. These agents are most commonly used as adjuvant treatment for hormone-positive breast cancer.

All AI versus tamoxifen trials showed no difference in the incidence of ischemic heart disease. However, the risk of VTE is reduced when comparing the AIs to tamoxifen [308–314].

Lipid metabolism changes occur in postmenopausal women due to reduction in estrogen levels. Tamoxiifen has a favorable profile lowering total cholesterol up to 20% and raising HDL. The AIs have a neutral effect on lipids. As a result of early trials, there was some thought that the AIs had a negative effect on lipids because of the changes in lipid levels when women were treated sequentially with tamoxifen followed by an AI. Letrozole was found to increase total cholesterol and LDL over 16 weeks in a small study of 20 patients with advanced breast cancer. Patients switched to anastrozole from tamoxifen in the ITA trial showed a higher incidence of lipid abnormalities (9.3%) than in the tamoxifen

alone arm (4.0%, $P = 0.04$). The difference was thought to be due to the discontinuation of tamoxifen (favorable effect) and the addition of anastrozole (neutral effect) [315–317].

> Tamoxifen lowers LDL cholesterol and raises HDL cholesterol while the aromitase inhibitors have a neutral effect on lipids.

5.19 5-HYDROXYTRYPTAMINE$_3$ RECEPTOR ANTAGONISTS

There are three 5-hydroxytryptamine$_3$ receptor antagonists available in the United States for the prevention and treatment of chemotherapy-induced and postoperative nausea and vomiting: dolasetron (Anzemet), granisetron (Kytril), and ondansetron (Zofran).

These agents are generally well tolerated with mild headache and diarrhea associated with their use. Studies in healthy subjects showed ECG changes (prolongation of PR, QRS and QT intervals) with the use of all three drugs. In less than 10% an asymptomatic and transient intraventricular conduction delay may be seen on the ECG as well as minor (<15 ms) prolongation of the QT interval that returns to baseline within 6–8 h after infusion. These changes are more prominent with dolasetron. Proarrhythmia is rare and clinical consequences of these cardiovascular changes have not been reported in practice [318–321]. Caution should be used in patients with pre-existing conduction delay and underlying heart disease and chemotherapy that can cause QT prolongation since most of the literature experience is based on events in healthy subjects.

5.20 SUMMARY

CT is a frequent complication of the treatment of both solid and hematologic malignancies. There is a large variation in quantitating the incidence due to a lack of standardization in definition, monitoring, reporting and grading.This is compounded by patient and tumor characteristics and the fact that most studies have excluded patients with pre-existing or known cardiac disease and/or risk factors.

Not withstanding these issues, the potential for chemotherapy-related CT is real. Clinicians should be vigilant and approach chemotherapy both in the context of a high-risk patient and a high-risk chemotherapy regimen with the knowledge that there is as much individual variation in CT incidence as there is in regimen efficacy.

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6

Diagnostic Aspects of Cardiovascular Toxicity of Antitumor Drugs

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6.1 INTRODUCTION

Anti-cancer drugs comprise a diverse group of agents that affect the cardiovascular system in a variety of ways. From the clinical perspective, these agents may be associated with temporary functional impairment or permanent loss of contractile elements, abnormalities of rhythm formation and/or conduction, as well as vascular effects that include vasospasms, alterations of blood pressure, thromboembolic sequelea, and, when radiation therapy is employed, pericardial alterations and disruption in the integrity of valvular and vascular structures. From the pharmacologic standpoint, these agents may be divided into the various sub-groups of chemotherapeutic agents, biological or immunological agents, or physical agents that include the various forms of radiation. Cardiovascular effects become much more complex when combinations of anti-cancer modalities are used, as additive or synergistic interactions may play a role, and timing is sometimes an important factor; other medications not related to malignancy that a patient may be taking can

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also contribute to the complexity of the clinical picture. Finally, the biologic diversity of our patients and the state of their health, both at the time of treatment and in their subsequent course, impact on the cardiovascular effects. When we attempt to place the effects of anti-cancer agents in perspective and to diagnose cardiovascular effects of anti-cancer therapy we must take a large number of variables into consideration and appreciate that many of these variables change as the malignancy progresses or goes into clinical remission, and as the effects of anti-cancer drugs cause damage that may or may not be permanent. Finally, the predictive value of many of the tools used to identify cardiac effects is sub-optimal, lack both sensitivity and specificity, and they may be impacted by inter-observer interpretative variation. This chapter will look at some of these factors in greater detail as they pertain to diagnosing as well as appreciating the progression of cardiac complications associated with cancer treatment, and will attempt to provide an overview that is useful to clinicians, scientists, and clinical researchers. The most important cancer treatments that affect the cardiovascular system are

Table 6.1 Dominant anti-cancer modalities associated with cardiovascular toxicity

I) Agents associated with impaired left ventricular contractility
  a. Agents associated with Type I treatment-related cardiac dysfunction (agents associated with cell death)
    i. Anthracyclines
      1. Doxorubicin
        a. Liposomal forumations
      2. Daunorubicin
      3. Epirubicin
      4. Idarubicin
      5. THP Adriamycin (Pirarubicin)
    ii. Other anthraquinones
      1. Mitoxantrone
  b. Agents associated with Type II treatment-related cardiac dysfunction (agents associated with potentially reversible dysfunction of the myocyte contractile elements)
    i. Trastuzumab
    ii. Lapatinib
    iii. Sunitinib
    iv. Gleevec
    v. (other monoclonal antibodies / tyrosine kinase inhibitors)
  c. Other cardiodepressant agents
    i. Cyclophosphamide (especially high dose)
    ii. α - interferon

Table 6.1 (*continued*)

II) Agents associated with ischemia
  a. 5-Fluorouracil (5-FU)
  b. Capecitabine
  c. Vinblastine
  d. Vincristine
  e. Bleomycin
  f. Cisplatin
  g. (Biological response modifiers)

III) Agents associated with hypertension or hypotension
  a. Bevacizumab
  b. Sunitinib
  c. Sorafenib
  d. Homoharringtonine
  e. Interleukin-2

IV) Miscellaneous cardiotoxic agents
  a. Paclitaxel (bradycardia)
  b. Arsenic trioxide (QT prolongation and torsades de points
  c. Radiation (Pericardial damage, but may damage all cardiac structures)

V) Cardioprotective agents
  a. Dexrazoxane (Cardioprotector for anthracyclines)
  b. Angiotensine converting enzyme inhibitors (ACE) (Probably cardioprotective for Type I damage and possibly protective for Type II dysfunction)
  c. β-adrenergic blocking agents (weak evidence for cardioprotective effects in cancer therapy)

listed in Table 6.1. Only those agents that result in toxicity that is clearly evident will be reviewed.

6.2 AGENTS THAT RESULT IN CONTRACTILE DYSFUNCTION

A number of different anti-cancer agents affect myocardial contractility, and various mechanisms contribute to contractile dysfunction. Categorization of these agents into various groups remains suboptimal as more than one mechanism may be involved in a single agent. Additionally, threshold effects for permanent damage are a consideration. Nevertheless, one may think of these agents as falling into one of two primary categories, whereby the distinction is clinically useful as it places the drugs

in groups with similar manifestations of toxicity, and similar long-term considerations regarding prognosis, monitoring, and treatment. The first, now often referred to as Type I treatment-related cardiotoxic agents, has, as a major characteristic, the potential to cause cell death [1]. Once threshold-exceeding damage has taken place, the injury progresses relentlessly to cell death. The ability of individual cells to remain potentially viable despite exposure to such agents depends on many factors that have not yet been adequately studied, but anti-oxidative capacity and vascular substrate availability may be especially important.

As the heart is a post-mitotic organ with no significant potential to regenerate lost cells, this form of injury should be considered permanent and the implications of life-long importance. However, the clinical manifestation of such toxicity frequently becomes manifest only years after the initial exposure. This is because compensatory mechanisms, such as survival factors and adaptive myocardial hypertrophy, can compensate for the initial loss of myocardium. However, over time these compensatory mechanisms may become exhausted and additional myocardial stresses can lead to further cell loss. This makes early diagnosis of cardiotoxicity challenging.

The various anthracyclines are the most frequently encountered agents in this category, with doxorubicin, epirubicin, and dauorubicin the drugs most often used. Mitoxantrone, an anthraquinone, while not a member of the anthracyclines, causes cardiotoxicity that is indistinguishable from that caused by the anthracyclines. The clinical characteristics of these agents includes the facts that the cardiotoxicity is cumulative-dose related, that structural abnormalities are characteristic, that risk factors exist which predict augmented toxicity, and that, at least to some extent, cardiotoxicity can be mitigated without compromising oncologic efficacy.

6.2.1 Type 1 Treatment-Related Cardiotoxic Agents

The following discussion pertains to agents that demonstrate cumulative-dose related cardiotoxicity in the form of myocardial dysfunction associated with a high degree of propensity for cell death following exposure.

6.2.1.1 Mechanism of Cardiotoxicity

The mechanism of anthracycline cardiotoxicity is complex, and, at least in part, different from that of oncologic efficacy; were this not the case, it

would not be possible to impact upon cardiotoxicity independently of tumor control. Cardiotoxicity is clearly related to iron-based oxygen free radical induced oxidative stress. The resulting free radicals induce peroxidation of the myocyte membranes, resulting in increased permeability of the inner cell membranes [2,3]. Depletion of a survival factor for differentiated postnatal cardiomyocytes, transcription factor GATA-4, may also be involved in anthracycline-induced cardiotoxicity [4]. The cytostatic effect of anthracyclines is related to inhibition of nucleic acid (DNA and RNA) and protein synthesis that triggers DNA cleavage by topoisomerase II.

There are undoubtedly other factors involved. Mitochondrial dysfunction correlates with structural changes seen in irreversible cardiotoxicity and could be related to accumulation of 8-hydroxyguanosine adducts in cardiac mitochondrial DNA [5]. Additionally, the doxorubicin metabolite, doxorubicinol, is associated with both functional and structural alterations consistent with the anthracycline lesion. The possibility has been suggested that prevention of the reductase pathway leading to doxorubicinol could selectively mitigate cardiotoxicity [6]. It is difficult to detect early anthracycline cardiotoxicity. However, since myocyte cell death is an important pathophysiological factor, elevation of cardiac biomarkers has been shown to be predictive for anthracycline cardiotoxicity.

6.2.1.2 The Cumulative-Dose Relationship of Type I Agents

The relationship of cardiotoxicity to the cumulative dose of doxorubicin has been known for more than three decades. The curve was initially proposed by Von Hoff and later modified by investigators at the University of Texas M. D. Anderson Cancer Center as well as by Swain *et al.* who suggested that there was little concern regarding cardiotoxicity at low cumulative doses, but that the curve ascended more rapidly as cumulative doses increased. The original curve suggested an incidence of congestive heart failure approximating 5% at a cumulative dose of 550 mg m^{-2}, and then rose sharply. Later review of the clinical data suggested that the doxorubicin was more cardiotoxic than had been appreciated, and that the cumulative dose corresponding to an incidence of cardiotoxicity of about 5% was closer to 400–450 mg m^{-2} (Figure 6.1) [7,8]. These analyses went far beyond the mere prediction of cardiotoxicity in that they recognized that in order to maximize survival for a cancer patient one had to balance cardiotoxicity with oncologic

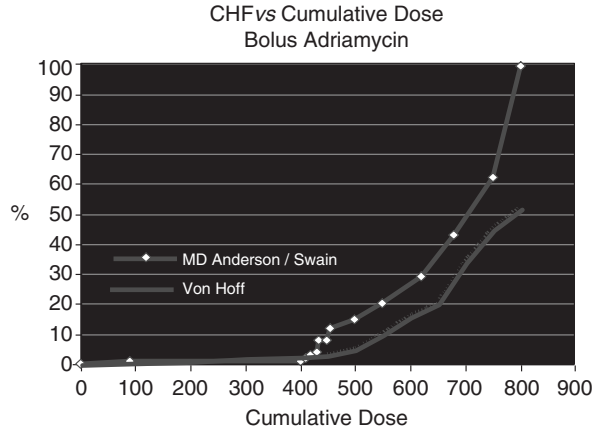


Figure 6.1 CHF versus cumulative dose for bolus adriamycin

efficacy; accepting a 5% incidence of congestive heart failure would result in a relatively low incidence of cardiac death along with aggressive, albeit perhaps not always maximal oncologic efficacy. The 5% rule for acceptable toxicity has served the oncology community very well during the intervening years.

Several other important insights can be derived from the concept of cumulative-dose related toxicity that distinguishes such agents from those without a clearly defined dose–toxicity relationship: the dose–response curve can be extrapolated to its origin and implies cell damage must start at dosages far below those at which we are able to appreciate clinical cardiotoxicity. Correlations with cardiac biopsy grades have confirmed that structural damage does, indeed, precede changes in systolic cardiac function [9]. Furthermore, a mathematical relationship is implied, and the likelihood of developing cardiotoxicity can be approximated [10].

6.2.1.3 The Characteristic Cardiac Structural Abnormalities Associated with Type I Agents

The typical electronmicroscopic changes associated with anthracyclines were first described by Billingham *et al.* [11,12]. The original grading scale incorporated grades of 0–3 based on progressively increasing structural abnormalities. Later the grading scale was expanded by Mackay to include the grades of 0.5 and 1.5 between the original grades proposed by

Table 6.2 Electronmicroscopic grading of cardiac biopsy specimens: the number of muscle fibers showing changes[a,b]

Grade	Vacuoles	Myofibrillar dropout	Necrosis
0.5	<4	0	0
1	4–10	<3	0
1.5	>10	3–5	<2
2	varies[c]	6–8	2–5
3[d]	varies[c]	>8	>5

From Mackay *et al.* [11,12] and Legha *et al.*
[a]Reflects average number of abnormal muscle fibers per grid based on an examination of a minimum of six grids obtained from six blocks.
[b]The highest biopsy grade based on any one category is assigned. If two or more categories indicate the same grade, the grade is increased by half a point.
[c]Any number of vacuoles.
[d]With present dosing and schedules, a biopsy grade of 3 is seldom achieved.

Billingham [13,14]. The earliest change associated with anthracycline administration is the appearance of vacuoles; at higher cumulative dosages myofibrillar disarray and necrosis appear. As the changes do not occur in all cells uniformly, grading is undertaken by looking at the average cells in a tissue block that exhibit the change. The grading scale is presented in Table 6.2; typical ultrastructural changes are depicted in Figure 6.2.

As implied above, cardiac biopsy changes are appreciated at much lower cumulative dosages than are decreases in systolic function, and the relationship is depicted in Figure 6.3. The ability to recognize cardiac damage at lower cumulative dosages than is possible with parameters that look at systolic function, such as the multi-gated (MUGA) scan or the cardiac ultrasound, also allows us to make more meaningful comparisons of the extent of cardiotoxicity associated with various anthracyclines, with different administration schedules, and with modalities intended to reduce cardiotoxicity. These comparisons have been explored, and are reproduced in Table 6.3.

While much information has been derived from endomyocardial biopsy examinations the use of this test in the day-to-day management of patients is no longer necessary. The invasive nature of cardiac biopsy procedure, as well as the fact that cumulative dosages higher than those associated with a 5% risk of cardiotoxicity are much less frequently used, has made biopsy less important in following the clinical course of patients. Additionally, the overall use of anthracyclines for the treatment of some malignancies has decreased.

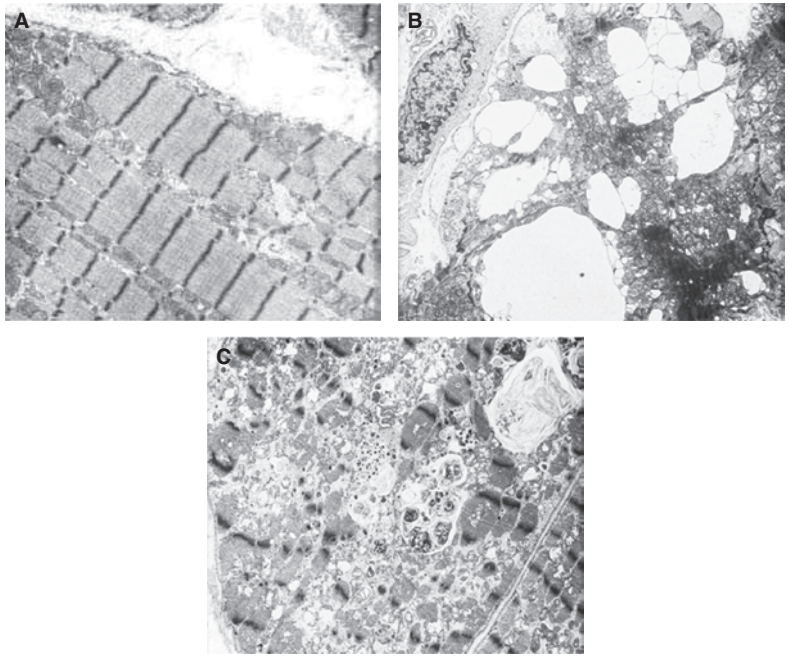
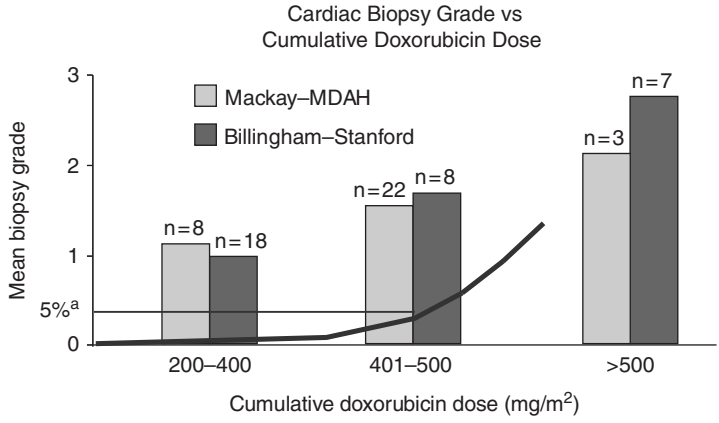


Figure 6.2 Electronmicrographs from patients who have received anthracyclines. A, demonstrates normal myocardium without evidence of anthracycline damage. B, demonstrates typical vacuoles, and represents typical electronmicroscopic changes that are considered moderate; it is possible that some of the cells demonstrating vacuoles may recover while others may proceed to cell death. C, demonstrates high-grade myocardial changes and necrosis. These images are representative, but in the grading of anthracycline damage the number of cells within an electronmicroscopic grid is a crucial component of assessing myocardial damage

6.2.1.4 The Clinical Spectrum of Cardiotoxicity of Type I Agents

Traditionally, anthracycline cardiotoxicity was thought to be either early, occurring within 3 months of the last administration, or late, occurring more than three months after exposure. However, frequently clinical signs or symptoms do not occur until years after exposure, since loss of myocardium is initially compensated and myocardial remodeling and additional cardiac stressors lead to myocardial remodeling. Most commonly, early toxicity presents in the form of non-specific electrocardiographic repolarization changes or myopericarditis. The sub-acute or late form has been studied more extensively, and incorporates myocardial dysfunction expressed as abnormal diastolic function, decreased systolic function, and, in severe instances, overt congestive heart failure that may progress to cardiac death.



[a]Risk of CHF.
Doxorubicin was given IV every 3 to 4 weeks.
Biopsy specimens were taken approximately 3 weeks following last therapy.
Adapted from Ewer et al. *J Clin Oncol*. 1984;2:112.

Figure 6.3 Cardiac biopsy grade versus cumulative doxorubicin dose. Doxorubicin was given IV every 3 to 4 weeks. Biopsy specimens were taken approximately 3 weeks after last therapy. Adapted from Ref. [9]

We now know that these forms of anthracycline cardiotoxicity constitute a continuum, in that the early manifestations, non-specific as they are, result from the damage to the myocyte. When injury to any given myocyte reaches a threshold, repair is no longer possible, and the cell dies. Cell death can be documented semi-quantitatively by measuring cardiac biomarkers, but is often under appreciated and difficult to correlate with clinical events [15]. Each administration of anthracycline adds to the cell destruction that preceded it, offering an explanation for the shape of the cumulative dose versus the congestive heart failure curve depicted in Figure 6.1; a typical curve of the $Y = X^2$ type.

As already mentioned, following the initial injury a period of compensation may be postulated, whereby remodeling occurs. This period of time may extend over months or years, and patients are generally asymptomatic during this period of stabilization. Interestingly, in most patients, compensation is such that the resting ejection fraction, the most widely used parameter for toxicity, is usually normal. Once the compensatory mechanisms are exhausted, the heart begins to fail; the ejection fraction decreases, symptoms may increase, and, in rare instances cardiac death may ensue many years after the initiating toxic anthracycline exposure. These events are illustrated in Figure 6.4.

Table 6.3 Relative toxicities of anthracycline: A comparison of relative toxicities of different cardiotoxic drugs, dosage schedules, and protective regimens

Drug	Schedule	Relative myelosuppressive potency of single dose compared with doxorubicin administered by standard schedule	Approximate relative cardiotoxicity[a]	Cardiotoxicity index compared with doxorubicin administered by standard schedule[b]	Recommended maximum dose (mg m^{-2})[c]
Doxorubicin	Rapid infusion (20 min)	1	1	1	400
Doxorubicin	Weekly	1	0.73	0.73	550
Doxorubicin	24-h infusion	1	0.73	0.73	550
Doxorubicin	48-h infusion	1	0.62	0.62	650[d]
Doxorubicin	96-h infusion	1	0.5	0.5	800-1000[d]
Epirubicin	Rapid infusion	0.67	0.67	0.44	900
Mitoxantrone	Rapid infusion	5	0.5	2.5	160

Daunorubicin	Rapid infusion	0.67	0.75[e]	0.5[e]	800[e]
Idarubicin	Rapid infusion	5	0.53	2.67	150
Pirarubicin	Rapid infusion	1	0.62	0.62	650[e]
Doxorubicin + Dexrazoxane	Rapid infusion	1[e]	0.5	0.5e	800-1000[e]
Doxorubicin, 300 mg m^{-2} + dexrazoxane	Rapid infusion	1[e]	0.73e	0.73e	550e

[a]Factor by which the cardiotoxic effects of the cumulative dose of rapid infusion doxorubicin can be compared with the cumulative dose of the agent, combination and schedule listed, when given at an equivalent myelosuppressive dose.
[b]Derived by dividing 400 mg m^{-2}, the recommended maximum dose of rapid-infusion doxorubicin, by the recommended maximum dose for the agent in question. The cardiotoxicity index represents a factor by which to multiply the cumulative dose of a drug administered to obtain an approximation of toxicity that might be expected had the resultant amount of doxorubicin been given by rapid infusion. For example, if a cumulative dose of 120 mg m^{-2} mitoxantrone had been administered, the patient would be expected to demonstrate cardiac damage approximately equal to 300 mg m^{-2} of doxorubicin given by rapid infusion ($120 \times 2.5 = 300$). This value is useful when changing from one cardiotoxic regimen to another. When the sum of the products of the indexes and the cumulative doses administered exceeds 400, the risk of clinically significant cardiotoxicity exceeds 5%.
[c]Dose producing clinically significant congestive heart failure in 5% of patients.
[d]Less toxic by endomyocardial biopsy.
[e]Inadequate data.

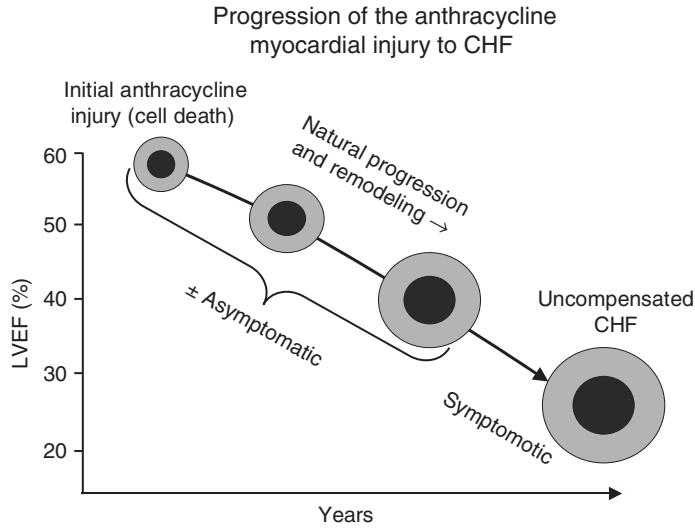


Figure 6.4 Progression of the anthracycline myocardial injury to CHF. Modified from Mann DL, Mechanisms and models in heart failure: a combinatorial approach, Circulation, 1999, 100, 999–1008

Symptoms of anthracycline-associated cardiotoxicity are not substantially different from heart failure of other etiologies involving diffuse (as opposed to regional) cell loss. While compensated patients may be asymptomatic; as decompensation ensues, patients may exhibit progressive tachycardia, fatigue, and varying degrees of shortness of breath. Physical examination may identify a diastolic gallop (S3), cardiomegaly, and evidence of pulmonary congestion; imaging studies show varying degrees of decreased systolic and diastolic function, and laboratory studies may show elevated levels of B-type natriuretic peptide (BNP). The symptoms of anthracycline-associated cardiotoxicity respond to the usual treatment modalities for congestive heart failure that include diuretics, angiotensin-converting enzymes (ACE), angiotensin receptor blockers, and β-adrenergic blocking agents. Digitalis preparations are used sometimes, and may result in symptomatic improvement, but probably do not extend life.

6.2.1.5 Risk Factors that Predict Augmented Toxicity of Type I Agents

A number of risk factors have been identified that, when present in a patient exposed to an anthracycline, predict that the patient will experience cardiotoxicity at a cumulative dose lower that that usually associated with any given degree of toxicity. These risk factors are listed in

Table 6.4 Anthracycline recognized risk factors, modified from Ref. [16]

Cumulative dose
Combination chemotherapy
Prior/concomitant mediastinal radiotherapy
Age (pediatric and elderly)
Previous cardiac disease (entities associated with increased LVEDP)
Hypertension

Table 6.4 [16]. Risk factors should be looked at with an understanding of what the anthracycline does to the heart and the natural history of the lesion. When taken in that context, risk factors can be thought of as either lesions that have previously affected the heart, thereby making it more vulnerable to the secondary insult – the anthracycline exposure – or to lesions that alter the threshold for toxic injury. In a number of instances both prior injury and increased sensitivity to toxic agents may play a role. Prior anthracycline or radiation exposure fit better in the first group, while the juvenile heart fits better with those who may have heightened sensitivity to ongoing toxic exposure. Patients with hypertension valvular disease, ischemic heart disease, and advanced age probably have both increased sensitivity and prior damage.

6.2.1.6 Mitigation of Cardiotoxicity Associated with Type I Agents

Because the mechanisms of cardiotoxicity differ from those implicated in tumor control or elimination, it is possible to alter some characteristics of an anthracycline or its administration so as to make it less cardiotoxic without altering oncologic efficacy. Cardiotoxicity can be reduced by altering the structure of the anthracycline, modifying the administration schedule, reducing the degree of oxidative stress or wall stress to which the heart is exposed at the time of anthracycline administration.

Altering the structure of the anthracycline. A number of doxorubicin analogs have been developed that, because of altered structure, appear to have reduced cardiotoxicity at equivalent myelosuppressive dosages. The most widely used is the 4'-epimer of doxorubicin, or epirubicin. In order to achieve comparable myelosupression with doxorubicin, larger dosages of epirubicin are needed; however, even at these increased dosages cardiotoxicity is probably lower. As with other anthracyclines, the cardiotoxicity of epirubicin is the dose-limiting factor. Patients experience a 5% likelihood of developing congestive heart failure at a cumulative dose of about 900 mg m^{-2}, or about twice that of the parent compound,

doxorubicin. This advantage is reduced, however, as the oncologic equivalency ratio with doxorubicin is about 4:3. Expressed in other words, 600 mg m^{-2} of epirubicin would have a similar oncologic effect as would 450 mg m^{-2} of doxorubicin, but demonstrates cardiotoxicity comparable with 300 mg m^{-2} of the parent compound.

Other anthracycline or related agents that show less cardiotoxicity than doxorubicin include pirarubicin, most commonly used in Japan for the treatment of lymphoma, and idarubicin, most widely used for the treatment of leukemia. Mitoxantrone, an anthracenedione rather than an anthracycline, exhibits similar cardiotoxicity, but is also less cardiotoxic than doxorubicin. The relative cardiotoxicities of these agents is summarized in Table 6.3.

Administration schedule modification. Prolonged infusion of doxorubicin selectively reduces cardiotoxicity. The effect was initially studied with weekly administration and later was demonstrated with infusions of 24, 48, 72, and 96 h. It is now recognized that cardiotoxicity is related more to peak plasma levels than is oncologic efficacy. Infusion durations longer than 96 h are associated with increasingly troublesome stomatitis, and are not recommended. Infusions of 96 h duration allow about twice as many cycles of doxorubicin to be administered as would be possible with rapid infusions; cumulative doses of up to 900 mg m^{-2} are often tolerated. Continuous infusion regimens usually require a central line for administration, and an infusion pump; nausea and vomiting are reduced.

The use of prolonged infusions has not been widely used for agents other than doxorubicin. Such administrative schedules peaked at the M. D. Anderson Cancer Center in the 1980s. Since that time the use of doxorubicin given at high cumulative dosages has decreased, and along with that the use of continuous infusion regimens.

Cardioprotective agents. A number of free radical scavengers have been tried in an effort to selectively mitigate the cardiotoxic characteristics of the anthracyclines. Dexrazoxane, a bisdioxopiperazine, is thought to undergo hydrolysis to form ADR-925, which is formed inside cardiac myocytes. ADR-925 removes iron from anthracycline complexes, thereby reducing the concentration of highly toxic iron–anthracycline complexes that damage myocytes [17]. A large number of pre-clinical and clinical studies have demonstrated cardioprotection of dexrazoxane, however, some concern has been raised that oncologic efficacy might be reduced [18, 19]. Dexrazoxane has been studied extensively when used to prevent the cardiotoxicity of doxorubicin, but it is cardioprotective with other anthracyclines as well [20].

As intimated above, the anthracycline injury probably starts with exposure. From a theoretical perspective, any modality that could either reduce the damage at the time of injury or slow the progression of remodeling might prove cardioprotective. With this in mind, reducing risk factors such as hypertension, or decreasing wall stress in cases that are associated with valvular disease, would seem to carry an empiric advantage. Where practical, patients should be optimized with regard to risk factors prior to receiving anthracycline-based chemotherapy [21,22].

Alternate delivery systems. Pegylated liposomal doxorubicin has been shown to be cardioprotective. Both the size of the molecule, a factor that selectively increases concentration to areas where altered vessels are encountered, and the prolonged half-life contribute to cardioprotection. The spectrum of diseases that respond to pegylated liposomal doxorubicin is somewhat different from that of the native molecule; the agent is widely used for the treatment of Kaposi's sarcoma and ovarian cancer. It is also used for the treatment of refractory multiple myeloma and has been widely studied as a cardioprotective anthracycline in the treatment of breast cancer. A non-pegylated form of liposomal doxorubicin is also available.

6.2.2 Type 2 Treatment-Related Cardiotoxic Agent

Type II agents do not cause cell death in highly selected patients such as those recruited in the adjuvant breast cancer trial. However, if combined with anthracyclines or used in high risk patients, type II agents might, at least in principle, induce some cell death. These possible exceptions having been said, type II agents are not usually associated with typical dose-congestive heart failure response, do not demonstrate characteristic structural abnormalities on electronmicroscopy, and are more likely to present as cardiac dysfunction that is reversible. A comparison of type 1 and type 2 agents is outlined in Table 6.5 [1].

6.2.2.1 Trastuzumab Cardiotoxicity

The first agent associated with myocardial contractile depression with characteristics that are fundamentally different from those of the anthracyclines was trastuzumab. The agent has impressive activity in the treatment of HER2 positive breast cancer, but initial studies demonstrated a high incidence of cardiotoxicity when the agent was administered

Table 6.5 Comparison of Type 1 and Type 2 treatment-related cardiac injury [1]

Type I (e.g., Doxorubicin)	Type II (e.g., Trastuzumab)
Predominantly cell death Damage starts with the first administration	Cellular dysfunction
Biopsy changes (typical of anthracyclines)	No typical anthracycline-like biopsy changes
Cumulative dose-related	Not cumulative dose related
Permanent cell damage (myocyte death; bad prognosis)	Predominantly reversible (my ocyte dysfunction; good prognosis)
Risk factors: Combination CT Prior/concomitant RT Age Previous cardiac disease Hypertension	Risk factors: Prior/concomitant anthracyclines or paclitaxel Age Previous cardiac disease obesity (BMI $> 25\ kg/m^2$)

concurrently with doxorubicin. Later, it became apparent that biopsy changes that were typically seen with the anthracyclines were not present, and it was ultimately noted that trastuzumab cardiotoxicity was largely reversible [23].

The initial pivotal study regarding trastuzumab noted that 29% of patients developed evidence of cardiac dysfunction or heart failure. However, in these initial trials trastuzumab was mostly used concomitantly with anthracyclines, likely worsening anthracycline-associated cardiotoxicity. Consequently, four major trials were initiated to evaluate the benefit of trastuzumab in the adjuvant setting, and as an additional endpoint, to evaluate the true incidence of cardiotoxicity. Several interesting findings emerged from these trials. Despite the fact that more than 10 000 patients were enrolled in the trastuzumab arms of these trials, there was only one cardiac death among the patients on the trastuzumab arms. Additionally reversibility was reported in two of the trials [24]. The number of patients who experienced cardiotoxicity was much less when trastuzumab was administered without an anthracycline. Thus, trastuzumab-associated cardiotoxicity is unusual in the absence of other cardiotoxic agents.

6.3 DIAGNOSTIC ASPECTS OF MYOCARDIAL DYSFUNCTION

Myocardial dysfunction is recognized as a continuum that ranges from very subtle and asymptomatic impairment of left ventricular function to

fulminate and life-threatening heart failure. In mild forms patients may be asymptomatic, physicians often remain unaware, and clinical indicators are frequently ambiguous. It is within this arena of clinical vagueness, with multiple sequential and contributing, related and unrelated factors, and imperfect tools for assessment, that the various cancer modalities enter the picture, cause cardiac damage, and contribute to impairment of systolic or diastolic function.

The tools we have at our disposal to quantitate cardiac damage include imaging techniques and quantization of chemical biomarkers. From an historical perspective, the cardiac parameter that has enjoyed a very long, but perhaps undeserved, tenure and loyalty among its advocates is the left ventricular ejection fraction. This value, expressed as a percentage, represents an indirect estimation of the amount of blood contained in the left ventricle at the end of systole as a percentage of what was contained in the left ventricle at the end of diastole (however, it does not represent myocardial contractility, which would be more useful to measure cancer drug associated cardiac toxicity). If half of the blood remained at the end of systole, the ejection fraction is reported as 50%. Ejection fractions can be estimated using a variety of techniques, but are most commonly derived from cardiac ultrasound (echocardiography) or multigated cardiac blood pool scans (MUGA). While, theoretically, the values obtained from estimations of ejection fractions should be identical, regardless of the technique employed, considerable experience has demonstrated that this, unfortunately, is not the case. On the one hand there are differences in the values reported for ejection fraction when different techniques are employed, and, on the other, these differences are not predictable. The more common techniques for estimating ejection fractions will be discussed in greater detail.

6.4 CARDIAC ULTRASOUND

Cardiac ultrasound or echocardiography was first introduced in the 1970s as m-mode, or motion studies. Early images provided a very narrow view through the myocardium, often referred to as an ‘ice pick; view. While m-mode studies have been superseded by more sophisticated imaging studies, the m-mode offers images of high resolution that is sometimes helpful in timing intervals during the cardiac cycle. Two-dimensional images expand the ice pick view and provide images of a planer cut through the heart in a plane determined by the operator. These

two-dimensional images are used most frequently to estimate ejection fraction, and several techniques have been used to arrive at a representative and reproducible estimation. Most studies incorporate some form of planimetry in the estimation, older methods using single or multiple measurements of fractional shortening are probably less accurate.

Modern cardiac ultrasound goes beyond assessing wall motion and cardiac contractility to incorporate directional and velocity of blood flow within the cardiac chambers and across the valves using shifts in the time that are proportional to velocity. These techniques, first described by Doppler with respect to astronomical movement of galaxies, have provided important information regarding cardiac relaxation (diastolic function) as well as intracardiac blood flow. A complete study generally incorporates m-mode, two-dimensional images, and Doppler flow patterns to provide an assessment of cardiac function. In some centers, two-dimensional studies can now be computer enhanced and rotated around an axis to provide three-dimensional images. It should be noted, however, that these methods are only indirect methods to assess myocardial contractility. Newer methods will likely improve the accuracy of cardiac ultrasound.

MUGA scans, unlike cardiac ultrasound that is imaged in real time, create an image over time by detecting the number of radioactive strikes in any particular position within a cardiac chamber during a short division of the cardiac cycle. These images are then assembled to generate a representative cardiac cycle. The end-systolic and end-diastolic frames of this representative cycle are then compared, whereby the number of radioactive counts is compared to estimate the ejection fraction.

While functional tests assess the ability of the heart to eject blood during contraction, this parameter does not take into account ongoing or prior damage to the myocyte. Attempts have been made to integrate changes in bio-markers into a broader view of myocardial damage. The brain natriutetic peptide (BNP) is a neurohormone secreted by cardiac chambers when the ventricular volume and pressure are elevated. BNP correlates with the left ventricular end diastolic pressure and the pulmonary capillary wedge pressure in patients with heart failure. The BNP would be expected to be elevated in any patient with any form of cardiac strain or dilation; anthracyclines or other agents that impact wall tension or volume are associated with BNP changes. Some of the difficulties with BNP as a parameter in following patients with cancer treatment-related cardiotoxicity are: (i) the BNP elevations are non-specific and may be caused by conditions not related to the toxic agent of concern; (ii) we do not yet have adequate criteria to identify those patients who should,

without other identifying characteristics, have alterations in their therapeutic regimen; and (iii) the BNP, at least in the case of anthracycline administration, is a late finding that implies that the heart has, or continues to undergo compensation for, sufficient damage that has altered either the volume or pressure within the cardiac chambers. Some suggest that significant BNP elevations per se are an indication for pharmacologic intervention.

In contrast, troponins are biomarkers of cell death. Troponin I has been shown to correlate with acute anthracycline toxicity [15]. Troponin I elevation early following the administration of anthracyclines has added to our understanding that myocyte destruction occurs much earlier than was previously appreciated. As myocyte death following anthracycline exposure probably takes place over a longer period of time than is the case with myocardial infarction, the troponin I levels are lower. As in the case with BNP, our knowledge of this marker is not yet sufficiently broad to fully understand the potential of this marker of cell death. In the future, troponin I screening could help to identify patients who have higher than anticipated levels (suggesting increased cell death) following administration so they could be followed with higher levels of scrutiny for early toxicity; such patients might be candidates for non-anthracycline regimens.

Bio-markers represent yet another area where the oncologist and cardiologist should work together, for only then can the isolated benefits of the interventions offered by the various specialties be balanced, not just with the risks limited to those of a limited area of interest or expertise, but the broader multidiscipline risks, for it is the impact of the global (all-inclusive) risks and the global benefits that will ultimately improve survival. The center that proudly announces that they have virtually no treatment-related cardiotoxicity may so under-treat the cancer as to have an overall sub-optimal outcome.

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7

Cardiovascular Toxicity of Antitumor Drugs: Translating Molecular Mechanisms into Clinical Facts

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7.1 CONTEMPORARY ISSUES IN CARDIOTOXICITY INDUCED BY ANTITUMOR DRUGS

The last few years have witnessed major changes in our perception of cardiotoxicity induced by antitumor drugs. In the past, cardiotoxicity was seen with drugs that lacked specificity and caused some type of damage to healthy tissues as well. This type of cardiotoxicity, best exemplified by that of the anthracyclines (doxorubicin (DOX), epirubicin (EPI), daunorubicin (DNR), idarubicin (IDA)), could only be managed through appropriate schedule modifications, intensified cardiac surveillance, or coadministration of preventative agents that it was hoped would mitigate the actions of drugs in the heart but not in tumor cells. Dose

Cardiotoxicity of Non-Cardiovascular Drugs Edited by Giorgio Minotti

reductions were also of help in diminishing cardiotoxicity induced by anthracyclines, but the same measure could not be adopted with antimetabolites or alkylating agents which caused cardiotoxicity without an obvious dose-dependence.

In more recent years, cardiotoxicity has also been seen with anticancer drugs that were designed to hit tumor-specific receptors and associated signaling moieties (antibodies, tyrosine kinase inhibitors (TKI)). The unexpected cardiotoxicity of such 'targeted drugs' uncovered the fact that the heart expresses non-negligible levels of the very same receptors or signaling moieties, which obviously play some key role in maintaining cardiac homeostasis.

Having said that, switching from the old-fashioned cytotoxics/cytostatics to the new targeted drugs did not abate the risk of cardiotoxicity. One should also keep in mind that the two kinds of drugs probably impose their cardiac effects by different mechanisms: whereas the former, or their metabolites, could cause cardiotoxicity by damaging or perturbing cardiomyocyte constituents and functions, the latter probably act by diminishing defenses against damage induced by comorbidities and/or other drugs that are given concomitant with the targeted therapy. Targeted drugs might also cause damage that develops at a distance from cardiomyocytes but causes hemodynamic effects that eventually affect the cardiac function.

In the light of the aforesaid premises one cannot escape the conclusion that the available preclinical models of cardiotoxicity are of limited value in defining the cardiotoxic potential of a given antitumor drug or combination of drugs. In the case of cytotoxics/cytostatics that gain cardiotoxicity after metabolic activation in the heart, there are potential pitfalls caused by the heterogeneous expression of drug metabolizing enzymes in one animal species or strain as compared with humans. In the case of targeted drugs, the available models might prove useless if the drugs were not probed in the presence of stressor agents such as other anticancer drugs, comorbidities, or experimentally induced hemodynamic alterations. All such concepts have obvious implications in the settings of drug development and preclinical toxicology; they also call for a cautionary reappraisal of current criteria for patient selection and recruitment in registratory Phase III trials which usually exclude subjects with comorbidities.

An additional emerging issue pertains to the lifetime risk of cardiotoxicity induced by antitumor drugs. Once again, the venerable 'old' anthracyclines offer quite an instructive example. In the case of DOX, the incidence of congestive heart failure (CHF) or other treatment-requiring

cardiac events averages well below 5% if the lifetime cumulative dose does not exceed ~450–500 mg m^{-2} [1]. In modern adjuvant therapy for breast cancer (240 to 360 mg m^{-2}), the incidence of CHF averages ~1.6 to 2.1 [2]. However, clinicians are facing unexpected problems, such as asymptomatic ventricular dysfunction in patients exposed to sub-threshold cumulative doses of DOX, or symptomatic CHF in 5 to 10 years survivors of operable breast cancer treated with adjuvant anthracycline [3,4]. Likewise, there is an impressive incidence of cardiovascular events in the long term survivors of childhood cancer treated with anthracyclines [4]. These observations suggest that there is no completely 'safe' dose of anthracyclines; moreover, the occurrence of delayed CHF suggests that anthracyclines introduce a lifetime risk of cardiotoxicity that may become symptomatic at any time after completing chemotherapy. How and when the asymptomatic damage progresses toward symptomatic CHF or other cardiac events remains to be established, but a possible synergism of the subclinical damage with overlapping comorbidites seems to be very plausible [2–4]. Again, these concepts call for a reappraisal of both preclinical models and study design of clinical trials. Less is known about delayed cardiovascular consequences of targeted therapy. One or two years follow-up of patients included in randomized controlled clinical studies can only provide partial information [5]; the apparently higher incidence of cardiac events outside such clinical trials raises concerns that targeted drugs might also conspire with prevailing comorbidities and introduce a lifetime risk of cardiac events [6].

In summarizing the aforesaid concepts, it may be said that basic and clinical scientists are faced with a number of unmet goals: (i) improving the translational value of preclinical models and making them more suitable to predict delayed cardiac events induced by, for example, anthracyclines; (ii) developing new models tailored to the unique characteristics of targeted drugs; (iii) filling the gap between cardiac safety signals in clinical practice as compared with clinical trials.

7.2 OF MODELS AND PREDICTIVENESS: LESSONS FROM ANTHRACYCLINES

The goal of improving preclinical models of cardiotoxicity has been partially met in the case of anthracyclines. These drugs have a pivotal role in the treatment of breast cancer and many other adult or childhood malignancies; therefore, elucidating the general mechanism(s) of

anthracycline-related cardiotoxicity has been the subject of countless investigations.

7.2.1 General Mechanisms of Anthracycline Cardiotoxicity: Preclinical Models and Clinical Readouts

In summarizing the prevailing hypotheses of anthracycline-related cardiotoxicity it can be said that anthracyclines may be toxic *per se* but gain manifold toxicity after one- or two-electron reductive activation. One-electron reduction of the quinone moiety of DOX results in the formation of a semiquinone free radical which regenerates its parent quinone by reducing molecular oxygen to superoxide anion ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2), members of the broad family of reactive oxygen species (ROS) that cause oxidative stress and energy collapse in cardiomyocytes. Two-electron reduction of the side chain carbonyl moiety converts anthracyclines to a secondary alcohol metabolite (doxorubicinol (DOXOL), epirubicinol (EPIOL)) that is slightly less active in redox cycling but remarkably more potent in dysregulating calcium and iron homeostasis. Oxidative stress, ion dysregulation, and concomitant alterations of the cardiac-specific gene expression and metabolic programs, eventually conspire to induce cardiomyopathy [7] (Figure 7.1).

The relative weight of ROS and secondary alcohol metabolite in anthracycline-related cardiotoxicity has been a matter of debate. Attempts to probe the role of ROS in clinical settings by means of antioxidant supplementation were quite unsuccessful. Out of four published trials only one study found that vitamin E could prevent a decrease in left ventricular ejection fraction (LVEF); however, the effect of vitamin E was confounded by concomitant administration of the calcium antagonist, nifedipine, which likely contributed protective effects such as, for example, reduced cardiac afterload [8]. Carvedilol, a mixed alpha-beta adrenoceptor blocker, has been shown to preserve LVEF in unselected patients exposed to anthracyclines, reportedly because carvedilol is also endowed with anti-oxidant properties [9]; however, confirmation of the possible beneficial effects of such antioxidant properties awaits comparisons of carvedilol with adrenolytic agents that share effects on heart rate and hemodynamic load but lack anti-oxidant properties [10].

The role of ROS has also been probed by means of the iron chelator, dexrazoxane, a bis-ketopiperazine that diffuses in cardiomyocytes and chelates iron before it reacts with the $O_2^{\bullet -}/H_2O_2$ couple and converts it to

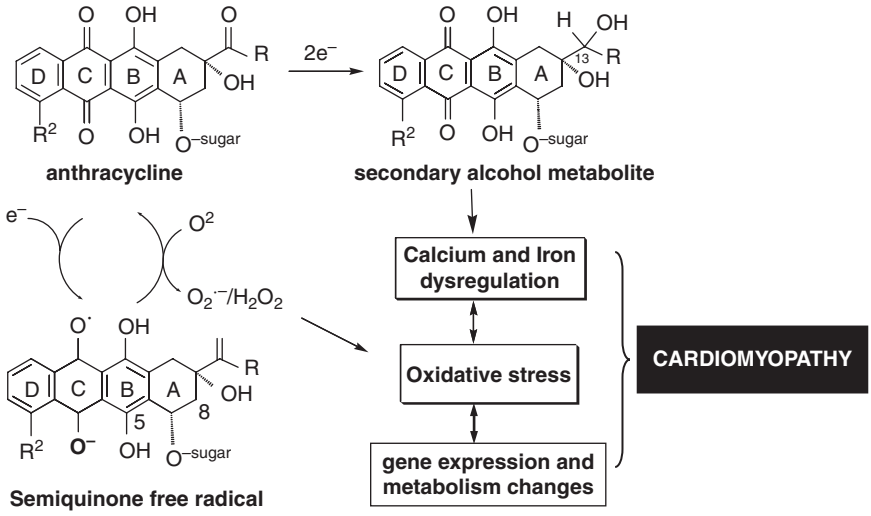

Figure 7.1 Metabolic determinants of anthracycline-induced cardiotoxicity. One-electron quinone reduction is accompanied by formation of O_2•– and H_2O_2, two-electron side chain carbonyl reduction causes formation of a secondary alcohol metabolite. The two pathways might conspire to induce cardiomyopathy

the more aggressive hydroxyl radical [11]. Unfortunately the widespread use of dexrazoxane was limited by early reports that it could interfere with anthracycline activity. Such concerns may have been overestimated, and more recently the preventative activity of dexrazoxane has been witnessed in very many clinical settings [10]. This said, there is little room to reconcile the lack of protection by antioxidants with the apparent protection afforded by dexrazoxane; accordingly, there is some evidence to suggest that dexrazoxane could protect by mechanisms other than iron chelation, for example, by interference with topoisomerase 2beta and antagonization of anthracycline-induced DNA damage in cardiomyocytes [12].

Attempts to probe the role of DOXOL or other anthracycline secondary alcohol metabolites proved to be even more difficult. Only a minor fraction of DOX (<1%) gets converted to DOXOL; thus, the role of DOXOL would be marginal or irrelevant, even if one assumed that DOXOL was 30–40 times more potent than DOX or ROS at inactivating ATPases, iron regulatory proteins (type 1), or plasma membrane ion exchangers [13]. Further complexity is introduced by the fact that two-electron reduction of the side chain carbonyl may be accomplished by a broad variety of cytoplasmic reductases: the superfamily of aldoketo

reductases (AKR) aldehyde reductase (AKR1A1), aldose reductase (AKR1B1), hydroxysteroid dehydrogenases (AKR1C1-AKR1C4) [14], and the superfamily of short chain dehydrogenases/reductases (carbonyl reductases, retinol/retinal dehydrogenases, sepiapterin reductases) [15]. Because the expression levels of such reductases may vary considerably across animal species and humans, the yield of DOXOL that is measured in a given animal (or cellular) model by no means anticipates the yield of DOXOL that actually forms in the human heart [16].

7.2.2 Need for a Translational Model of Human Heart

All such problems have been tentatively obviated by developing a translational model of human heart; this consists of human myocardial strips incubated in plasma and exposed to clinically relevant concentrations of DOX [17–19]. Such samples retained their viability and functions throughout the incubation time, as evidenced by: (i) bidirectional movements of anthracycline molecules across the plasma and the strips always followed rigorous polarity/apolarity rules; (ii) the strips responded properly to inhibitors of mitochondrial electron transport and of many other enzymes; (iii) the strips never released myoglobin, troponin T or MB-creatine kinase during the course of incubations. Thus, the system is well prepared to probe the cardiac pharmacokinetics of anthracyclines without confounding effects due to acute damage and consequent perturbations of membrane permeability and drug metabolization [17–19]. An appropriate exploitation of this model uncovered that DOX was lipophilic enough to diffuse from plasma to cardiomyocytes and back, while DOXOL generated inside cardiomyocytes was too polar to diffuse toward plasma. It follows that DOXOL accumulates in the heart, such that the product of [potency] and [accumulation] makes DOXOL become more toxic than DOX over months or years (Figure 7.2). In other words, DOXOL forms a long-lived anthracycline reservoir which may help to understand how anthracyclines introduce a lifetime risk of cardiotoxicity.

7.2.3 From a Translational Model of Human Heart to Clinical Facts

Does this preclinical rumination fit the clinical practice? Several results suggest that this might be the case. A retrospective nested case-control

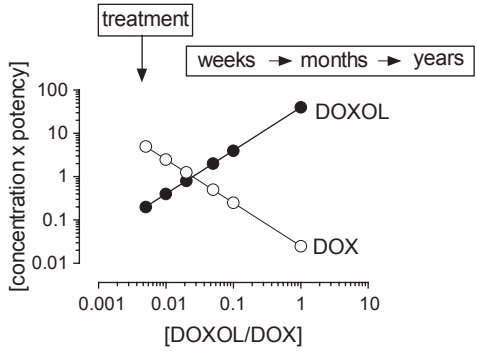

Figure 7.2 Toxicokinetics of DOXOL in human myocardium. DOXOL/DOX ratios were plotted by assuming an immediate post-administration concentration of 10 or 0.05 μM for DOX or DOXOL in the heart, respectively. [Concentration × potency] factors were calculated by assuming that DOXOL was 40-times more potent than DOX toward ATP-dependent Ca^{2+} handling proteins. Simulation based on data in references [17–19]

study of patients enrolled in the Childhood Cancer Survivor Study indicates that the V244M polymorphism of carbonyl reductase (CBR) 3 introduces a hazard ratio for CHF as high as 10.2 compared with properly matched controls [20]. It seems that the $methionine_{244}$ isoform of CBR3 shows a greater catalytic specificity toward carbonyl substrates [21]. Similarly, valine to isoleucine polymorphism (V88I) of CBR1 facilitates reduction of DNR to its alcohol metabolite, DNROL [22]. The V88I CBR1 polymorphism has been detected with a high frequency in individuals of African ancestry; this is highly suggestive of a possible link with anthracycline cardiotoxicity and delayed cardiovascular events, as the African American race represents an independent risk factor of CHF in women who received anthracyclines for the (neo)adjuvant treatment of breast cancer [3].

Reconstitution and simulation of anthracycline metabolism in a translational model of human heart thus identified DOXOL as the possible major determinant of cardiotoxicity in cancer survivors with a history of prior DOX. On practical grounds, the observation that gain-of-function polymorphisms of CBR1 or CBR3 could retrospectively be associated with higher hazard ratios for cardiotoxicity also rationalizes the idea that CBR inhibitors could be of value in preventing formation of long-lived anthracycline metabolites in the human heart [23]. Nevertheless, general conclusions seem to be premature at this time. Inhibitor studies show that human myocardium generates DOXOL and EPIOL through the activity of AKR1 rather than CBR1 or CBR3 [17–19]; the latter would primarily

metabolize DNR or IDA [16, 24, 25]. There is also a possibility that certain reductases, for example AKR1A1, contribute to reducing both DOX and DNR to their corresponding alcohols, albeit with very different kinetic efficiencies [26]. These observations raise the need for caution when considering the clinical implications of the aforesaid CBR polymorphism(s), as the available data do not tell us whether the apparent linkage of CHF with CBR1 or CBR3 polymorphisms was contributed by subsets of patients who had been exposed to an anthracycline or another.

Distinguishing the contribution of individual reductases to the total yield of alcohol metabolite will be a difficult task; the next few years will tell us whether inhibiting alcohol metabolite formation is possible and therapeutically advantageous. Here we would like to focus on some unquestionable results that the translational model of human heart can offer at this time. A very instructive example is offered by the results of studies that probed EPI in this model. Epirubicin only differs from DOX in an axial-to-equatorial epimerization of a hydroxyl group in the aminosugar bound to the anthraquinone chromophore. Such a minimal modification makes EPI ~1.5 times less active than DOX against tumors, which is explained by the unusual susceptibility of EPI to glucuronidation in liver and improved body clearance [27]); interestingly, however, EPI induces CHF at cumulative doses $\geq$2 times higher than DOX [28]. The cardiotoxicity of EPI is therefore approximated to ~66% of that of DOX. Clinical studies did not show differences between the C_{max} values of DOX and those of equiactive doses of EPI [29,30], thus showing that the reduced cardiotoxicity of EPI cannot be attributed to a reduced cardiac exposure to the drug; moreover, studies in laboratory animals did not always show a reproducible reduction in the cardiotoxicity of EPI as opposed to DOX [31,32]. Molecular determinants of the reduced carditoxicity of EPI could only be identified by taking advantage of the human myocardium model. This showed that EPI underwent sequestration in cytoplasmic acidic organelles and, hence, it failed to reach mitochondrial reductases that promoted its one-electron bioactivation to ROS. Epirubicin also exhibited an impaired catalytic efficiency toward cytoplasmic two-electron reductases, such that the levels of formation of EPIOL averaged 40–50% of those of DOXOL [18,19]. The reduced cardiotoxicity of EPI therefore seems to correlate much better with a ~50% reduction of EPIOL formation rather than with a complete elimination of ROS formation, as one would expect if the time-related sequelae of anthracyclines were determined by the size of a cardiac pool of long-lived secondary alcohol metabolites.

In providing novel formal explanations about how EPI causes cardiomyopathy and CHF at doses higher than equiactive to DOX, these results implied that anthracycline cardiotoxicity would be aggravated by any concomitant chemotherapeutic that increased the levels of DOXOL or EPIOL in the human heart. This latter possibility was successfully probed in the human heart model by exploring pharmacodynamic interactions of DOX or EPI with the tubuline-active taxanes, paclitaxel (PTX) or docetaxel (DCT). Combining anthracyclines and taxanes has long been considered a logical opportunity for improving the treatment of breast cancer and other solid tumors; unfortunately, however, pivotal trials showed that DOX immediately followed by PTX caused cardiomyopathy and CHF in a higher than expected number of patients, as if PTX accelerated the dose-related progression of anthracycline cardiotoxicity [29]. A trend toward a higher than expected incidence of cardiotoxicity was also seen with DOX–DCT combinations [33], while neither PTX nor DCT proved able to aggravate the clinical pattern of cardiotoxicity induced by EPI [34,35]. Studies with the translational model of human heart showed that adding plasma with 6 μM PTX or DCT, similar to the C_{max} value of either taxane in patients' plasma, caused a peak of formation of DOXOL but not of EPIOL [19] (Figure 7.3). Next, mechanistic exploration revealed that PTX or DCT acted as allosteric modulators of DOX-reductase complexes, while the affinity of EPI for the reductases was too low to favor such a modulation [19] (Figure 7.4). Thus, modulation of DOXOL but not EPIOL in the translational model of human heart offered a novel and formal explanation to recapitulate the cardiac safety of EPI–taxane schedules as opposed to the toxicity induced by equivalent DOX–taxane doublets. Needless to say, studies with laboratory animals did not characterize any such effect of PTX or DCT on anthracycline secondary alcohol metabolites [36,37]. In confirming the existence of species-related differences in anthracycline bioactivation, these latter findings demonstrated that the translational model of human heart was of value in providing quantitative information on the yield of secondary alcohol metabolites; they also showed that combination therapies could easily pass safety assessment in animal models while causing cardiac events in clinical practice. The approval of combination therapies should therefore be preceded by a judicious scrutiny of drug interactions in the translational model of human heart, but too often this methodological requirement is neglected by drug developers and regulatory agencies.

On a different note, the lessons from EPI in a translational model of human heart anticipate that anthracycline analogs forming fewer or no alcohol metabolites would be very useful in improving the therapeutic

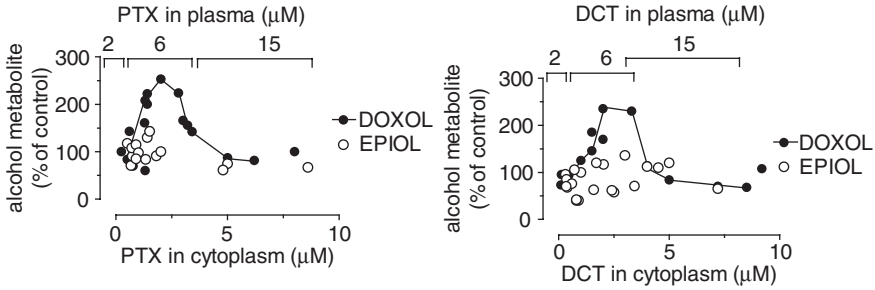

Figure 7.3 Effects of taxanes on the levels of DOXOL or EPIOL in the cytoplasm of human myocardial strips. Human myocardial strips were incubated in plasma that contained 10 μM DOX or EPI, and 2 to 15 μM PTX or DCT cosolvent formulations. After 4 h the cytoplasm of strips was assayed for the levels of taxanes and anthracycline secondary alcohol metabolites (percent of increase versus strips without taxanes). Left panel shows the effect of PTX and DOXOL or EPIOL; Right panel shows the effect of DCT on DOXOL or EPIOL. Modified after reference [19]. Reprinted with permission from the American Society for Pharmacology and Experimental Therapeutics

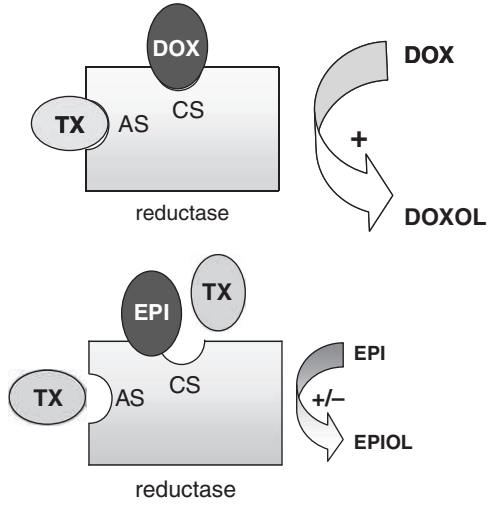

Figure 7.4 Kinetic simulation of active or defective taxane modulation of anthracycline metabolism in human myocardial strips. Upper panel shows that taxanes caused an accelerated conversion of DOX to DOXOL by binding with high affinity to the allosteric site of cytoplasmic aldehyde reductases. Bottom panel shows that taxanes failed to stimulate EPIOL formation because of the low affinity of EPI for the catalytic site and the possible competition of taxanes for such a site. Based on reference [19]. TX, taxane; AS, allosteric site; CS, catalytic site (see Color Plate 2)

index of anthracycline-based regimens. This concept also depends on the result that secondary alcohol metabolites did not always contribute to, or sometimes diminished, the antitumor activity of their parent anthracycline [38], the only noticeable exception being that the secondary alcohol metabolite of IDA proved to be more active than IDA itself [39]. Analogs of DOX or EPI that were not converted to alcohol metabolites were therefore anticipated to kill tumor cells while also sparing cardiomyocytes from a lifetime risk of toxicity. A DOX analog that lacks the carbonyl group liable to reduction to a secondary alcohol metabolite (C-13 deoxy-DOX) has been developed. When probed in limited clinical studies C-13 deoxy-DOX caused on-treatment CHF at cumulative doses of 900 mg m^{-2}, which was the required dose to achieve equiactivity to DOX [40]. These findings should not be interpreted as disproving the secondary alcohol metabolite hypothesis of cardiotoxicity; instead, they demonstrate that high cumulative doses of novel anthracyclines, as required to achieve equiactivity to DOX, may abrogate the beneficial effects of a defective conversion to one toxic species or another. The unfortunate clinical experience with C-13 deoxy-DOX thus indicates that a cardiac accumulation ratio of 2:1 versus DOX would make investigational anthracyclines induce cardiotoxicity by alcohol metabolite-independent mechanisms such as chaotropic effects on mitochondria, altered electrophysiological responses of cardiac plasmalemma, or inactivation of numerous energy-related enzymes [41–43]. All such effects may be caused by anthracyclines not only through an excessive formation of ROS, which becomes high enough to replace alcohol metabolites in inducing cardiac damage [40], but also through direct mechanisms that do not involve either ROS or secondary alcohol metabolites [44].

The aforesaid concepts can be retrospectively applied to further reappraisal of the reduced cardiotoxicity of EPI as opposed to DOX. By having shown that chronic cardiomyopathy correlated with alcohol metabolite formation, and that EPI formed ~50% less alcohol metabolite than DOX, one would approximate the cardiotoxicity of EPI to be ~50% of that of DOX; unfortunately, however, we mentioned that the cardiotoxicity of EPI could be firmly approximated to 66% of that of DOX [45]. So, what is counterbalancing the benefit of a defective conversion of EPI to EPIOL? By adapting the translational model of human heart to measure anthracycline uptake versus anthracycline release we determined that EPI exhibited a higher uptake and reached myocardial levels two times higher than those of DOX; however, EPI was found to operate a unique pathway of myocardial clearance that diminished the final cardiac ratio of EPI to DOX to 1.5:1 [46]. This was safer than a 2:1

ratio but certainly contributed to increasing the cardiotoxicity of EPI from a theoretical value of 50% to the observed level of 66% [46].

The unique pathway of EPI clearance from human myocardium depended on the conversion of a sizeable pool of EPI to EPIOL aglycone: this was not seen with DOX. Such formation of EPIOL aglycone occurred independently of EPIOL formation and several lines of evidence suggested that it could be viewed as a sort of salvage pathway. First, EPIOL aglycone formation occurred neither through sequential formation and hydrolytic deglycosidation of EPIOL nor is reflected EPI hydrolysis followed by carbonyl reduction; instead, EPI converted to an intermediate which could not be identified because of its rapid and complete biotransformation to EPIOL aglycone. Second, EPIOL aglycone formation was kinetically favored over EPIOL formation. Third, EPIOL aglycone was lipophilic enough to diffuse from myocardial samples to plasma, which was contrary to the polar character of EPIOL and its tendency to accumulate in the strips. And finally, and perhaps more importantly, the efflux of one molecule of EPIOL aglycone caused permeation effects that favored the efflux of many more molecules of EPI, such that the accumulation ratio of EPI to DOX quickly decreased from a potentially toxic value of 2:1 to a 1.5:1 value which still allowed the gain in cardiac tolerability observed with EPI as compared with DOX (Figure 7.5).

Characterization of the tendency of EPI to accumulate in human myocardium, and of the efficacy of EPIOL aglycone in improving elimination of excess EPI, thus helps to precisely quantify the cardiotoxic potential of EPI as opposed to DOX. Moreover, these findings introduce new concepts and guidelines that should be considered in developing noncardiotoxic anthracycline analogs. Pharmaceutical efforts are usually concentrated on eliminating or modifying the quinone or carbonyl moieties that enable anthracyclines to form toxic ROS or secondary alcohol metabolites; in principle, diminishing the formation of secondary alcohol metabolite would prove more advantageous in abating the lifetime risk of cardiotoxicity introduced by anthracycline regimens. This said, it is evident that investigational anthracyclines should be probed for their tendency to reach cardiac levels that are high enough to mitigate the benefit of a defective conversion to secondary alcohol metabolites; hence, new anthracyclines should also be probed for their formation of diffusible metabolites that eliminate excess anthracycline by mechanisms similar to those described for EPIOL aglycone. The next few years will tell us more about the analog-specificity of such a mechanism of metabolism-dependent anthracycline elimination.

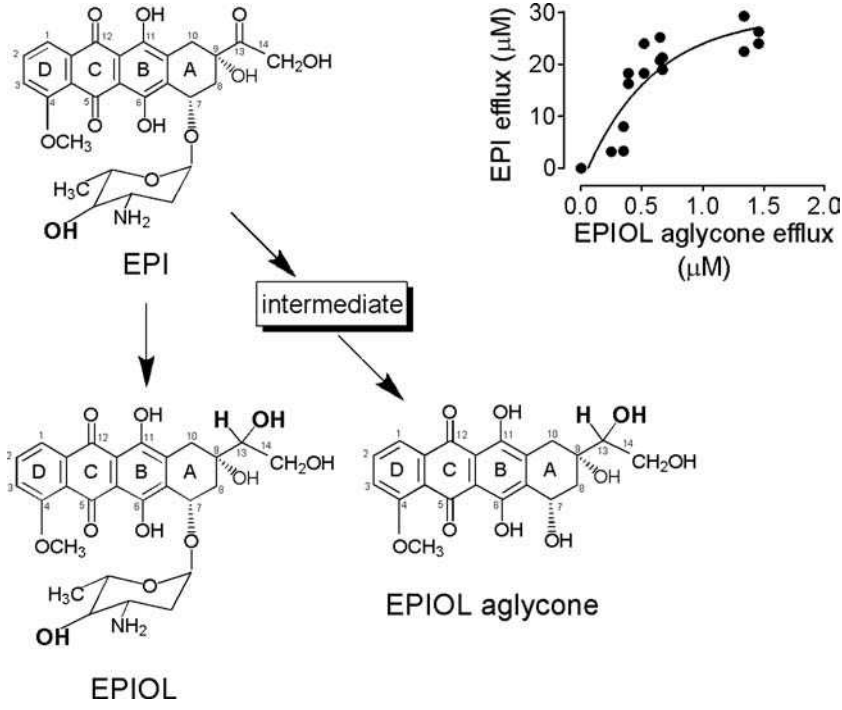

Figure 7.5 Metabolism-dependent elimination of EPI from human myocardium. The arrows indicate the metabolic conversion of EPI to EPIOL and its concomitant independent conversion to EPIOL aglycone. This latter metabolite then undergoes efflux from human myocardium and causes membrane permeation effects that mobilize many more molecules of EPI from human myocardium. Metabolism-dependent elimination of excess EPI is important to prevent EPI from accumulating high enough to cause cardiotoxicity in the face of its defective conversion to EPIOL. Adapted with modifications from reference [46], reprinted with permission from the American Society for Pharmacology and Experimental Therapeutics

7.2.4 Overall Assessment of the Translational Model of Human Heart and Its Applicability to Research Issues

An overview of the experimental settings developed with the translational model of human heart is reported in Table 7.1, together with the experiment-oriented information that is obtained in predicting toxicity induced by anthracyclines as single agents or in combination with other chemotherapeutics.

Although successfully exploitable in such defined settings, the translational model of human heart has its inherent experimental limitations that need to be carefully evaluated. Human myocardial strips are tissue

Table 7.1 Applicability of the translational model of human heart to research issues in anthracycline cardiotoxicity.

Experimental setting	Translational information
Passive uptake and efflux	Prediction of toxicity by excess anthracycline
Metabolism-dependent efflux	Mitigation of toxicity induced by excess cardiac anthracycline
Sequestration in cytoplasmic acidic organelles	Inactivation of ROS formation
Formation and retention of secondary alcohol metabolites	Analog-specific lifetime risk of cardiotoxicity
Allosteric modulation of cytoplasmic reductases	Increased cardiotoxicity of anthracycline-containing multiagent therapies

Source: Based on references [17–19,37,46].

blocks composed of cardiomyocytes, endothelial cells, and fibrocytes (~20% of tissue mass). Whereas incubation of such samples in human plasma may help to account for PK factors such as drug–protein binding and consequent modulation of drug partitioning from the plasma into the strips, the gross composition of the tissues strips does not completely explore the relative contribution of individual cell types to drug accumulation and/or metabolization. Fibroblasts, endothelial cells, as well as smooth muscle cells and adipocytes, provide structural and trophic support to the myocytes, and some of these cells (endothelial cells and fibroblasts) may actually prove to be more sensitive to the toxic effects of DOX or other anthracyclines than are cardiomyocytes. Thus, cardiomyocyte deterioration, as anticipated from the metabolic profiling of the translational model of human heart, might well be preceded by alterations in matrix composition, paracrine signals, and anthracycline distribution across extracellular fluids and cardiomyocytes [47].

Another important issue pertains to the possible role of pluripotent cardiovascular progenitor cells that can differentiate into a functional cardiomyocyte phenotype [48]. Although much debate exists about whether these progenitor cells could repopulate anthracycline-induced foci of myocyte damage or affect, if irreversibly poisoned, progressive anthracycline cardiotoxicity [49], the role of such cells in the observed distribution and metabolization of anthracyclines in the translational model of human heart will need to be investigated. This might be particularly relevant to long-term survivors of childhood cancer, as anthracycline-associated cardiomyocyte loss leads to inadequate compensatory left ventricular hypertrophy and hence, chronic afterload excess that progressively impairs ventricular function [50]. Rat-derived neonatal cardiomyocytes or embryo myocytes, such as the widely used H9c2 cell

line, might be considered suitable for exploring the effects of anthracyclines on dividing progenitor cells [51]; unfortunately, however, the available evidence shows that the metabolic machinery of such cells is largely inadequate to simulate the clinical conditions of anthracycline administration and metabolization [18,37].

Other issues worthy of consideration pertain to the mechanism(s) by which test compounds partition from plasma into myocardial strips. Whereas myocardial strips may be of immediate value in exploring the passive diffusion of anthracyclines, they may be much less so in exploring, for example, the relative contribution of gp60/caveolin 1-mediated endothelial transcytosis. This latter route can be particularly important in the case of drugs formulated in complex vehicles or nanoparticles, as is the case for a recently developed and approved albumin-PTX formulation [52]. The role of gp60/caveolin 1-mediated endothelial transcytosis might be explored by probing the strips with methyl-β-cyclodextrin, a compound known to disrupt cholesterol-dependent mechanisms of caveolar transport [53].

On balance, there are some unavoidable limitations to the translational model of human heart, especially if one considers exploiting this model to monitor the diffusion and metabolic fate of newly engineered nanoparticles or drugs reconstituted in vehicles that are more complex than traditional water–cosolvent systems. This said, we suggest that the translational model of human heart, as developed and exploited so far, provides the most valuable and reliable tool for deciphering the metabolic determinants of cardiotoxicity prospectively induced by investigational anthracyclines/multiagent therapies.

7.3 UNFILLED: THE GAP FROM TRANSLATIONAL MODELS FOR THE MULTIPLE-HIT MECHANISM

The translational model of human heart, as described in the preceding sections, defines PK and metabolic events which occur at each cycle of anthracycline administration. Because anthracycline cardiotoxicity is a cumulative effect that develops from the summation of one exposure with another, understanding how human myocardial strips respond to one anthracycline or another provides important information for predicting whether and how that particular anthracycline introduces a lifetime risk of cardiotoxicity. Moreover, the system can be powered by adjusting anthracyclines to reproduce their clinical C_{max} value, the PK parameter

which best correlates with the risk of anthracycline-related cardiotoxicity [54]. Needless to say, however, the model cannot be tailored to incorporate the comprehensive picture of cardiotoxicity that is currently referred to by the name 'multiple-hit hypothesis'. This latter concept is based on the notion that every single exposure to anthracyclines or any other anticancer drug will most likely overlap with sequential or concurrent insults due to comorbidities or unfavorable lifestyle, that would be harmless to the general population but gain a magnified cardiotoxic potential in the heart of cancer patients (2–4, 55–60). This concept, therefore, encircles the epidemiologic evidence for asymptomatic cardiac dysfunction or symptomatic CHF in childhood or adult cancer survivors exposed to reportedly safe cumulative doses of anthracyclines or other chemotherapeutics.

The current and future consequences of the 'multiple-hit hypothesis' are potentially devastating, as we currently lack reliable tools to predict which patients are, or will be, at an increased risk of late-occurring cardiac events. Full-blown CHF induced by antitumor drugs does not always respond, or only partially responds, to standard medications, particularly in patients with a clinical history of cumulative anthracycline regimens; in many cases heart transplant is the only option for patients who survived cancer but then experienced delayed full-blown CHF [61]. The lack of a suitable long-term, multifactorial model of cardiotoxicity, therefore, contributes to a lack of knowledge in clinical settings and to the social and economic consequences of the high prevalence of treatment-requiring cardiotoxicity in cancer survivors.

Chronic cases of anthracycline cardiotoxicity require weeks of treatment as well as weeks or months of post-treatment monitoring; furthermore, cardiotoxic manifestations that resemble those seen in humans may eventually develop in only a few drug-treated animals. Adequate studies require large numbers of animals to be monitored for extended periods, increasing costs and exceeding the capacity of a single laboratory.

7.3.1 One Step Forward: Anthracyclines in the Spontaneously Hypertensive Rat

In spite of these limitations, the first step to be taken in filling the gap between translational models of cardiotoxicity and the complex pathobiology of the multiple-hit hypothesis must consist in giving absolute

precedence to a chronic model over acute models of cardiotoxicity [62]. As an example of such a compelling need, we draw the reader's attention to the fact that cardiomyocyte apoptosis is seen in acute cardiotoxicity induced by short-term administration of DOX [63,64] but not so often in the progressive cardiotoxicity induced by long-term DOX treatment [65]. This general guideline must incorporate a thoughtful analysis of the route of administration. Anthracyclines should always be administered intravenously as subcutaneous, intramuscular, or intraperitoneal administrations are confounded by localized tissue damage that could well modify local and systemic PK. Similarly, the effect of any prospective cardiac protectant should be assessed under such defined conditions [10].

7.3.2 Another Step Forward: Combining Anthracyclines with Nonanthracycline Chemotherapeutics

The second step toward a better exploitation of chronic animal models of cardiotoxicity should consist of adopting strains that carry at least one of the several comorbidities implicated in the multiple-hit hypothesis. This is accomplished by means of spontaneously hypertensive rats (SHR). In comparison with normotensive rats, SHR show a greater sensitivity to chronic DOX and exhibit histological and functional cardiotoxicity very similar to that seen in cancer patients, including noticeable elevation of circulating troponin (Tn) I and decrease in mean arterial pressure [66]. Of particular relevance is the recurring increase of TnI in these animals: small increases in the serum concentration of cardiac troponin T in children after the first dose of DOX predicts subsequent risk for left ventricular dilatation and wall thinning [67], and transient increase of TnI in adults receiving high dose chemotherapy predicts subsequent decrements of LVEF [68]. Thus, SHR reproduce clinical settings of increased susceptibility to anthracycline-related cardiotoxicity, as is the case for early age or high dose chemotherapy.

The SHR model is a good example of how the multiple-hit hypothesis could be tentatively explored in preclinical settings. One such approach would not obviate the aforesaid differences in drug metabolism that can only be obviated by the translational model of human heart; however, one needs to compromise between the pros and cons. Combining one model with the other would be most satisfactory but, sad to say, funding agencies and regulatory bodies pay little attention to these issues. There is an unmet need for studies that explore how drug metabolism and

hypertension influence anthracycline damage to sarcomeric or cytoskelatal proteins such as titin [69] or dystrophin [70]. The time and effort required to address these points show the need for coordinated efforts by several laboratories, each specializing in more than one mechanism of toxicity and studying one or more such mechanisms in robust cohort of both human myocardial samples and animals. Regrettably, we are not yet at this point of a concerted action.

The third step towards a better exploitation of chronic animal models of cardiotoxicity should consist of combining anthracyclines with other chemotherapeutics, thus reproducing the clinical settings of multiagent therapies. We have described how combining DOX or EPI with PTX or DCT in the translational model of human heart helped to decipher the metabolic determinants of cardiotoxicity induced by anthracycline–taxanes regimens. Surprisingly, however, very few studies have been done exposing rats or rabbits to cumulative doses of anthracyclines in combination with other chemotherapeutics. This is a 'black box' in preclinical research, as anthracyclines are combined with alkylating or antimetabolite agents in very many protocols for the treatment of breast cancer, lymphomas, and other malignancies.

Non-anthracycline chemotherapeutics cause independent mechanisms of cardiac damage that overlap with anthracyclines and make cardiotoxicity even more complex and pleiotropic, as is usually the case in cancer patients. For example, alkylators such as cyclophosphamide and ifosfamide and platinum compounds, or antimetabolites such as fluoropyrimidines or methotrexate, cause endothelial dysfunction and/or coronary spasm, accompanied by silent ischemia, arrhythmias, angina, or acute myocardial infarction in the most severe cases [71–73]. In accordance with this notion and with the possible overlap of ischemic disease with contractile failure, we now know that cardiac ischemic disease is a cause of excess mortality in previously non-ischemic patients with a history of anthracycline-based multiagent therapies [74]. On the other hand, anthracyclines have long been known to induce myocardial Ca^{2+} overload and stiffness by very many mechanisms, such as, (i) direct inhibition of the Ca^{2+}-ATPase that sequesters Ca^{2+} in the sarcoplasmic reticulum, (ii) reduced expression levels of Ca^{2+}-ATPase, (iii) inhibition of the energy build-up that assists Ca^{2+} loading in mitochondria [7]. Myocardial stiffness primes the heart to diastolic dysfunction, which often precedes systolic dysfunction or sometimes represents a neglected but most insidious form of cardiotoxicity in cancer patients with normal LVEF [75–77]; moreover, myocardial stiffness and diastolic dysfunction increase interstitial pressure and reduce coronary conductance [78],

thereby causing ischemia that overlaps with that induced by non-anthracycline chemotherapeutics. In providing a typical example of how ischemia begets ischemia, this scenario ramifies in further vicious loops: ischemia would be an independent determinant of cardiac Ca^{2+} overload and stiffness [78] and, by doing so, it would also aggravate cardiotoxicity induced by anthracyclines [4].

In the light of these considerations, treating animals with only anthracyclines would be of limited value in describing and interpreting the cardiotoxicity attributed to multiagent therapies that incorporate anthracyclines. The conspiracy of anthracyclines with non-anthracycline chemotherapeutics thus extends beyond the canonical picture of systolic dysfunction and leads to a reciprocal potentiation of diastolic dysfunction with ischemia. Such a scenario, as sketched in Figure 7.6, clearly needs to be brought into a research focus. In looking at this tentative circuit of pathological events, the substantial limitations of using only imaging, functional, or biohumoral indices of systolic function and cardiomyocyte viability become apparent. In preclinical settings there is, therefore, a need to implement the monitoring of, for example, B-type natriuretic peptides that herald ventricular wall tension and diastolic dysfunction in animals with otherwise normal LVEF; in such apparently 'healthy' animals TnI (or TnT) would most probably look normal, as it only heralds cardiomyocyte irreversible damage and necrosis.

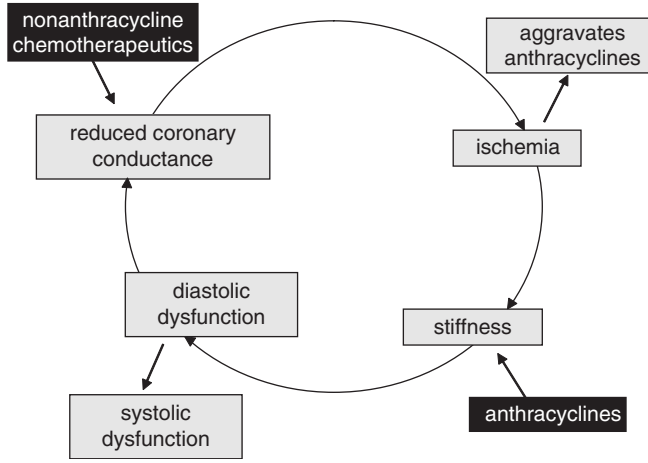

Figure 7.6 Multiple links between cardiotoxicity induced by anthracyclines and nonanthracycline chemotherapeutics. Based on references [4,70–78]

7.3.3 Towards an Integrated Model of Multiple Hits

One disturbing aspect of the multiple-hit mechanism of cardiotoxicity is that the impact of such a mechanism on the life expectancy and quality of life of cancer survivors rests with primarily epidemiologic evidence. Hypertension, dislipidemias, pre-existing arrhythmias and valvular or coronary diseases, and endocrine disorders, all have been suspected of dissipating cardiac defenses against subthreshold cumulative doses of anthracyclines, thereby leading to the onset of cardiac events in cancer patients; however, how precisely one comorbidity compares with another, or how precisely one or more comorbidities overlap with subchronic damage induced by, for example, DOXOL, has not been determined.

Certain aspects of the multiple-hit hypothesis are perceived in clinical practice but lack experimentation in animal models. For example, reduced physical activity and overweight are often neglected when evaluating the cardiovascular consequences of anthracycline-based therapies. Early breast cancer patients decrease their physical activity by a couple of hours per week, with greater decreases occurring in those women who receive combined/multiagent therapies rather than single agent/modality therapies [79]. Furthermore, very many breast cancer patients are found to gain body weight during chemotherapy [80]. Both physical inactivity and weight gain are independent predictors of cardiovascular mortality in noncancer adults; hence, the clinical value of these factors as independent predictors of cardiotoxicity in the cancer population should be considered and incorporated in the available preclinical models. Moderate dietary restrictions and/or physical exercise prevent anthracycline cardiotoxicity to some extent in laboratory animals [81,82], but these are limited experimental approaches that should be exploited more systematically in preclinical models of cardiotoxicity.

Figure 7.7 summarizes our personal view of what should be done to bring the multiple-hit hypothesis of cardiotoxicity from the clinical arena into the laboratory. Animal models should be developed that carry a cardiac pool of the long-lived DOXOL, the concomitant damage induced by nonanthracycline chemotherapeutics, and no less than two or three biochemical/functional abnormalities to replicate the comorbidities/unfavorable lifestyles that concur at inducing cardiotoxicity in cancer survivors. These things are easier said than done, but efforts in that direction would be beneficial in the long run.

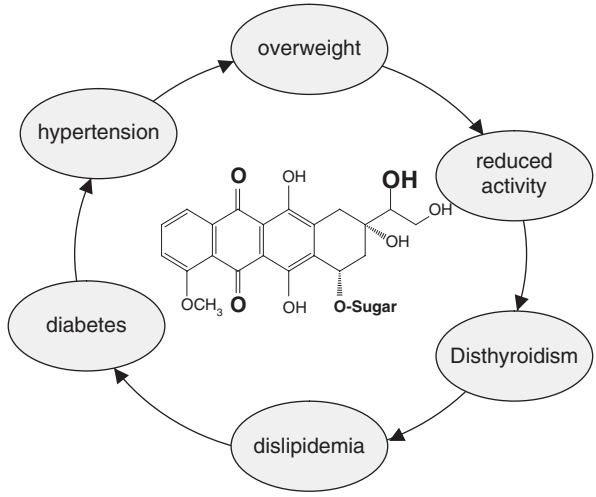

Figure 7.7 Anthracyclines and the multiple-hit hypothesis of cardiotoxicity. Proposal for animal models that carry a cardiac pool of long-lived DOXOL, the damage induced by nonanthracycline chemotherapeutics, and a constellation of comorbidities or unfavorable lifestyles

7.4 PREDICTING CARDIOTOXICITY FROM TARGETED DRUGS: LESSONS FROM TASTUZUMAB

Cardiotoxicity proved to be an unanticipated complication in the clinical use of antibodies or TKI which were designed to target and influence tumor-specific processes. The best known example of cardiotoxicity induced by 'targeted' drugs is offered by the clinical experience with Trastuzumab (Herceptin®), a humanized monoclonal antibody directed against the ERBB2 (HER2) receptor that is overexpressed in approximately 25% of human breast cancers. The pivotal trial of Trastuzumab in women with metastatic breast cancer clearly showed that combining Trastuzumab with DOX-cyclophosphamide caused severe symptomatic CHF in an unacceptably high number of patients, even though the vast majority of such patients had received a reportedly safe cumulative dose of the anthracycline [83]. Because Trastuzumab had safely passed preclinical screens of cardiovascular liability, the higher than expected cardiotoxicity of combining Trastuzumab with safe doses of DOX raised novel issues in the field of anticancer drug development: what can be done

to predict cardiotoxicity from 'magic bullets' that were purportedly designed to spare cardiac cell constituents and functions?

7.4.1 Trastuzumab: How Could It Pass Preclinical Screens of Cardiovascular Liability?

The preclinical and clinical development of Trastuzumab relied on the use of animal models. On the one hand, the pivotal role of HER2 in neoplastic transformation, growth, and metastatization, was characterized by means of rats that developed carcinogen-induced brain tumors or transgenic mice that developed breast tumors due to the overexpression of HER2; next, a murine subrenal capsule fresh human tumor explant assay was utilized to probe murine monoclonal anti-HER2 antibodies. Such a laborious screening paved the road to engineering humanized anti-HER2 monoclonal antibodies and testing its efficacy, dose–response relationships, and interactions with chemotherapeutics [84].

This was the brilliant story of Trastuzumab, a blockbuster that changed the life expectancy of countless women with HER2 positive breast tumors. Regrettably, however, none of such thoughtful preclinical trials detected excess cardiac toxicity that could be attributed to Trastuzumab or its combination with chemotherapeutics [85]. How could this be? The answer is disappointingly obvious: the heart does express significant levels of HER2 but Trastuzumab does not recognize the ectodomain of HER2 which is expressed in murine heart; hence, Trastuzumab did not cause cardiotoxicity in rats or mice [84]. Trastuzumab does bind to primate HER2, but the preclinical toxicology studies were done with a few healthy primates that had not been treated with anthracyclines or other chemotherapeutics.

Studies conducted across the pivotal clinical trial (prior to, during, or after it) uncovered that HER2 and its neuregulin-1-dependent dimerization with the companion receptor, HER4, governed cardiac development and function: thus, germline deletion of either one of these three players (HER2, HER4, or neuregulin-1) was found to disrupt the embryonic development of the heart, while the cardiac-specific deletion of HER2 late in development not only caused post-natal dilated cardiomyopathy but reduced cardiac resistance to anthracyclines or other stressor agents [86–89]. This latter finding shed light on Trastuzumab pathobiology and suggested that Trastuzumab might eliminate survival pathways that help the adult heart to withstand a variety of stimuli. Clinical experience

confirmed that this was the case. In women with breast cancer Trastuzumab alone caused a contractile dysfunction that developed dose-independently, showed reversibility upon medication or Trastuzumab withdrawal, did not relapse upon rechallenge, and only occasionally induced ultrastuctural damage at endomyocardial biopsies [90,91]. The characteristics of Trastuzumab-related cardiotoxicity therefore proved to be opposite to those of DOX, and this concept formed the basis to distinguish the cardiotoxicity of anthracyclines (type I) from that of Trastuzumab (type II) (see also Chapter 6).

In the light of the 'mild' cardiotoxic profile of Trastuzumab, how can this be reconciled with its strong aggravation of anthracycline-related cardiotoxicity? The current thinking is that HER2–HER4 heterodimerization and receptor phosphorylation activate cardiac signal pathways (Grb2, ras, Raf, MAPK, P13K, Akt) that modulate gene expression, cell growth, glucose uptake, and turnover of sarcomeric proteins [69]. Thus, the cardiotoxic synergism of Trastuzumab with anthracyclines can be explained by the two-hit mechanism: anthracyclines cause damage, while Trastuzumab abolishes signals that help the heart to survive and repair such damage.

7.4.2 Trastuzumab Cardiotoxicity: A Matter of Two Hits or Multiple Hits?

Several clinical studies lend support to the aforesaid concept of 'two-hit damage'; in fact, the cardiac toxicity of anthracycline–Trastuzumab regimens could be prevented by (i) replacing free DOX with liposomal DOX, which is small enough to cross the discontinuum endothelium of tumors but is too large to cross the endothelium of coronary vessels [92]; (ii) replacing DOX with EPI, which causes less cardiac toxicity because of its defective conversion to EPIOL or ROS [93]; (iii) giving anthracyclines first and Trastuzumab second, with the safest results occurring when the two agents were separated by a washout of ~90 days [94].

Regrettably, not all that gleams is gold. As also discussed elsewhere in this book (see Chapter 5) a review of the major trials that adopted anthracyclines and Trastuzumab in a sequential manner shows that a disappointing incidence of serious/symptomatic CHF may still occur [95]. Moreover, there is growing evidence that, in the general population, the cardiotoxicity of Trastuzumab may be higher than was seen with the well-selected patients in clinical trials [96]. It therefore seems that a

constellation of individual risk factors (comorbidities, borderline cardiac parameters, etc.) might prime Trastuzumab to cause more cardiotoxicity. Along this line of reasoning it is worth noting that neuregulin-1 was recently shown to counteract adrenergic stimuli and to facilitate an obligatory interaction of cardiomyocytes with the muscarinic cholinergic system, diminishing mechanical stress to the contractile apparatus [97]. This finding anticipates that blocking the neuregulin/HER2-HER4 machinery with Trastuzumab might precipitate cardiac events in a number of patients presenting with clinical or subclinical evidence of activation of adrenergic stimuli (hypertension, hyperthyroidism, etc.). Finally, studies of a frequent HER2 gene polymorphism (Ile655Val) suggest that the presence of a Val allele significantly increases the risk of cardiac events while not having clinically detectable impact on tumor response and survival [98]. The benefit : risk ratio of Trastuzumab could therefore be less benign in patients with the Val allele. This said, should we stay with the two-hit hypothesis or should we move to embrace the more canonical multiple-hit hypothesis?

7.4.3 Do We Have a Model to Probe Trastuzumab in a Multiple-Hit Scenario?

We do not know much about the long-term cardiac outcome of patients exposed to Trastuzumab. Cardiac safety assessments of sequential anthracycline–Trastuzumab regimens are confined to data from one or two years of follow-up. Given that long-term Trastuzumab therapy offers excellent improvements in disease-free survival and life expectancy, should we prepare to see delayed cardiac events that denote a Trastuzumab blockade of defense mechanisms in patients with comorbidities, adrenergic activation, or polymorphisms?

Putting Trastuzumab in a multiple hit scenario calls for proper investigations in the next few years. Preclinical models of aging, polymorphism, and comorbidities (with or without concomitant moderate cardiac dysfunction) would be much needed to better decipher the beefit : risk ratio of Trastuzumab in the general population. Murine or primate models would be useless or too laborious and demanding, respectively. Alternative approaches might rely on anti-HER2 antibodies that cross-react with the murine protein (possibly at the same juxtamembrane epitope that is recognized by Trastuzumab). Doing this in the SHR would be magnificent but we are unaware of any such ongoing approach.

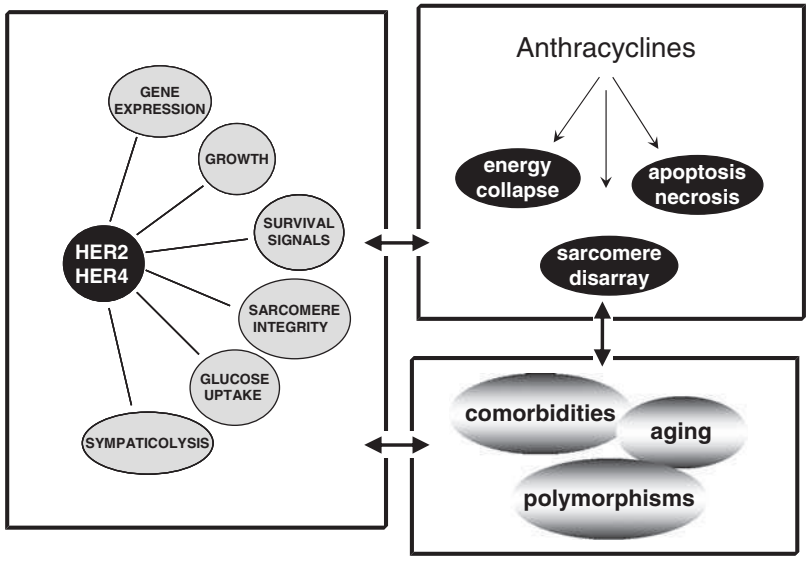

Figure 7.8 Trastuzumab and anthracyclines in the multiple-hit scenario. Proposal for animal models that probe the salvage pathway sustained by Trastuzumab against anthracyclines and the influence of cofactors such as aging, comorbidities, polymorphisms

Figure 7.8 shows our personal view of how the survival/repair signals sustained by HER2 should be probed in a multiple-hit scenario against anthracyclines and a constellation of comorbidities, aging, and polymorphisms.

7.5 CARDIOTOXICITY FROM OTHER TARGETED DRUGS

Most of the concepts illustrated for Trastuzumab may well apply to many other targeted drugs, primarily TKI.

Lapatinib, that inhibits the kinase domain of both EGFR (HER1/ERBB1) and HER2, is suspected to cause contractile dysfunction and QT prolongation; hence, lapatinib has been labelled to be avoided in patients with borderline myocardial function, other risk factors, previous or concurrent exposure to cardiotoxic chemotherapeutics [99]. Nonetheless, two Phase I studies of heavily pretreated patients with advanced malignancies failed to document symptomatic CHF or other serious

cardiac events, even though the entry criteria were quite permissive in terms of cardiac function (LVEF $\geq$40%) and separation between chemotherapy and lapatinib (which could be as short as 4–6 weeks). A similar safety profile was demonstrated in a Phase III study that probed lapatinib and capecitabine versus capecitabine alone in women with HER2-positive breast cancer that had progressed after anthracyclines, taxanes, Trastuzumab [100]. Thus, there is some controversy about whether lapatinib should be considered less cardiotoxic than Trastuzumab.

Surprisingly, very few studies challenged lapatinib and anthracyclines by a straightforward approach. Limited studies show that the lapatinib analog GW2974, but not Trastuzumab, activates a metabolic stress response that protects cardiomyocytes against tumor necrosis factor α (TNFα)-induced cell death [101]. Generalizations should nonetheless be avoided since the cardiac stress induced by TNF α cannot be compared to that induced by anthracyclines. Short- or long- term cardiac tolerability of lapatinib should be probed in the context of experimental therapies that adopted concomitant or sequential administration of anthracylines and lapatinib, possibly in SHR or integrated models that incorporated the weight of comorbidities. We are unaware of any such study that addressed these issues, even though a TKI like lapatinib poses many fewer technical difficulties than does a species-specific antibody like Trastuzumab.

Other TKI that call for a deeper reappraisal in experimental models include sunitinib and sorafenib, inhibitors of the kinase domain of the receptor for vascular endothelial growth factor. Both are plagued by cardiovascular effects ranging from cardiomyopathy and hypertension to QT prolongation; sorafenib also causes disthyroidism that aggravates cardiotoxicity. Sunitinib and sorafenib are said to be 'specific' in their mechanisms of action, but a more prudent analysis shows that at therapeutic plasma levels sunitinib probably inhibits some 90 kinases; inhibition of which kinase, or even which combination of kinases, is responsible for cardiovascular side effects may be very difficult to ever definitively identify [102]. Perhaps more importantly, the registratory trials of sunitinib and sorafenib reported cardiotoxicity in approximately 10% of the patients but more recent observational studies of the general population show that cardiac events may occur in as many as $\sim$40% of patients [103]. Thus, cardiovascular toxicity from sunitinib and sorafenib seems to have been largely underestimated, presumably because of a limited appreciation of the concurring weight of risk factors. Reassessing either TKI in models like those sketched in Figures 7.6–7.8

would be very appropriate for redefining the prospective toxicity of these TKI in a general population.

Other TKI that call for more suitable preclinical models of cardiac toxicity include imatinib (Gleevec®) and back-up cogeners like nilotinib and dasatinib, all of them inhibiting the unphosphorylated kinase domain of the BCR-ABL fusion protein encoded by the Philadelphia chromosome (Ph) of leukaemic cells. The tremendous impact that these TKI had on the curability of chronic myeloid leukemia was counterbalanced by concerns on the possible incidence of CHF in a significant number of patients [104–106]. The actual incidence and molecular mechanisms of cardiotoxicity from ABL kinase inhibitors are under scrutiny. The ABL kinase usually mediates apoptosis and, hence, one would not expect cardiotoxicity from ABL kinase inhibitors [106]. Moreover, some believe that the incidence of CHF in patients taking imatinib might have been overestimated, while others believe that the discrepancies could be explained by the methodological approaches adopted in the reporting of CHF (low rates of CHF in retrospective reports versus high rates in prospective evaluation) [106]. This latter observation indicates that the actual incidence of cardiotoxicity from such drugs may depend on cofactors that need evaluation in animal models of multiple hits.

7.6 CONCLUSIONS

Large pharmaceutical companies are developing new chemotherapeutics, new selective TKI, new multikinase or pankinase inhibitors. The experience gained with the available prototypes cautions that cardiotoxicity might complicate the clinical use of forthcoming new chemical entities. There is an unmet need for preclinical screens of cardiotoxicity that help to prevent undesired new paradigms of cardiotoxicity or even the loss of human lives.

The translational model of human heart will remain of great value in deciphering the safety or toxicity of drugs that undergo bioactivation by species-related enzymes or multienzymatic systems. Animal models of multiple hits will also need to be brought center stage. Whether such models are possible is uncertain at this time, but their conceptual foundations look robust and well justified. In a broader perspective, concerns about the validity of preclinical models should be extended to questioning the entry criteria in registratory clinical trials, which too often exclude patients carrying those risk factors or comorbidities that eventually determine the incidence of cardiac events in the general population.

Chemistry and drug design are proceeding faster than toxicology. The safety of new drugs and the quality of life of cancer patients will depend on our ability to make preclinical toxicology run shoulder to shoulder with the new drugs.

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8

NSAID Action and the Foundations for Cardiovascular Toxicity

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8.1 INTRODUCTION

The rapid development, marketing and ultimate reassessment for the uses of COX-2 selective inhibitors in humans has underscored the need to fully understand the physiological and pathophysiological roles of a desired molecular target and to identify the patient population that will benefit from the drug. COXIBs were developed to ease the burden of a sizeable population of NSAID-sensitive patients who could not tolerate the gastrointestinal toxicity associated with dual inhibition of COX-1 and COX-2 in the GI tract. Due to its involvement in inflammation and disease progression, COX-2 was viewed as the 'bad' COX isoform and this relatively broad assumption was inevitably short-sighted as COX-2 has now been shown to have physiological roles in the cardiovascular, renal, and central nervous systems. Resultant cardiovascular side effects in patients taking COX-2 inhibitors for long periods of time led to the withdrawal of all but one COXIB from the US market and a reassessment of the use of these drugs.

Cardiotoxicity of Non-Cardiovascular Drugs Edited by Giorgio Minotti

In researching the mechanism-based causality between COX-2 inhibition and cardiovascular risk, researchers have learned a great deal about COX-2 and the contribution of its prostaglandin products (e.g. PGI_2) in normal biology and also the pathophysiological responses of the microvasculature to inhibition of this enzyme and suppression of its resultant prostaglandin products. As researchers begin to understand the mechanisms behind COX-inhibition-associated toxicities in the cardiovascular/renal, neurological and gastrointestinal systems, we will continue to see a critical discussion about the balance between efficacy and safety and between benefit and risk. This chapter will attempt to summarize the clinical history of NSAIDs and COXIBs and the molecular foundations of their cardiovascular toxicity.

8.2 HISTORY OF NSAIDs

Non-steroidal anti-inflammatory drugs (NSAIDs) and their COX-2 selective counterparts (the COXIBs) have a long history that dates back to ancient civilizations. The isolation, synthesis and development of the first known NSAIDs is largely a result of a combination of natural product isolation, medicinal chemistry, the progressive development of animal models of *in vivo* pharmacology and efficacy, and serendipity [1]. Prior to the development of animal models of inflammation, compounds were tested directly on human subjects with the resultant benefits and side effects being largely unknown until use. Salicylic acid (and aspirin), phenacetin and phenylbutazone (Figure 8.1) were members of the first three classes of traditional NSAIDs discovered and marketed for human consumption. Following World War II, the use of *in vivo* inflammatory animal models led to the rapid development and marketing of other 'traditional' NSAIDs, including the oxicams, fenamates, profens, indomethacin, and arylacetic acids like diclofenac. Subsequent to their marketing, concurrent studies by Vane and Smith and Willis established, in 1971, that NSAIDs inhibited an enzyme that synthesized prostaglandins from polyunsaturated fatty acids [2,3]. The identification of a plausible molecular target for the action of NSAIDs led to a flurry of literature on the precise molecular and kinetic determinants for the inhibition of this cyclooxygenase (COX) enzyme by the wide spectrum of available NSAIDs.

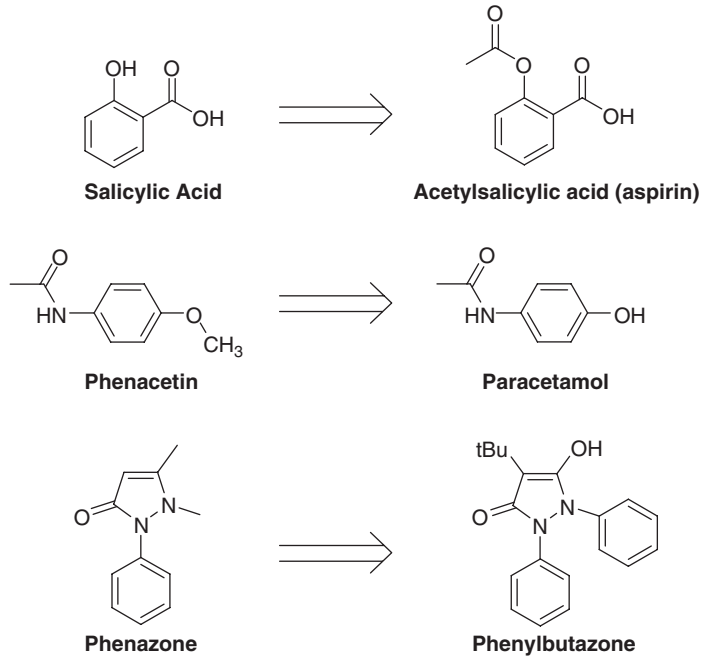

Figure 8.1 Evolution of the first three classes of traditional NSAIDs

8.3 COX AND NSAID ACTION

Prostaglandin H synthase, or COX, is a homodimeric protein that is responsible for the *bis*-dioxygenation of arachidonic acid to prostaglandin H_2 (PGH_2). COX enzymes have two spatially distinct but mechanistically coupled active sites – the cyclooxygenase active site that binds both substrates and inhibitors (NSAIDs or COXIBs), and the peroxidase active site that contains the heme moiety and serves as the initiation site in the COX reaction (Figure 8.2A) [4,5]. As arachidonic acid and NSAIDs share the same active site, binding of an inhibitor will preclude substrate binding and prevent oxygenation of arachidonic acid. The majority of NSAIDs and COXIBs are non-covalent inhibitors and exhibit what would be referred to as competitive inhibition, although many inhibitors in both classes can exhibit binding to the enzyme that is so tight that it appears functionally irreversible [6, 7]. The product of substrate oxygenation, PGH_2, is converted by multiple isomerases or a reductase to one of five potential prostaglandin products: PGE_2, PGD_2, $PGF_{2\alpha}$, PGI_2, and TxA_2 (Figure 8.2B) [8]. These products target a family of G-protein-coupled

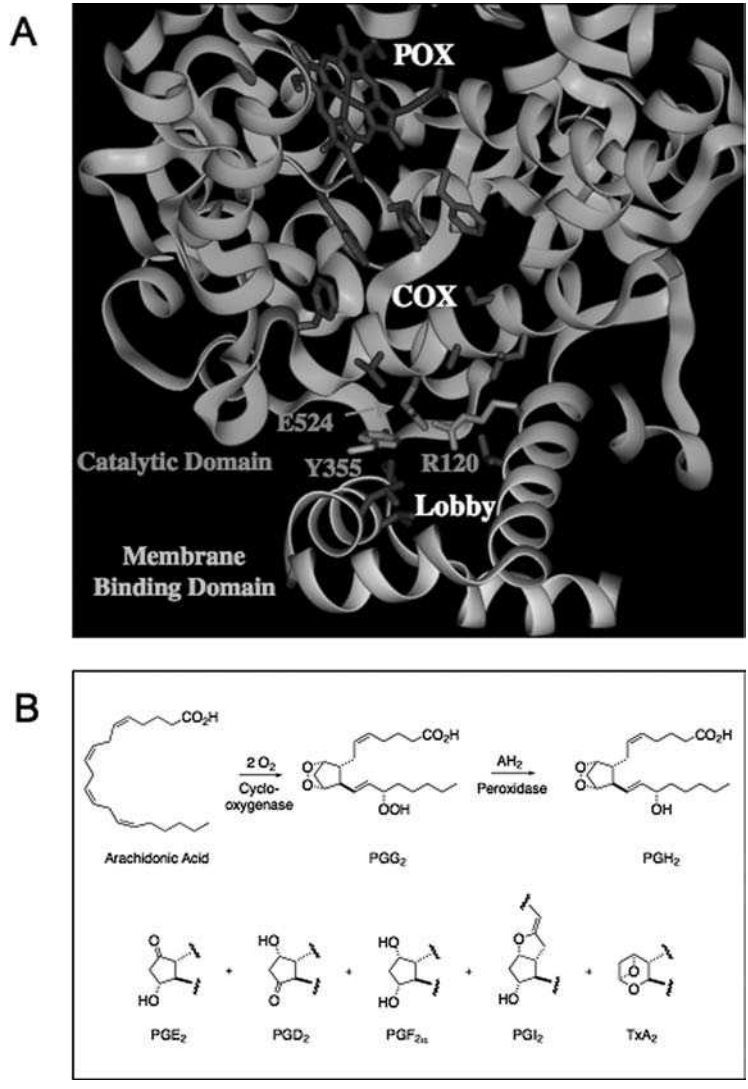

Figure 8.2 COX structure and reaction mechanisms. (A) The cyclooxygenase monomer is depicted with the membrane-binding domain in yellow and the catalytic domain in green. Residues that line the cyclooxygenase (COX) active site are shown in purple with the constriction site residues that separate the active site from the lobby shown in gray with labels. Residues in the lobby are shown in red. The peroxidase active site (POX) is identified by the presence of the heme. (B) The two sequential reactions of the cyclooxygenase enzymes are shown with the ultimate products of PGH_2 metabolism (see Color Plate 3)

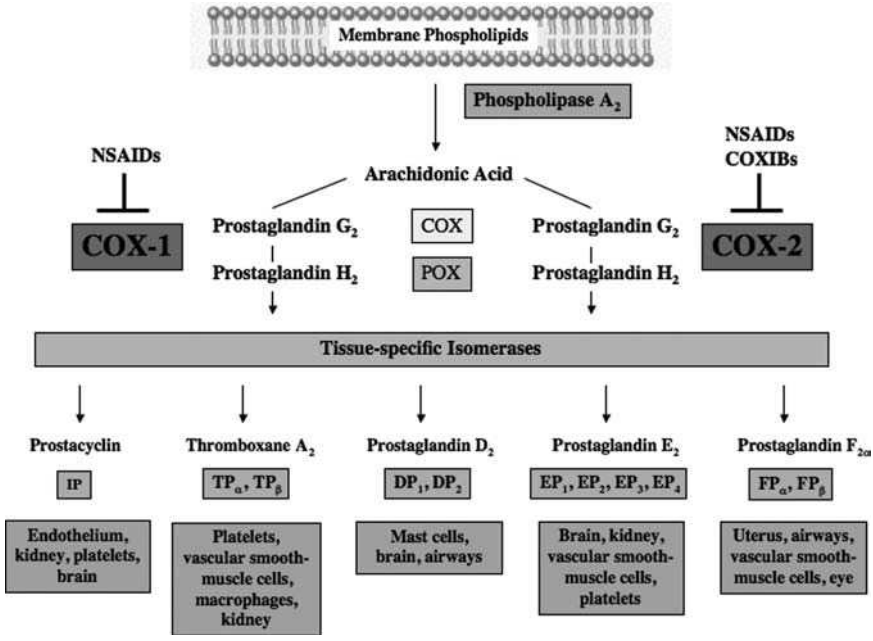

Figure 8.3 The arachidonic acid (AA) cascade beginning from liberation of AA from the membrane by phospholipase A_2 and ending with the action of each prostaglandin at specific G-protein coupled receptors in cells and tissues to elicit physiological or pathophysiological effects. Figure adapted from FitzGerald and Patrono [62]. Copyright 2001, Massachusetts Medical Society

receptors that trigger a variety of physiological and pathophysiological responses (Figure 8.3) [9]. Cells and tissues are fairly distinct in the prostaglandins that they produce – for example, the vascular endothelium makes PGI_2 and PGE_2, whereas platelets primarily make TxA_2. The unique tissue distribution of PGs and their receptors has helped to elucidate the role that each plays in normal/basal cell functions and in inflammatory or diseased states.

Although NSAIDs had long been used to treat inflammation, the discovery that COX was the molecular target for NSAID action and that the inhibition of COX results in a decrease in prostaglandin production underlined a mechanism for the action of NSAIDs and strengthened the rationale for their use as anti-inflammatory agents. However, in addition to their beneficial analgesic and anti-inflammatory effects, a wide range of side effects became associated with the long-term use of NSAIDs. These included liver and kidney toxicity, central nervous system toxicity and the formation of gastric ulcers and extensive GI toxicity and bleeding. Some of these side effects (GI toxicity) have been directly linked to

COX inhibition while others are off-target effects. The concentrations of drug that are necessary to engage these off-target non-COX effects are frequently higher than those that inhibit COX activity; therefore, their utility is primarily restricted by the mechanism-based, COX-dependent side effects. In addition, there is great interindividual variation with regard to both the efficacy of the drug and the side effect profile and tolerance with different NSAIDs [10]. A sizeable population exists that is so sensitive to the GI toxicity of NSAIDs that they are unable to take this class of drug at all and represent a target group for which a new, GI safe anti-inflammatory drug would be extremely beneficial.

8.4 THE DISCOVERY OF COX-2 AND THE DEVELOPMENT OF COXIBS

The 'original' COX (COX-1) was purified in 1976 from bull seminal vesicles [11] and was shown to be widely distributed in different mammalian tissues. The discovery of a second cyclooxygenase isoform (COX-2) [12,13] that demonstrated differential gene regulation and protein expression as well as a unique tissue distribution was the basis for what was called the 'COX-2 hypothesis' – that is, that this new enzyme, whose protein expression was induced by cytokines, growth factors, mitogens, and so on, provided a molecular target for compounds with analgesic and anti-inflammatory activity, but reduced GI toxicity [14]. The widely popular COX-2 hypothesis assumed that the COX-2 isoform only played a role in the pathophysiology of inflammation and certain disease states and that it did not have a role in normal physiology. In addition, the hypothesis was also based on the notion that all prostaglandins functioning in a cytoprotective role in the GI tract are specifically derived from the 'constitutively' expressed COX-1 isoform. Although initially supported by *in vitro* data and some *in vivo* animal models of inflammation (rat air pouch and foot pad) [14], the assumptions of the COX-2 hypothesis would prove short-sighted in the long-term. In fact, key inconsistencies with the COX-2 hypothesis existed prior to the launch of several human clinical trials that may have predicted the ultimate problems associated with this theory. For example, basal expression of COX-2 can be detected in the hippocampus, vascular endothelium and kidney, suggesting potential physiological roles for this enzyme in these areas. Interestingly, neither COX-1 or COX-2 knockout mice are resistant to inflammation and COX-1 null mice do not develop spontaneous gastrointestinal lesions

[15–17]. Finally, inhibitors that are selective for the COX-1 enzyme do not lead to GI ulcers or toxicity on their own, but will induce GI lesions when combined with a COX-2 selective inhibitor [18, 19]. Many of these data were not published until after the drug discovery efforts in the COX-2 field were begun, but represent insights into the underlying problems with the COX-2 hypothesis.

The identification of a COX-2 protein that had 60% overall sequence similarity to COX-1 and over 85% active site similarity, led to the rapid discovery, optimization, commercial development and eventual success of the COX-2 selective inhibitors (known collectively as the COXIBs) through multiple efforts in medicinal chemistry and pharmacology within the pharmaceutical industry. The existence of multiple NSAID scaffolds that dually inhibited both COX enzymes provided an initial starting point for the synthesis and screening of potential COX-2 selective inhibitors. The most successful scaffold that eventually led to the development and marketing of celecoxib (Celebrex) and rofecoxib (Vioxx) was the diarylheterocycle skeleton derived from Dup697, originally conceived from the phenylbutazone lineage (Figure 8.4). When Dup697 was first reported in the literature nearly 20 years ago, it was shown to inhibit prostaglandin production in macrophages but not in platelets, and to exhibit reduced GI toxicity in rodent models – confusing data that upon the discovery of COX-2 could be well-explained [20]. Extensive structure–activity analyses of the Dup697 framework distinguished the key determinants that resulted in COX-2 selectivity – two aromatic rings that were attached to either a carbocycle or a heterocycle and a para-substituted methylsulfone or sulfonamide on one of the rings [21–23]. These diarylheterocycles were able to inhibit COX-2 selectively by exploiting a pocket in the active site of the enzyme that was partially blocked in COX-1 due to a Val-523 to Ile-523 amino acid substitution (Figure 8.5) [24, 25]. Importantly, the selectivity of these inhibitors also derives from their kinetic behavior as slow, time-dependent and functionally irreversible inhibitors of COX-2, but not COX-1 [26–28]. Although multiple NSAID scaffolds were explored for the purposes of making novel COX-2 selective inhibitors [29], the only non-diarylheterocycle scaffold that resulted in a successfully marketed drug was lumiracoxib, an arylacetic acid derived from the non-selective COX inhibitor diclofenac, that had an entirely unique inhibitory and kinetic binding mode [30]. With a molecular basis for selectivity in hand and supportive cellular and animal data, the diarylheterocycles were advanced through short-term and long-term clinical trials to establish their potency and efficacy.

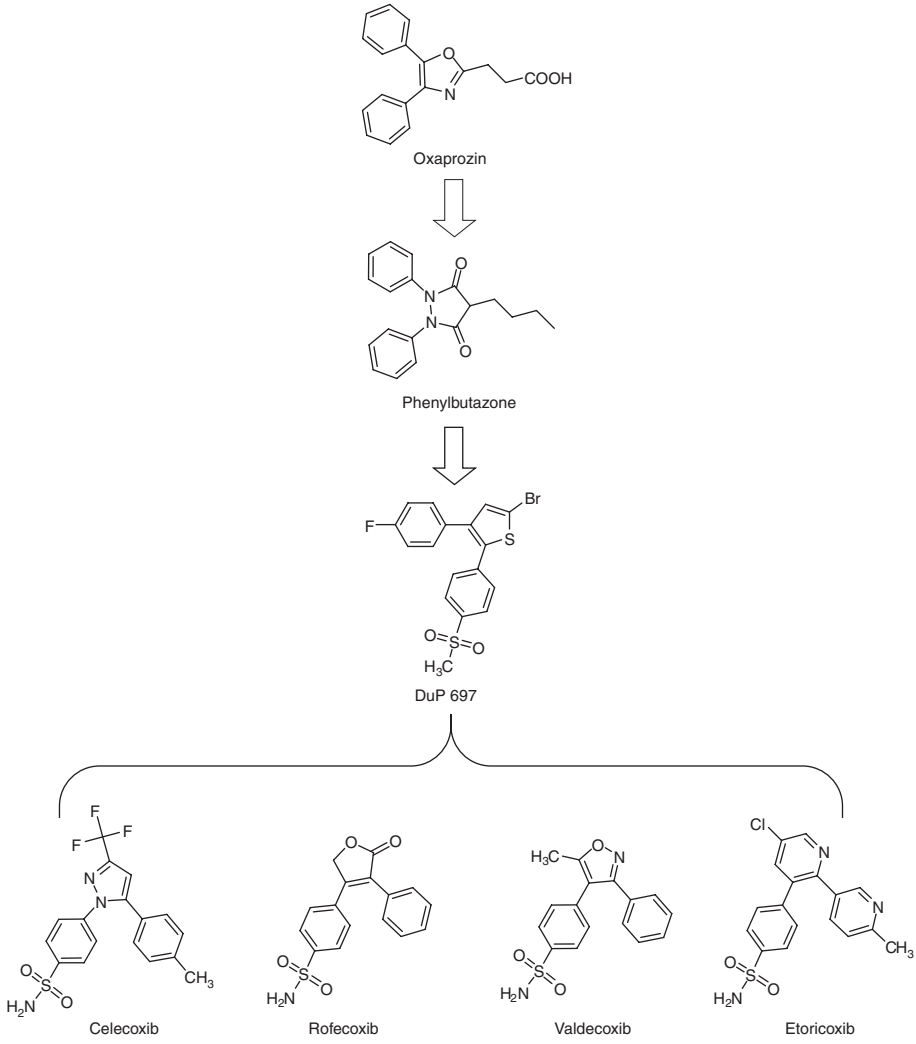

Figure 8.4 Evolution of the COX-2 selective diarylheterocycle inhibitor class from oxaprozin, phenylbutazone, and DuP 697

8.5 COX-2 AND ARTHRITIS TRIALS

Two 12 month-long arthritis trials in humans (the VIGOR and CLASS trials) were conducted to test the COX-2 hypothesis with Vioxx (rofecoxib) [31] and Celebrex (celecoxib) [32] in sufferers of rheumatoid arthritis (RA) and osteoarthritis (OA), respectively. In each of the trials, the dose of the

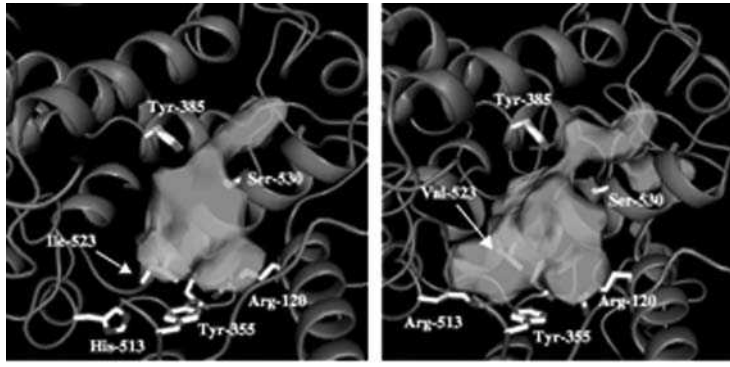

Figure 8.5 The solvent accessible space in the active sites of COX-1 and COX-2. A single amino acid substitution in the primary shell (Val-523 in COX-2 and Ile-523 in COX-1) along with several changes in the secondary shell allow for a larger active site in COX-2 and the existence of a "side pocket" where the diarylheterocycle COX-2 selective inhibitors bind [6]. Reprinted with permission, copyright 2007 The American Chemical Society (see Color Plate 4)

COX-2 selective inhibitor was two to four times higher than the dose recommended for anti-inflammatory activity and each inhibitor was evaluated against a comparator NSAID and not a placebo (i.e. naproxen for VIGOR; diclofenac and ibuprofen for CLASS), typical of clinical trials in arthritis. Each of the trials was designed to compare the anti-inflammatory activities of the COX-2 selective inhibitors with their ability to cause significantly less gastrointestinal toxicity than a traditional NSAID.

The basic tenets of the COX-2 hypothesis were supported in both the preliminary and long-term results of the VIGOR trial. Vioxx proved to be a successful anti-inflammatory agent in patients with rheumatoid arthritis and resulted in a significantly lower incidence of gastrointestinal ulceration compared with its traditional NSAID comparator, naproxen (Figure 8.6). Interestingly, a surprising result from the 12 month-long VIGOR trial was that treatment with naproxen resulted in a *lower* number of cardiovascular events than Vioxx (0.1% versus 0.4%, relative risk of 0.25) [31]. At the time, the study authors attributed this result to a cardiovascular protective effect of naproxen (akin to aspirin) and its ability to reduce TxA_2 synthesis in platelets and the associated platelet aggregation [31,33]. Preliminary data (at 6 months) from patients with osteoarthritis in the CLASS trial supported the use of Celebrex as an anti-inflammatory agent with a superior GI safety profile against comparator NSAIDs. However, at the 12-month conclusion of the study, there was no

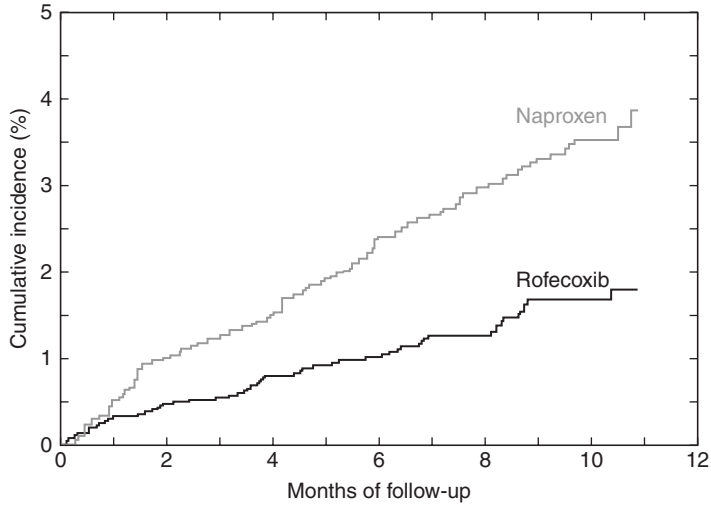

Figure 8.6 Results from the VIGOR study with naproxen and rofecoxib. Shown is the cumulative incidence of gastrointestinal lesions induced by either the traditional NSAID (naproxen) or the COX-2 selective inhibitor (rofecoxib) over a 12-month period [93]

significant difference in the incidence of gastroduodenal ulcers between Celebrex and the traditional NSAID diclofenac (Figure 8.7), suggesting that there was no GI benefit to the long-term use of this particular COX-2 selective inhibitor relative to diclofenac. In addition, no differences were observed between Celebrex and ibuprofen or diclofenac with regards to cardiovascular events [32], unlike what was observed in the VIGOR trial.

The results of the VIGOR and CLASS trials were interpreted to validate the COX-2 hypothesis and led to the approval of these drugs as anti-inflammatory agents and, in the case of rofecoxib, as an analgesic agent. Vioxx and Celebrex immediately became blockbuster drugs. Although they were intended for a target population of NSAID-intolerant patients, direct-to-consumer marketing allowed these inhibitors to spread into the general population where there really was no significant benefit to those patients who were not NSAID-sensitive. Postmarketing population-based studies examined the actual benefit of COXIBs relative to traditional NSAIDs in the realm of ulcer incidence and gastrointestinal side effects and found that chronic use of the COXIBs is actually associated with increases in GI lesions (albeit lower than comparable NSAIDs) [34,35]. In fact, studies in rats have indicated that it is the total reduction of PG biosynthesis that leads to gastrointestinal toxicity and not necessarily the selective inhibition of either COX-1 or COX-2 [18].

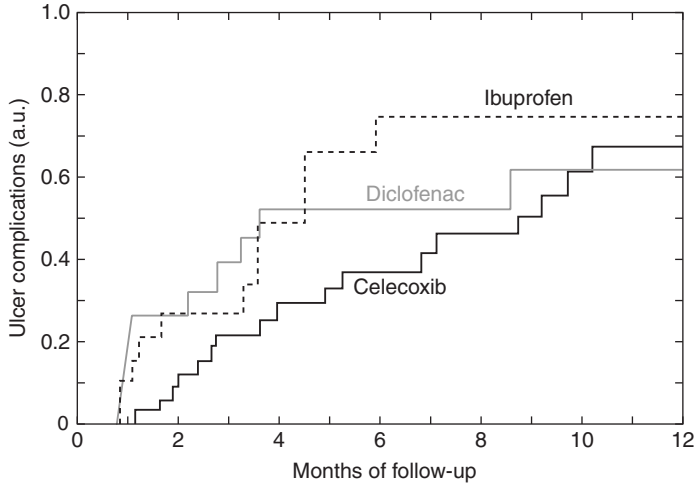

Figure 8.7 Results from the CLASS study comparing celecoxib with ibuprofen and diclofenac. Shown is the incidence of ulcer complications induced by the traditional NSAIDs (ibuprofen and diclofenac) or the COX-2 selective inhibitor (celecoxib) over a 12-month period [93]

8.6 COX-2 AND CANCER, COLON POLYP RECURRENCE TRIALS

The importance of COX-derived prostaglandins in the development and progression of a variety of cancers has been recognized for some time [36], with an important contributor being PGE_2. Multiple mechanisms exist for the role COX plays in tumorigenesis, including enhancing tumor promotion and metastasis, resistance to apoptosis, and angiogenesis. A strategy for cancer prevention via COX inhibition was first proposed based on the discovery that individuals who regularly take aspirin exhibit lower mortality from colon cancer [37]. With the knowledge that COX-2 is highly expressed in benign polyps and human colon cancers, but not normal tissue, COXIBs were tested in several animal models of tumorigenesis with great success. Further, the idea was proposed that the COXIBs could be chemopreventive agents in the progression of cancers, specifically colon cancer. Several human clinical trials were designed to test this hypothesis in patients at elevated risk for the development and progression of colon cancer. Three parallel long-term colon polyp recurrence trials were conducted over a 36-month period in which individuals who had a benign polyp removed following colonoscopy were administered a COXIB. Each trial was designed with a placebo arm and only

varied in the identity of the COXIB and the dose(s) of the drug – APPROVE (Adenomatous Polyp Prevention on Vioxx), APC (Adenoma Prevention with Celecoxib) and PreSAP (Prevention of Colorectal Sporadic Adenomatous Polyps).

In the APPROVE trial, over 2500 patients of both sexes aged 40 and above were administered a once daily dose of rofecoxib (25 mg) following pre-study polyp removal [38]. Patients were randomized to a placebo control group and were excluded based on perceived cardiovascular risk factors such as angina and hypertension. In support of the involvement of COX-2 in the progression of colon cancer, the rofecoxib-treated group exhibited a 24% reduction in the recurrence of colon polyps compared to placebo near the end of the three year study [39]. However, this same group also demonstrated an almost two-fold increase in the occurrence of cardiovascular events such as myocardial infarction and stroke (rofecoxib 1.50 events versus placebo 0.78 events per 100 patients). Interestingly, there was no difference in the occurrence of cardiovascular events in the rofecoxib- and placebo-treated groups up to the first 18 months of the study, but then a clear and statistically significant divergence emerged for the remainder of the trial (Figure 8.8A). The correlation between the long-term use of rofecoxib and the occurrence of cardiovascular events (heart attack and stroke) was clear and supported earlier data from the VIGOR trial, leading to the early termination of the APPROVE trial and the removal of Vioxx from the market by Merck.

The APC trial enrolled just over 2000 patients and randomized them into three treatment groups, including a placebo arm and two different doses of twice-daily celecoxib (200 and 400 mg) [40]. The ability of celecoxib to act as a chemopreventive agent in the recurrence of colon polyp formation was substantial and greater than that exhibited by rofecoxib in the APPROVE trial. At the three year conclusion of the trial, the percentages of patients presenting with an adenoma were 37.5% in the high-dose group, 43.2% in the low-dose group, and 60.7% in the placebo group [41]. Further, celecoxib at the 400 mg/2x daily dose was able to reduce colon polyp recurrence by 67% in patients with advanced adenomas (polyps >1 cm). The impact of celecoxib in cancer chemoprevention was diminished due to the dose-responsive increases in cardiovascular events between groups that was also observed over the three year trial period. Cardiovascular events increased 2.6-fold and 3.4-fold in the low and high dose groups, respectively (Figure 8.8B); values that were comparable to the results with rofecoxib in the APPROVE trial. Consistent with the results of the APC trial, the PreSAP trial also demonstrated celecoxib's effectiveness at reducing colon polyp formation (by 36% at 400 mg/1x and

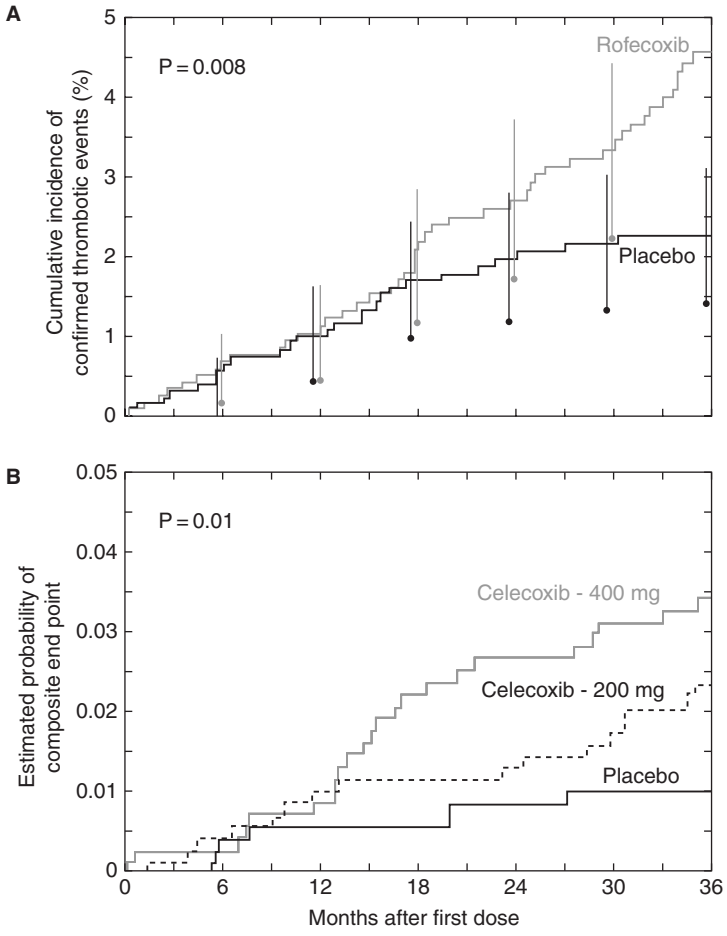

Figure 8.8 Results from the APPROVE and APC studies with rofecoxib and celecoxib. Shown is the cumulative incidence of cardiovascular events induced by rofecoxib in the APPROVE trial (A) or celecoxib in the APC trial (B) over a 36-month period [93]

by 51% in the advanced adenoma patients) [42]. However, this benefit also came with the development of cardiovascular events in the celecoxib arm of the trial relative to the placebo (1.3-fold increase).

The dose–response data from each of the three polyp recurrence trials supports a role for COX-2 in colon cancer development and progression and validates this enzyme as a molecular target for chemoprevention of colonic neoplasia. At the same time, these trials revealed a dose-responsive induction of cardiovascular events with prolonged administration of

the COX-2 inhibitor, independent of the COXIB structure. This suggested that the cardiovascular events were dependent on inhibition of the COX-2 enzyme and were, therefore, mechanism-based and represented a class effect. Support for this hypothesis was garnered from two brief trials that investigated valdecoxib/parecoxib for the relief of post-surgical pain resulting from coronary artery bypass graft surgery (CABG) and from the Multinational Etoricoxib and Diclofenac Arthritis Long-term (MEDAL) trial. In the CABG patients, valdecoxib (with pretreatment of its injectable prodrug, parecoxib) increased the number of cardiovascular events after only 10–14 days compared with the placebo [43,44]. It was obvious from these data that inhibition of COX-2 was causal in this rapid and three-fold increase in cardiovascular events with the drug-treated arm of the study. Comparisons of the cardiovascular outcomes with etoricoxib and diclofenac (Figure 8.9) in patients with osteoarthritis and rheumatoid arthritis confirm the observation that inhibition of COX-2 and the resulting cardiovascular toxicities occur irrespective of COX inhibitor structure [45]; etoricoxib is a diarylheterocycle whereas diclofenac is an arylacetic acid.

An important question that emerged from the clinical trials examining COX-2 selective inhibitors is why the magnitude of the cardiovascular events required more than 18 months to achieve a statistically different result from the placebo arm. It is possible that it is related to the magnitude of the actual cardiovascular challenge in the patient. For example, individuals with a history of, or genetic pre-disposition to,

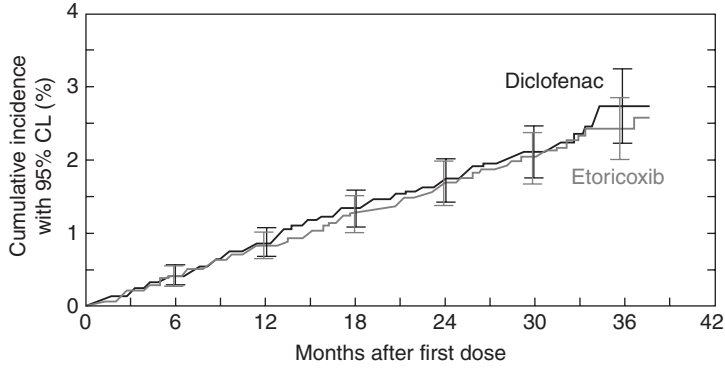

Figure 8.9 Results from the MEDAL study with diclofenac and etoricoxib. Shown is the cumulative incidence of cardiovascular events (myocardial infarction, stroke or death) with either diclofenac or etoricoxib over a 38-month period [93]

cardiovascular disease were excluded from the APPROVE trial and the treatment group was primarily made up of healthy individuals. Evidence for this theory of cardiovascular challenge is apparent in the CABG studies with valdecoxib where the individuals receiving drug treatment following coronary artery bypass grafts exhibited a significant increase in heart attacks and strokes in only 10–14 days. Hypothetically, the lengthy delay in cardiovascular side effects in the colon polyp recurrence trials could revolve around a chronic reduction in the biosynthesis of PGI_2, which may over time predispose an individual to atherosclerosis or plaque rupture.

Another important, and perhaps related, question resulting from the polyp prevention studies is why the percentages of individuals experiencing adverse cardiovascular events with the COXIBs are relatively low (2–3% of the total number of patients taking the inhibitors over 3 years). While other factors may be at play, the answer to this low-incidence question may be a reflection of the low cardiovascular challenge in these individuals or it may be a representation of the interindividual variation in the magnitude of response to inhibition by COX-2 selective inhibitors [10].

A classic conflict currently exists in the COX-2 field between benefit and risk with regard to colon cancer chemoprevention. Because the progression of the disease is so slow and would require long-term drug dosing, and because the risk of development of and progression is not 100% in this population, many have concluded that the risk of cardiovascular side effects, like myocardial infarction and stroke, outweigh the benefit of polyp prevention. Benefit to risk assessments can be calculated for a specific subset or population of patients to reflect a different outcome. For example, the use of celecoxib or the NSAID sulindac in the treatment of individuals with familial polyposis, a genetic disorder with large tumor burden and high progression rate, would yield a different benefit to risk profile in this patient population because of the large adenoma burden and the high probability of conversion to cancer.

8.7 SPECIFIC EVIDENCE FOR COX-2 DERIVED PGI_2 SUPPRESSION AND CARDIOVASCULAR TOXICITY

A mechanism-based causality of the cardiovascular side effects (i.e. resultant from COX-2 inhibition) appeared evident from comparisons of the various GI toxicity, arthritis, polyp recurrence, and pain trials mentioned above. Various groups have attempted to identify and explain how the

inhibition of the COX-2 enzyme led directly to the development of cardiovascular problems in the short-term or long-term. There are strong data from FitzGerald and coworkers that suggest that COX-2 is inhibited in the vessel wall and results in a reduction in levels of COX-2 derived PGI_2, and to a lesser extent PGE_2, that play physiological roles in the vasculature [46]. PGI_2 plays a role in reducing the response of platelets to pro-aggregatory substances and is a potent vasodilator [47]. In hyperlipidemic mice, PGI_2 appears to reduce vascular inflammation, thrombosis and atherosclerosis [48], and inhibition of its biosynthesis by COXIBs in humans could represent a significant cardiovascular risk factor.

Substantial *in vitro* and *in vivo* data exist to support the proposal that the COXIB-dependent suppression of COX-2 derived prostacyclin (PGI_2) can act biologically to initiate and promote thrombosis and atherogenesis. FitzGerald and coworkers found that both rofecoxib and celecoxib were able to suppress, in humans, the major urinary metabolite of PGI_2 (PGIM or 2,3-dinor 6-keto $PGF_{1\alpha}$) in a manner that was comparable to traditional non-selective NSAIDs without the simultaneous inhibition of COX-1 derived platelet TxA_2 [46]. Early hypotheses saw an 'imbalance' between these two molecules as a possible trigger for the cardiovascular side effects of the COXIBs (Figure 8.10).

The notion that the COX-2 enzyme could be playing a physiological role in the vascular system was also supported by the finding that despite the presence of only COX-1 in endothelial cells *in vitro*, PGI_2 appeared to derive largely from COX-2 under physiological conditions in humans [49,50]. In fact, COX-2 was shown to be upregulated in cultured endothelial cells exposed to laminar shear flow [51]. It follows from

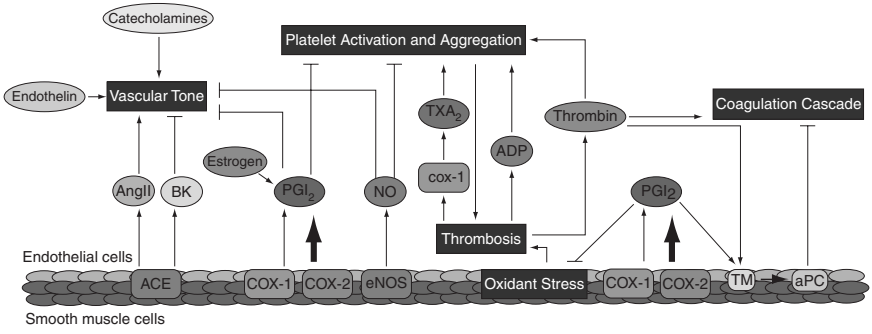

Figure 8.10 Complexities of COX-dependent control of vascular function and the influence of other proteins and signaling molecules on vascular tone, platelet activation/aggregation, thrombosis, and coagulation Figure was adapted from [65]. Copyright 2006, American Society for Clinical Investigation

these observations that COX-2 is likely induced in the vascular endothelium in humans in response to blood flow.

Animal models assessing the consequence of reduced PGI_2 levels *in vivo* were limited during the development of the COXIBs. Subsequent experiments showed that deletion of the PGI_2 receptor (IP) in mice augmented the response to an exogenous thrombogenic stimulus [52] and limited the effect of endogenous TxA_2 on vascular proliferation and the activation of platelets in response to injury [53]. Additional experiments revealed that the deletion of IP predisposed mice to thrombosis and atherosclerosis. Aside from the induction of endothelial COX-2 under physiological conditions (blood flow in the vasculature), it is also possible that inflammatory cytokines and products of activated platelets could induce COX-2 in the vessel wall. Similar events occur in atherosclerotic lesions in humans and both PGI-M (2,3-dinor 6-keto $PGF_{1\alpha}$) and the corresponding TxA_2 metabolite are increased in patients with this disease [54,55]. Recent work with COXIBs in a variety of animal models has clarified the role of COX-2 and PGI_2 in cardiovascular and renal biology.

In a rodent model under conditions of hypoxia-induced pulmonary hypertension, the selective inhibition of COX-2 suppressed PGI_2 levels and led to platelet activation and arterial thrombosis [56]. PGI_2 levels were also suppressed by a COX-2 selective inhibitor in hamster cheek pouch arterioles and led to platelet adhesion and enhanced platelet–vessel wall interactions [57]. Deletion of the IP receptor for PGI_2 removed the atheroprotective effect of estrogen in ovariectomized female mice and suggests that chronic treatment with COXIBs could undermine protection from cardiovascular disease in women [48]. Celecoxib was shown to abate the anti-thrombotic effect of aspirin in a dog model by suppressing PGI_2 in the vasculature [58]. COX-2-derived PGI_2 afforded protection against oxidant injury *in vivo* and in endothelial cells was shown to stimulate thrombomodulin [59], a restraint on thrombin activation. Taken together, these data support the hypothesis of there being one major mechanism at play leading to cardiovascular events when COXIBs are administered to humans (especially those who would be predisposed to cardiovascular risk) for the short- or long-term: suppression of COX-2 derived PGI_2 levels in the microvasculature. It is quite possible that aside from an immediate effect of PGI_2 suppression in high-risk patients taking COXIBs, treatment with COX-2 selective inhibitors and the resultant inhibition of COX-2 derived PGI_2 may predispose low-risk patients to a more gradual elevation of cardiovascular risk during long-term dosing with the inhibitor. The role

of NO and other prostaglandin products as contributing factors to COXIB-induced cardiovascular effects remains to be elucidated in full and may be considered as additional factors to be combined with the effects of PGI_2 suppression on atherogenesis and blood pressure in low- and high-risk patients.

An important consideration within the realms of this hypothesis deals with the actual selectivity of the COXIB attained at the site of action in the microvasculature and the possibility of large interindividual variations in drug response [10]. The precise enzyme selectivity of traditional NSAIDs and COXIBs *in vitro* and *in vivo* has long been disputed and depends heavily on the assay or model used to report inhibition [60]. For example, most COX-2 selective inhibitors were developed and selected using assay screens for slow, tight-binding inhibitors. However, at low substrate concentrations, most of these inhibitors also show some nonselective competitive inhibition of COX-1. The effective concentration of arachidonic acid in cells is variable and remains uncertain, so even weak competitive inhibitors may inhibit COX-1 to some extent [60]. This has been widely demonstrated for some diarylheterocycles, like celecoxib, and it is now understood that the COX-2 selectivity of a given inhibitor will change depending upon the conditions for screening [61]. As a result, one must exercise caution when extrapolating the results of *in vitro* inhibitor screens to *in vivo* COX inhibition.

Using celecoxib as an example, this inhibitor displays very high and selective inhibitory activity against purified COX enzymes *in vitro*, but lower selectivity in an *ex vivo* human whole blood assay [62]. In fact, celecoxib appears comparable to the traditional NSAID diclofenac in the whole blood assay, a result that was unexpected considering the *in vitro* differences between these two compounds. However, these data may help to explain why there was no difference in overall GI toxicity or cardiovascular toxicity between these two inhibitors in human clinical testing. In the CLASS arthritis trial, data published after 6 months of the study indicated that celecoxib treatment was associated with a reduced incidence of gastrointestinal events when compared to diclofenac [32]. However, the data from the full 12-month length of the trial revealed no statistical difference in the GI events between the two inhibitors [63]. In addition, a long-term study has confirmed cardiovascular risk with diclofenac [45], suggesting that COX-2 derived PGI_2 suppression is a clinical factor with both COX-2 selective inhibitors and traditional NSAIDs and that this is not simply a risk associated only with COXIBs.

8.8 ADDITIONAL MECHANISMS FOR CARDIOVASCULAR EVENTS RELATED TO COX-2 INHIBITION

The complexity of signaling molecules and mediators of vascular physiology and their interplay with one another has led to other explanations and additions to the current interpretation of the mechanism-based effect of COX-2 inhibition leading to cardiovascular toxicities (Figure 8.10). For example, in mice, treatment with COXIBs and their subsequent reduction of PGs does not increase vascular contractility or cause an elevation of blood pressure unless the animals are pretreated with inhibitors of nitric oxide (NO) biosynthesis [64]. An explanation of this observation is that the primary mediator of vascular reactivity is nitric oxide and the role of PGI_2 is consequential only when levels of NO are significantly reduced. This could translate directly to humans and specifically to the results of the APC polyp prevention trial as the majority of cardiovascular events in the celecoxib arm of the trial occurred in patients with conditions that could reduce nitric oxide biosynthesis (diabetes, smoking) and predispose those individuals to cardiovascular disease.

Another possible addition to the COX-2/PGI_2 inhibition and CV toxicity causal relationship mechanism arises from the ability of the COX-2 isoform to selectively oxygenate the endocannibinoid 2-archidonoylglyerol to its prostaglandin derivative PGI_2-G [65]. This glycerol prostaglandin has been reported to activate PPARδ, which in turn would repress the synthesis of the prothrombotic, tissue factor, and protect against thrombosis [66]. Inhibition of PGI_2-G synthesis with COXIB treatment allows elevated synthesis of tissue factor, which predisposes to thrombosis, and increases the possibility of cardiovascular events.

It remains to be established to what extent the inhibition of other prostaglandins (other than PGI_2) might contribute to a cardiovascular hazard of COXIBs. It is thought that COX-2 is the predominant source of PGE_2 and PGD_2 under physiological conditions and deletion of the receptor for PGE_2 (EP2) results in a salt-sensitive hypertension [67]. Macrophage activation in early atherosclerotic lesions results in abundant COX-mediated production of PGE_2, which acts on EP2 and EP4 receptors [68]. The EP4 receptor has been reported to be the primary mediator of the anti-inflammatory effects of PGE_2 [69] and signaling through this receptor may increase matrix metalloproteinases in macrophages and lead to unstable plaques [70]. Given that mice with a targeted deletion of EP4 die soon after birth [71], it has been difficult to study the

effects of PGE_2 signaling through this receptor in *in vivo* models. Recently, Linton and coworkers reported the use of fetal liver cell transplantation to create LDLR (–/–) mice chimeric for expression of EP2 and EP4 in hematopoeitic cells for the study of the roles of these receptors in apoptosis and atherogenesis *in vivo* [72]. The absence of EP4-promoted macrophage apoptosis through the down-regulation of NF-κB and PI3K signaling pathways, which correlated to a suppression of early atherosclerosis in LDLR (–/–) mice. Thus, EP4 deficiency compromises macrophage survival and suppresses early atherosclerosis *in vivo*. It is plausible that the selective inhibition of COX-2 could lead to increased apoptosis in atherosclerotic plaques (resulting in plaque rupture) through the inhibition of PGE_2 production and its EP4-mediated pathways and provide an additional mechanism for an increase in cardiovascular events.

Arachidonic acid, the substrate for COX, can also be converted to leukotriene products by lipoxygenases and there are data supporting a role for the 5-lipoxygenase (5-LO) enzyme and its enzymatic products in inflammation and cardiovascular disease [73]. Recently, Duffield-Lillico *et al*. reported that increased levels of urinary metabolites of the COX-2 and 5-LO pathways, PGE-M and LTE_4, are found in human smokers. Celecoxib treatment, through inhibition of COX-2, reduces levels of PGE-M in smokers, while increasing levels of 5-LO derived LTE_4 [74]. Taken together, these data represent the first clinical evidence for the existence of an arachidonic acid shunt between the COX and LOX pathways. In smokers, celecoxib treatment shunts arachidonate from COX-2 to the 5-LO pathway and results in an increase of 5-LO-derived products. These results may help to explain the cardiovascular toxicity associated with COX-2 selective inhibitors since 5-LO and its leukotriene metabolites have been previously suggested to play a role in inducing cardiovascular disease [73,75,76].

The COX-2 derived product, PGH_2, is converted by catalytic isomerization to PGE_2 by prostaglandin (PG) E(2) synthases (PGES). Deletion of microsomal PGES (mPGES) retards atherogenesis [77] and limits aortic aneurysm formation [78] in hyperlipidemic mice. Interestingly, deletion of mPGES does not predispose to thrombogenesis and has a limited impact on blood pressure compared to inhibition of COX-2 by NSAIDs and COXIBs. A consequence of the rediversion of PGH_2 to other synthases has been augmented formation of PGI_2 and PGD_2, which may be cardioprotective. Over the last several years, the design and development of mPGES inhibitors has been a major focus of pharmaceutical development [79]. The inhibition of the formation of PGE_2 is a key factor determining their therapeutic potential in cardiovascular inflammation and cancer.

8.9 COXIBS VERSUS NSAIDS AND CARDIOVASCULAR RISK

An unresolved question and one for which limited long-term clinical data are available is whether all NSAIDs induce the same cardiovascular side effects as COX-2 selective inhibitors and, therefore, whether this is simply a COX-2 selectivity problem? All NSAIDs will inhibit both COX enzymes and could also result in a decrease in the PGI_2 and PGI_2-G prostaglandins in the vasculature. Nonselective inhibitors of COX may also have the benefit of reducing TxA_2 levels to balance out the reduction in PGI_2 [65]; however, aspirin and naproxen are really the only NSAIDs that inhibit COX-1 in platelets strongly enough and for a prolonged time frame to significantly reduce TxA_2 levels [80]. Although long-term trials have not been conducted with the traditional NSAIDs to monitor their effect on the cardiovascular system, it follows from available *in vitro* and *in vivo* data that any NSAID other than aspirin or naproxen would not depress TxA_2 levels enough to be cardioprotective or to set off the decrease in PGI_2 via COX-2 inhibition in the vessel wall. Results from the MEDAL trial comparing diclofenac and the COX-2 selective inhibitor, etoricoxib, are the only human clinical trial data to confirm an increased cardiovascular risk for any NSAID (Figure 8.9) [45]. This year-long study with etoricoxib and diclofenac raised interesting questions regarding COX inhibition and toxicity in the GI tract versus the cardiovascular system. Etoricoxib was found to be significantly safer on the gastrointestinal system compared to diclofenac in the 'total' incidence of GI events, but was equal to diclofenac in causing 'serious' GI events. Interestingly, diclofenac was also found to have an increased cardiovascular toxicity profile. These data would suggest that inhibition of COX-1 and COX-2 simultaneously in the GI tract is required for ulcerogenesis and that *any* inhibition of COX-2 in the cardiovascular system results in toxicity or adverse events.

The only data on nonselective NSAIDs and cardiovascular toxicity comes from GI toxicity studies comparing COXIBs and NSAIDs with trial lengths usually less than one year and with low statistical significance. A meta-analysis of all of these studies has suggested that even within this short duration (1 year), traditional nonselective NSAIDs like diclofenac, ibuprofen, and indomethacin are associated with an increased cardiovascular toxicity profile [81]. Interestingly, naproxen was the only NSAID in the meta-analysis to be associated with a decrease in cardiovascular risk when compared to other NSAIDs and COXIBs, which may

be a reflection of its ability to repress TxA_2 levels in platelets and suppress platelet aggregation over a prolonged time [33]. A systematic review that combined data from independent case-control and cohort studies investigating COXIBs and NSAIDs and the occurrence of cardiovascular events contradicted the cardioprotective effect of naproxen and cited only diclofenac as a risk in the development of cardiovascular events (relative risk of 1.40) compared to COXIBs like rofecoxib (1.33) [82]. Interestingly, celecoxib was not associated with an increased risk in this review (1.06). These data, although supportive of the notion that any inhibition of COX-2 will result in cardiovascular toxicity irrespective of structural, molecular and kinetic differences in the inhibitor itself, must be validated in long-term safety and efficacy trials. It is also important to note that meta-analyses and epidemiological studies have relatively poor statistical power and can confound the results and interpretations of clinical trials.

8.10 SUMMARY AND PERSPECTIVES

In the last 60 years and even today, NSAIDs are among the most widely used prescription and non-prescription drugs in the world for analgesia and inflammation. The discovery in 1971 of COX as the molecular target of NSAID action and the subsequent discovery of two differentially regulated and expressed isoforms of COX, led to the development of the 'COX-2 hypothesis' whereby selective inhibitors of COX-2 would retain the beneficial effects of more traditional NSAIDs while sparing the toxicity to the GI tract. This hypothesis was born out in clinical trials of arthritis and the first COXIBs to market had great commercial success and proved safe and efficacious in their target populations over the short-term. However, colon polyp chemoprevention studies with rofecoxib and celecoxib revealed cardiovascular side effects associated with the drug-treated arms compared to the placebo. These cardiovascular events have proven to be mechanism-based (i.e. dependent on COX-2 inhibition) and may reflect an overall risk associated with all COX inhibitors, possibly including the traditional NSAIDs. From the standpoint of a discussion on the benefit-to-risk profiles of drug treatment, COX-2 inhibitors still have the ability to make a substantial difference in a subset of patients.

One population that would benefit from COXIB treatment is individuals severely sensitive to the GI effects of NSAIDs, and for which the benefit of the COX-2 selective inhibitor may justify its long-term

cardiovascular risk. Another clinical area where COXIBs will find use is in the prevention and treatment of certain cancers. There is no doubt that COX-2 regulation and expression has an enormous impact in the etiology of many cancers and COXIBs would be the preferred drug choice in cancer patients because of the high risk of bleeding disorders already present in this group. One result of the APC trial that was overshadowed by the appearance of cardiovascular toxicity, was the impressive efficacy of celecoxib in reducing the recurrence of polyp formation in high-risk patients [41]. Celecoxib is not the only COX inhibitor that has shown this chemopreventive effect; sulindac, in combination with an ornithine decarboxylase inhibitor, demonstrated, in a three-year trial, that polyp recurrence could be reduced by 70% (and in advanced adenomas by 92%) [83]. Sulindac itself is also of interest in that either the parent molecule or its metabolites have off-target effects from COX that are related to the induction of apoptosis and may contribute to the growth reduction of neoplastic cells in the colon [84,85]. Thus, in patients with high-risk for cancer development, such as familial polyposis patients, the long-term benefits of the COX inhibitor would outweigh the associated risk. In fact, currently both celecoxib and sulindac are approved for use in patients with familial polyposis.

An additional group that may benefit from therapy with COX-2 selective inhibitors are patients with non-small-cell lung cancer (NSCLC); those with advanced disease rarely survive beyond 2 years, even when treated with current therapies [86–88]. PGE_2 production from upregulated COX-2 in the lung is thought to contribute to the pathogenesis and progression of NSCLC and strategies designed to reduce PGE_2 levels are thought to represent a new therapeutic option in the treatment of the disease [89–91]. Recently, Edelman *et al.* reported the results of a clinical trial in which patients with advanced NSCLC were randomly assigned to receive platinum-based chemotherapy with either celecoxib or zileuton (a 5-lipoxygenase inhibitor) [92]. Patients with tumors that expressed high levels of COX-2 and treated with a combination of chemotherapy and celecoxib effected a higher survival rate than patients given chemotherapy alone. Additional trials must be performed to validate COX-2 inhibitors as a treatment option in NSCLC, but combination therapy with selective COX-2 inhibitors may be a successful treatment strategy for a variety of malignancies.

Since the discovery of COX-2 in the early 1990s, the importance of this enzyme as a drug target in inflammation and multiple chronic diseases has been at the forefront of both academic and industrial research. The rapidity of the development of COX-2 selective inhibitors and their

marketing showcased all the best tools in drug discovery, medicinal chemistry and pharmacology. With the possibility that both COXIBs and their nonselective NSAID precursors behave similarly with respect to cardiovascular risk, researchers, clinicians and government regulatory agencies must attempt to balance risk and benefit to formulate strategies to treat patients with disorders caused by excessive COX-2 function.

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9

Cardiovascular Toxicities of NSAIDS: Epidemiologic Aspects

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9.1 INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are possibly one of the most frequently used therapeutic groups worldwide. Under these circumstances, the potential implications from a public health perspective of virtually any adverse reaction associated with these drugs are enormous. Until recently the major concern related to NSAID use was its gastrointestinal (GI) safety. NSAIDs have been repeatedly shown to cause GI events ranging from dyspepsia to serious upper GI bleeding [1,2]. Although this appears to be a class effect, not all NSAIDs exhibit the same degree of GI toxicity at therapeutic doses. Thanks to the vast body of research in this area the GI safety profile of most NSAIDs has been described in detail and clinicians may now make better informed decisions on which NSAID to use according to accumulated evidence. Furthermore, the use of different strategies to minimize this risk (such as the concomitant use of PPI or misoprostol) has been proved to be useful

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and has been introduced as standard in high-risk population (patients with ulcer antecedents, elderly and chronic NSAID users) [3].

Nevertheless, NSAIDs still cause GI events, and the very appealing notion of attaining the same analgesic and anti-inflammatory effect but limiting the undesirable GI effects led to the development of a novel class of NSAIDs. Inhibition of cyclooxigenase 2 (COX-2) was thought to drive all the beneficial effects of NSAIDs, eliminating the unintended negative effects on gastrointestinal mucosa mediated by the constitutive isoform COX-1. The introduction of selective COX-2 inhibitors (commonly known as coxibs) almost a decade ago was a major breakthrough thought by many to solve all these problems at once. Thanks to a smart pharmacological design, selective inhibition of this inducible enzyme was achieved. But often nature is far more complex than human created and over-simplified models. Thus, although coxibs demonstrated to be effective and showed a better gastrointestinal safety profile than traditional NSAIDs (tNSAIDs), the suspicion that these newly designed drugs could increase the risk of acute myocardial infarction (AMI) soon jeopardized their place in the therapeutic arsenal. An interesting twist in this story came when this hypothesis, initially focused exclusively on coxibs, motivated an exhaustive scrutiny of the cardiovascular safety profile of non-selective or traditional NSAIDs. As a consequence of this still on-going scrutiny, now all NSAIDs, and not just coxibs, are thought to increase, to different degrees, the risk of ischemic cardiovascular events in general, and AMI in particular. In fact the initial hypothesis for this evaluation of the risk of tNSAIDs was quite different to what it turned out to be. Acetyl salicylic acid (ASA) – commonly known as aspirin and a member of the NSAID drug class – is widely used in the prevention of coronary heart disease by virtue of its anti-thrombotic properties, mediated by the complete and irreversible inhibition of COX-1 enzyme in platelets. When the first report of an increased AMI incidence associated with rofecoxib appeared, the authors discounted the risk suggesting that naproxen, a common tNSAID used as a comparator in the study, was actually cardioprotective. Thus, the fivefold increased risk observed in rofecoxib users in the VIGOR trial would not be due to rofecoxib itself, but to an intrinsic and previously unnoticed major cardio-preventive effect of naproxen, which is a potent suppressor of COX-1 [4]. This hypothesis did not receive much support for two main reasons. First, although naproxen certainly inhibits to a major extent the production of COX-1, this blockade, unlike that produced by ASA, is reversible. Second, even in the unlikely case that naproxen shared the same effect as ASA (which is estimated to reduce the risk of a secondary AMI by between 20 and

30%) it could never explain the fivefold increased risk observed in rofecoxib users.

In order to refute or confirm this hypothesis, an increasing number of observational studies and several RCTs evaluating the CV risk profile of tNSAIDs started appearing. As we will see, this ultimately led to considering that all NSAIDs, not just coxibs, could be cardiotoxic.

In this chapter we will try to review different aspects of NSAID-related CV toxicity, such as the observed heterogeneity between the NSAID class and its determinants, the time course of the effect, and the potential interaction between ASA and other NSAIDs.

9.2 PHARMACOLOGY

Although the molecular foundations of NSAIDs have been covered in detail in the previous chapter, here we would like to discuss briefly some specific aspects of the cardiovascular toxicity of NSAIDs and how they might help in reconciling the different results observed in epidemiological studies.

Although NSAIDs have been used for centuries the actual mode of action of these drugs was not unveiled until 1970s. It was Professor Vane who proposed the inhibition of prostaglandin synthesis as the mechanisim of action of 'aspirin-like drugs' [5], a discovery for which he was awarded the Nobel Prize in 1982. An enzyme called cyclooxigenase is responsible for the crucial step in this pathway. This enzyme mediates the conversion of arachidonic acid into PGH2. Within tissues PGH2 is later converted into other eicosanoids such as prostacyclin, thromboxane (TXA2) and other prostaglandins, each with a broad range of biological activities. There are at least two isoforms of cyclooxigenase. The constitutive isoform is called COX-1 and is located in virtually all human tissues. The expression of COX-1 is regulated by hormonal signals and is involved in regulation of homeostasis. The other isoenzyme, COX-2, is considered to be inducible (although it can be constitutive in certain tissues) and is found to be upregulated under endogenous stimuli such as aggression, trauma, and so on.

Although both isoforms are involved similarly in some of the prostaglandin-mediated processes, there are other processes that are almost exclusively regulated by one of them. As an example, in the thromboxane-mediated platelet aggregation it is COX-1 that plays a key role. In contrast the involvement of COX-2 is essential in inflammatory processes.

The mechanism underlying ASA anti-aggregant properties has been studied in detail and it is key to a clear understanding of the entire relationship between NSAIDs and cardiovascular risk. As we have previously noted, COX-1 is involved in the production of thromboxane in platelets, which plays a crucial role in the platelet aggregation process. Interestingly, platelets are small anucleated cytoplasmic bodies derived from blood cells. As such, they are completely unable to produce new proteins by themselves. While many NSAIDs can reversibly block COX-1 (and thus TXA2 production), only ASA can attain an irreversible blockade by acetylating a serine residue at the COX-1 binding site. Once acetylated, the enzyme loses all its functionality. In a regular nucleated cell *de novo* production of COX-1 can compensate this loss. However, as noted before, that is not the case with anucleated platelets. The lifespan of platelets is 7–10 days (i.e. roughly 10% of them are replaced every day). Thus, their inability to produce new COX-1-dependent thromboxane after permanent inhibition in ASA exposed platelets lasts (even if ASA is discontinued shortly after) until the platelet is destroyed by the spleen, which can take up to ten days. This ability to inhibit permanently TXA2 production in platelets places ASA in a unique position to be used in coronary heart disease prevention. Moreover, the ASA dosage needed to effectively block platelet aggregation is considerably lower than the dose used as an analgesic or in anti-inflammatory therapy, so the dose-dependent risk of GI toxicity associated with ASA is minimized.

Other non-selective NSAIDs can also inhibit COX-1 and therefore block TXA2 production in platelets. However, their inhibition is not irreversible but rather they compete with the substrate (arachidonic acid) for the binding site. In order to succeed in this competition high blood levels of the NSAID must be constantly maintained, as any attained inhibition would be reversed the moment drug intake is discontinued and blood levels drop [6].

However, TXA2 is not the only prostanoid involved in platelet aggregation and hemodynamic processes. Prostacyclin, whose production in endothelial vessel cells is catalyzed by COX-2, prevents the formation of blood clots and, at the same time, acts as a vasodilator (as opposed to TXA-2 which collaborates actively in the platelet function of aggregation). Since one enzyme compensates the effects of the other, one might think that the right balance between the effects of these two prostanoids is basic to a regular functioning of cardiovascular homeostasis. And here is precisely the problem with coxibs and, to a certain extent, all NSAIDs. On the one hand they block prostacyclin synthesis, which causes vasoconstriction and favors the formation of blood clots. On the other hand,

unlike aspirin, they do not block platelet function, which again favors platelet aggregation. This 'unopposed' blockade of COX-2 is seen as the underlying cause behind the elevated proportion of CV events, such as AMI, among patients using NSAIDs.

A recently published study by our group provides some useful insight into this issue. The study included 8852 cases of non-fatal MI and 20 000 population controls and was performed using THIN, a British database [7]. We found that among the different pharmacodynamic properties of NSAID, the degree of inhibition of COX-2 was the major predictor of AMI risk. These results seem to confirm the suspicion that cardiotoxicity in NSAIDs is common to almost all but in different degrees depending on their ability to block COX-2. Previous studies had tried to correlate the ratio of COX-1/COX-2 with CV risk, but the reason why this ratio is not such a good predictor is a pharmacological fact: In order for COX-1 inhibition to have an effect on TXA2 production, COX-1 suppression must be complete (i.e. the degree of inhibition must be 95% or more). At therapeutic doses only two of the studied NSAIDs can achieve this level of inhibition: ASA and Naproxen. While the NSAID CV effect via COX-1 presents a clear threshold effect, COX-2 inhibition resulting in reduced prostacyclin levels seems to follow a clear dose-effect pattern (i.e. the greater the degree of COX-2 inhibition the less favorable the cardiovascular profile). Thus, when we plot the risk of AMI against the degree of COX-2 inhibition at average plasma concentrations we find a pretty good correlation once ASA and Naproxen have been removed from the plot. This suggests that the degree of COX-1 inhibition becomes irrelevant when it does not reach a certain level (around 95%), and under these conditions it is COX-2 inhibition that explains the risk.

9.3 RISK ESTIMATE (MAGNITUDE OF THE PROBLEM)

There are several factors that we should take into account when describing an association between the use of a drug and the occurrence of an untoward effect. Probably the measure that often gets the most attention is the relative risk (RR), which describes how the background risk of a disease is increased (or decreased) in a population exposed to the drug under study. This estimate can be obtained from randomized clinical trials (RCTs) or observational studies. Estimates from RCTs are hypothetically less prone to bias and, therefore, more valid. Unfortunately, and due to several reasons, (including costs, and ethical reasons) it is not

always feasible to obtain estimates from RCTs. In these cases we have to rely on observational studies. The study of NSAID-related cardiotoxicity is not an exception. Whereas we do have some information from RCTs regarding the risk estimates of coxibs and some of the most frequently used non-selective NSAIDs, for the majority of NSAIDs all we have (and not always) is evidence from observational studies. Furthermore, many of the available RCTs are not sufficiently powerful to detect a variation in CV risk. Only a subset of long-term RCTs with large sample sizes are useful in this regard. In fact drug safety experts often complain that drug approval is based on the assessment of evidence from RCTs that are frequently too underpowered to detect many relevant adverse events. The approval program of coxibs is in some ways paradigmatic in this regard. The time elapsed from the discovery of the COX-2 isoform to the introduction in clinical practice of the first COX-2 inhibitor was just around ten years, an incredibly short interval for a member of a therapeutic group not used to treat cancer or HIV/AIDS [8]. One of the factors that determined this celerity was the combination of sound pharmacological principles with impressive preclinical and clinical results. All this seemed to provide a compelling simple model that latterly has been proven to be oversimplified. Although most early studies included in drug approval documentation were clearly underpowered to detect uncommon adverse events, the ambitious and praiseworthy comprehensive post-authorization development plan of these drugs included several long term RCTs. These trials were focused on quantifying the hypothesised improved safety profile (focusing mainly on GI safety) among long-term users of these drugs compared to selected tNSAIDs, with an average follow-up duration of one year. The control group consisted of naproxen, ibuprofen or diclofenac and/or placebo if feasible (in some instances when patients required a background anti-inflamatory treatment, like RA (rheumatoid arthritis) patients, the use of a placebo was not an option). The first study to show an increased risk of myocardial infarction with a coxib was the VIGOR trial. This trial belongs to the class just described, initially designed to compare the GI safety of rofecoxib (50 mg) and naproxen (500 mg bid) in patients with RA. Although rofecoxib demonstrated a lower risk of GI events, the finding of a four- to fivefold increased risk of myocardial infarction among users of rofecoxib marked an inflection bent in the assessment of the safety profile of these drugs [4]. As mentioned earlier, in the original paper the authors pointed to a potential cardioprotective effect of naproxen (which suppresses COX-1 to a similar extent to that of ASA though in a reversible fashion) as the underlying explanation for the observed increased risk of MI rather

than to an adverse effect of rofecoxib. This hypothesis could not stand the reasonable criticism that, even in the unlikely event that naproxen would attain a similar cardioprophylactic effect to ASA (about 25% reduction in ischaemic coronary events), other alternative explanations were required to account for the remaining two thirds of the increased risk associated with rofecoxib use.

The overall results of CLASS, a similar study aimed at comparing the GI safety of celecoxib (400 mg bid), diclofenac (75 mg bid), and ibuprofen (800 mg tid) failed to detect a significant difference in GI or CV events between treatment arms [9]. This study was subject to many methodological criticisms that could explain these results.

The TARGET trial (that was actually comprised of two sub-studies) is one other example of these trials and recruited around 18 000 osteoarthritis patients randomized to lumiracoxib (400 mg), naproxen (500 mg bid), or ibuprofen (800 mg tid) for one year. Overall this study was unable to find significant differences in MI risk, neither between lumiracoxib and ibuprofen (0.66, 0.21–2.09) nor between lumiracoxib and naproxen (1.77, 0.82–3.84). However, these results were based on a limited number of events (a total of 33 cases) [10].

The MEDAL program comprised three similar RCTs (MEDAL, EDGE I, and EDGE II) comparing overall GI and CV toxicity of etoricoxib 60 or 90 mg versus diclofenac 150 mg [11]. More than 30 000 people were included in these studies that did not find differences in thrombotic CV events (0.95, 0.81–1.11) or complicated upper GI events, although etoricoxib was associated with a greater number of discontinuations due to heart failure, edema, and hypertension. A common criticism of this study is the choice of diclofenac as comparator [12]. It is known that diclofenac is both a weak COX-1 inhibitor and one of the most potent COX-2 inhibitors at average therapeutic dose. In fact its COX-2 selectivity has been compared to some coxibs [13]. Furthermore, evidence from observational studies has shown that diclofenac was consistently the tNSAID with the greatest increased risk of MI. Therefore, the failure to detect a difference between etoricoxib and diclofenac could be interpreted as a confirmation of diclofenac potential cardiotoxicity rather than a reassuring result on the safety of etoricoxib.

Besides these safety trials, a different type of long-term trial was planned for coxibs. Previously, other NSAIDs had been shown to be associated with a reduced risk of different cancers (especially colorectal cancer) and Alzheimer's disease [14,15]. While most evidence for this hypothesis comes from observational studies, some RCTs have been conducted with variable results [16–18]. Yet, the risk/benefit ratio of

long-term use of these drugs was questioned from the outset, especially in a population with low background risk of these conditions. The hypothesized mechanism for the reduction of these two outcomes was the suppression of COX-2 mediated prostanoids and, partly because of the expected favorable safety profile of coxibs compared to tNSAIDS, they were considered good candidates as therapeutic tools to reduce the risk of colorectal cancer and Alzheimer. Given the incidence of these conditions and their etiology (most likely requiring persistent exposure in order to translate into a clinical effect), studies with longer treatment durations and follow-up were planned in order to assess the potential of coxibs as chemopreventive agents. Thus, although not primarily designed to this end, these studies were more powerful than others to assess CV toxicity. In fact, preliminary results of two of these trials suggesting an increased CV risk associated to coxibs caused a generalized early termination of the four major trials of this kind.

One of these is the APPROVe study, with three years of planned follow-up, assessing as primary endpoint the recurrence of adenomatous polyps in patients with antecedents of colorectal adenomas in patients receiving rofecoxib (25 mg) or placebo. The study was terminated prematurely due to elevated incidence of CV events in the rofecoxib arm (1.92, 1.19–3.11) [19]. Two similar studies were conducted for celecoxib, the APC and the Pre-SAP, comparing varying doses of celecoxib (APC: 200 mg bid and 400 mg bid; Pre-SAP: 400 mg od) with placebo [20,21]. The intended follow-up was also about three years. However, some preliminary results of APC, suggesting a dose-related twofold increased CV mortality with celecoxib compared to placebo motivated the termination of APC when the study was close to completion (77% of the patients had already completed the study and at least 2.8 years of follow-up were accrued for the remaining patients). Although preliminary data from Pre-SAP did not show this trend, APC suspension caused the early termination of this study.

Another study of celecoxib (200 mg bid), naproxen (220 mg bid) and placebo, this time in the prevention of Alzheimer's disease (AD) in patients 70 years or older with family history of AD was also suspended on the same day that the APC and Pre-SAP trials were stopped, based on the APC results. This early termination precluded a formal analysis and preliminary results cannot be regarded as conclusive. These preliminary data were published two years after the trial suspension and showed no signs of an increased risk of CV events (death, MI, stroke, CHF, or TIA) on the celecoxib arm compared to placebo. Intriguingly, naproxen, an NSAID whose weak COX-2 inhibition and strong COX-1 inhibition made some

authors postulate it could have an aspirin-like effect, showed an increased risk at the time of trial premature termination (1.63, 1.04–2.55) [22].

Finally, a meta-analysis, inclusive of these and other RCTs not considered here, with either shorter follow-up, smaller sample sizes, or both, estimated that coxibs were associated with a 42% increased risk of serious vascular events compared to placebo (1.42, 1.13–1.78) [23]. In a specific analysis that included only trials with duration of follow-up longer than one year the estimate remained virtually unchanged (1.45, 1.12, 1.89). The estimate comparing coxibs with tNSAIDs was not statistically significant (1.16, 0.97–1.38) although heterogeneity between individual tNSAIDs was detected. The main source of heterogeneity was naproxen. Thus, among studies with naproxen as comparator, coxibs presented a RR of 1.57 (1.21–2.03) though it should be noted that VIGOR results accounted for a significant proportion of the naproxen comparisons. On the other hand, when non-naproxen comparators were used the estimate dropped to 0.88 (0.69–1.12). Some studies included as comparator both a tNSAID and placebo. Individual estimates for the most commonly used tNSAIDs compared with placebo are therefore available from these studies. Kearny *et al.* provided overall estimates for naproxen (0.92, 0.67–1.26), ibuprofen (1.51, 0.96–2.37), and diclofenac (1.63, 1.12–2.37). The effect of dose was also explored in this meta-analysis only for celecoxib, detecting a significant trend towards an increased risk with higher doses, although this observation was mainly driven by the results of the APC trial. Also, it should be noted that daily doses for tNSAIDS chosen in these trials are those considered high dose as used in the general population.

One interesting point raised by this meta-analysis of RCTs is that despite including a total of 138 RCTs (both published data and unpublished data provided to the authors by pharmaceutical companies) irrespective of the drugs, the total number of cardiovascular events occurring in coxib and tNSAID users were only 340 and 211 cases, respectively [23].

While RCTs are useful in characterizing the CV risk of coxibs, observational studies are helpful for both replicating with larger samples sizes the coxib effects seen in RCTs, and assessing whether tNSAIDs share this effect and to what extent. A large number of observational studies have explored this issue. Epidemiologists like to think that designing a good observational study is far more complex than a good RCT, and in some respects this statement is probably true. Since observational studies, unlike RCTs, are not immune to bias, careful design and analysis become the only possible way to sort out the different problems that may arise. Potential threats in observational studies stem mainly from improper

ascertaining of the case status and/or exposure status, and from the existence of confounding factors.

Among observational studies assessing NSAIDs and CV risk we find a small number of hospital-based case-control studies, but the vast majority are conducted in large cohorts using automated databases as the primary source of information. The preponderance of studies carried out using automated databases is certainly remarkable. These studies present several advantages, such as the ability to identify population-based controls from the underlying study cohort, the large sample sizes available, and the absence of recall bias since exposure is ascertained prospectively (i.e. before the event has actually occurred). However, these studies have the limitation of not being able to capture over-the-counter (OTC) drug use. This can certainly become an issue when studying NSAIDs since OTC use of these drugs is fairly common as short-term treatment. However, since there is no reason to believe that OTC use is more common in cases than controls, not including these data would result in a non-differential misclassification that would tend to slightly bias results towards the null. Ilkanoff *et al*. studied this issue in detail and estimated that the exclusion of users of OTC NSAIDs from the unexposed group would result in around 10% change away from the null [24].

Among the few observational studies that did not use automated databases we find two studies conducted in a network of 36 hospitals in the Philadelphia area [25,26] and a reanalysis of data from a clinical trial, the Physician Health Study (PHS), carried out in the 1980s to evaluate the overall efficacy of ASA in reducing the incidence of primary MI. Even though these three studies could include information regarding OTC use, none of them can represent the paradigm of exposure ascertainment in observational field studies. On the contrary, they highlight some of the greatest threats to the validity of these studies. The two hospital-based studies, for instance, ascertained NSAID exposure via telephone interview in both cases and population controls. The problem is that while controls were asked about their current use of NSAID at the time of the phone interview, cases were asked about NSAID use prior to their MI hospitalization. Considering that the telephone interview was made up to four months after the episode of MI had occurred (an event that itself may have affected the patient's ability to recall), NSAID use in cases is likely to be underestimated while NSAID exposure in controls is much less prone to recall bias. This differential misclassification would tend to underestimate the true effect, possibly resulting in a protective effect of virtually any drug exposure being studied. Among the non-database studies we also find the study by Kurth *et al*., in which exposure was ascertained by

means of a mailed questionnaire that was completed and returned by the participants once a year [27]. Based on their yearly responses NSAID exposure was estimated for the whole preceding year, which again comprises a significant amount of misclassification.

Three different meta-analyses have been published to date summarizing the results from observational studies [28–30]. Table 9.1 shows all observational studies included in these meta-analyses [25–27,31–52] showing several characteristics of these studies (including study population, design, type of endpoint, exposure ascertainment method and meta-analyses that used estimates from each study).

As noted before, the vast majority of studies included in any of these three meta-analyses were conducted using automated databases such as the General Practitioner Research Database (GPRD), QResearch and MEMO from the UK [31,34,35,38,47,43,51], Medicaid or Medicare-based databases from the US [32,33,36,39,45], Kaiser Permanente also from the US [40], databases from the Canadian provinces of Ontario and Quebec [37,41,52], and other European databases from Denmark [42,48,49] and the Netherlands [46]. Most of them used a nested case-control study design (the most efficient when using these databases) whereas some used a cohort design.

The remaining four studies were the two previously mentioned hospital-based case-control studies from the Philadelphia area [25,26], the observational reanalysis of the PHS also mentioned [27], and finally a hospital-based case-control study from Australia [44].

Most studies analyzed first MI or first MI hospitalization as the main endpoint, but we find some studies that either did not exclude cases with antecedents of MI prior to the study period [32,36–40,44–46,52] or specifically followed patients from a first MI to a second CV event or death [49–51].

Although the three meta-analyses used heterogeneous inclusion and exclusion criteria, most of the evidence is common to all of them and, therefore, their results and conclusions are similar. Thus, depending on the meta-analysis, the relative risk associated with tNSAIDs ranges from 1.08 (0.95–1.22) to 1.19 (1.08–1.31). The corresponding estimate for coxibs is only presented in two of them and exclusively for celecoxib and rofecoxib. The latter was associated with an overall 30% increased risk whereas celecoxib conferred no apparent risk (See Table 9.2). Among tNSAID the estimates for naproxen were essentially null, ranging from 0.97 to 0.99. The estimates for ibuprofen were somewhat different since Singh *et al.* reported a statistically significant estimate (1.11, 1.06–1.17) contrasting with non-significant estimates calculated by Hernández-Díaz

Table 9.1 Details of observational studies included in one of the three published meta-analyses

Author, year	Study population (study period)	Sample size (age)	Design	Cardiovascular endpoint	Exposure	Metaanalyses
García Rodríguez, 2000 [31]	GPRD, UK (1991–1995)	164 769 women (50–74)	Nested CC	First MI and CV death	Rx in database	HD,S, MG
Ray, 2002 [32]	Tennessee Medicaid, USA (1987–1998)	262 644 (50–84)	Cohort	MI hospitalization and CV death	Rx in database	HD,S, MG
Solomon, 2002 [33]	Medicare – Medicaid, USA (1991–1995)	22 225 (>64–<85)	Nested CC	First MI hospitalization	Rx in database	HD,S,MG
Watson, 2002 [34]	GPRD, UK (1988–1999)	16 937 with RA (40–79)	Nested CC	First MI	Rx in database	HD,S, MG
Schlienger, 2002 [35]	GPRD, UK (1992–1997)	16 454 (<75)	Nested CC	First MI	Rx in database	HD,S, MG
Ray, 2002 [36]	Tennessee Medicaid, USA (1999–2001)	453 962 (50–84)	Cohort	MI hospitalization and CV death	Rx in database	HD,S, MG
Mamdani, 2003 [37]	Database in Ontario, Canada (1998–2001)	166 864 (≥65)	Cohort	MI hospitalization	Rx in database	HD,S, MG
Kurth, 2003 [27]	PHS, USA (1982–1988)	22 069 (40–84)	Cohort	First MI	Annual questionnaire	HD
Kimmel, 2004 [25]	36 Hospitals, USA (1998–2001)	4859 (40–75)	CC	First MI non-fatal	Telephone interview up to 4 months later	HD, MG
García Rodríguez, 2004 [38]	GPRD, UK (1997–2000)	404 183 (50–84)	Nested CC	MI hospitalization and CV death	Rx in database	HD,S, MG
Solomon, 2004 [39]	Medicare - Medicaid, USA (1999–2000)	251 298 (>64–<85)	Nested CC	MI hospitalization	Rx in database	HD, MG
Graham, 2005 [40]	Kaiser Permanente, USA (1999–2001)	1 394 764 (18–84)	Nested CC	MI hospitalization and CV death	Rx in database	HD,S,MG

Kimmel, 2005 [26]	36 hospitals, USA (1998–2002)	8518 (40–75)	CC	First MI non-fatal	Telephone interview up to 4 months later	HD, MG
Lévesque, 2005 [41]	Database in Québec, Canada (1999–2002)	113 297 (≥66)	Nested CC	First MI hospitalization	Rx in database	HD,S, MG
Johnsen, 2005 [42]	North Jutland, Vigor and Aarthus, Denmark (2000–2003)	102 557 (>20)	CC	First MI hospitalization	Rx in database	HD,S, MG
Hippisley-Cox, 2005 [43]	QRESEARCH, UK (2000–2004)	95 567 (25–100)	Nested CC	First MI	Rx in database	HD,S, MG
McGettigan, 2006 [44]	Hospital in Australia (2003–2004)	806 (50–78)	CC	Non-fatal MI & unstable angina	Personal interview.	MG
Singh, 2005 [45]	Medicaid California, USA (1999–2004)	76 715 (>18)	Nested CC	Fatal and non-fatal MI	Rx in database	MG
Sturkenboom, 2005 [46]	Dutch database (1999–2004)	1482 (>45)	Nested CC	Thrombotic event	Rx in Database.	MG
Fischer, 2005 [47]	GPRD, UK (1995–2001)	42 611	CC	First MI (fatal & non-fatal)	Rx in database	MG,S
Bak, 2003 [48]	Danish database (1994–2000)	42 717 (≥65)	Nested CC	First ischemic CVA	Rx in database	MG
Gislason, 2005 [49]	Danish database (1995–2002)	66 701	Cohort	Death or reinfarction after a first MI	Rx in database	MG
Curtis, 2003 [50]	Medicare, USA (1994–1996)	70 316	Cohort	Death within 1 year of MI	Rx in database	MG
MacDonald, 2003 [51]	MEMO, Scotland (1989–1997)	7107 (27–100)	Cohort	CV death following CVD hospitalization	Rx in database	MG
Rhame, 2002 [52]	Database in Québec, Canada (1992–1994)	18 323 (≥65)	CC	MI hospitalization	Rx in database	S

Abbreviations: PHS: Physicians' Health Study, CC: case-control, MI: myocardial infarction, CV: cardiovascular, CVA: cerebrovascular accident, Rx: prescriptions. HD: Hernández-Díaz *et al*., S: Singh *et al*, MG: McGettigan *et al*.

Table 9.2 Summary estimates from published meta-analyses studies

	Hernández-Díaz *et al.* [28]	**Singh et al. [29]**	**McGettigan *et al.* [30]**
Rofecoxib	1.27 (1.12–1.44)	NA	1.35 (1.15–1.59)
Celecoxib	0.97 (0.86–1.08)	NA	1.06 (0.91–1.23)
tNSAID	1.08 (0.95–1.22)	1.19 (1.08–1.31)	1.10 (1.00–1.21)
Diclofenac	1.39 (1.18–1.64)	1.38 (1.22–1.57)	1.40 (1.16–1.70)
Ibuprofen	1.01 (0.89–1.15)	1.11 (1.06–1.17)	1.07 (0.97–1.18)
Naproxen	0.98 (0.87–1.11)	0.99 (0.88–1.11)	0.97 (0.87–1.07)
Meloxicam	NA	NA	1.25 (1.00–1.65)
Indomethacin	NA	NA	1.30 (1.07–1.60)
Piroxicam	NA	NA	1.06 (0.70–1.59)

NA: not available.

et al. and McGettigan *et al.* (1.01,0.89–1.15 and 1.07, 0.97–1.18, respectively). The estimates for diclofenac, however, were virtually the same, ranging from 1.38 (1.22–1.57) to 1.40 (1.16–1.70). This result comes with no surprise since all but one of the nine studies that assessed the effect of diclofenac observed an increased risk of MI among users of this drug (most of them with a statistically significant effect).

McGettigan *et al.* reported individual summary estimates for indomethacin (1.30, 1.07–1.60), piroxicam (1.06, 0.70–1.59), and meloxicam (1.25, 1.00–1.65), although these estimates were based on a limited number of published studies (7, 4, and 3, respectively). The effect of dose is assessed only in a few studies and it is difficult to draw conclusions from these sparse data. The most studied, however, has been rofecoxib and all studies that looked into this found that higher doses (>25 mg) were associated with higher risks. The corresponding estimates for low and high rofecoxib dose presented by Hernández-Díaz *et al.* based on data from four studies were 1.18 (1.07–1.31) and 1.78 (1.36–2.34).

Several observational studies have been published after these three meta-analyses were conducted. Again most of these additional studies used automated databases like the GPRD and THIN from the UK [53,7], the database from the Canadian province of Saskatchewan [54], the claims data base from the insurance company United Health from the US [55], a cohort of RA patients from PharMetrics also from the US [56], and Medicaid and Medicare databases from the states of Tennessee and Pennsylvania respectively [57,58]. Among all these additional studies that were conducted applying somewhat similar methodology but with different data sources, there is one salient study because of its enormous sample size. This is the study by Helin-Salmivaara *et al.* that used a

nationwide Finnish database of discharge summaries and included a total of 33 309 incident cases of MI [59]. This number of cases affords the calculation of individual estimates for virtually all NSAIDs. Overall the results of this study are congruent with previously detailed summarized data. The risk of MI associated with individual NSAIDs went from 1.06 (0.83–1.34) for celecoxib to 2.21 (1.18–4.14) for etoricoxib. Among tNSAIDs those with the lowest risk were ketoprofen (1.11, 0.94–1.31) and naproxen (1.19, 1.02–1.38), and those that exhibited the greatest risk were nimesulide (1.69, 1.43–1.99) and indomethacin (1.56, 1.21–2.03). Diclofenac was associated with a RR of 1.35 (1.18–1.54). By the time this study appeared the meta-analysis by McGettigan *et al.* was already under review. However the authors include an addendum at the end of the meta-analyses mentioning that even though they did not formally take into account this study in their meta-analyses, given the magnitude of the study they performed an alternative meta-analysis with this study and summary estimates remained unchanged.

9.4 DURATION

For a good understanding of the NSAID-related cardiotoxic effect a clear analysis of the role of duration of use is needed. This has been explored in a number of studies. Probably the most notorious evidence came from a RCT. When investigators of the APPROVe trial studied time to event associated with coxib use compared to placebo they found evidence suggesting that the deleterious effect appeared only after 18 months from the start of treatment [19]. The survival curve accompanying this result was compelling as it was the significance of the statistical test of this hypothesis. This finding generated a great deal of controversy because previous studies with shorter follow-up had been able to detect an increased risk [4]. The fact that the analysis considered only events occurring while treatment was ongoing or within 14 days of discontinuation was also criticized [60]. If treatment (rofecoxib in this study) caused a side effect that increased the likelihood of both the discontinuation of treatment and the outcome event, then censoring the follow-up just 14 days after discontinuation might cause a differential exclusion of events in the two groups that could result in a failure to detect increased risks associated to the treatment arm. The rate of discontinuation was in fact higher in the rofecoxib arm than in the placebo arm, suggesting that the risk estimate could have been underestimated. In a later report the

authors acknowledged that the statistical test of time dependence of the association had been done without taking into account a log transformation of time. When this was taken into account (as it said it would be in their methods section) the test did not reach statistical significance ($p = 0.07$), which to some extent weakened the hypothesis of a delayed effect with continuous use [61].

New data from an extended follow-up of the APPROVe study have been published recently [62]. This analysis considered CV events occurring during treatment and at least one year after discontinuation, avoiding the potential problems of differential exclusions described earlier. Overall estimates of the effect were similar to the censored report with a HR for APTC events of 1.72 (1.13–2.62). However, the survival plot of this endpoint (not exactly the same as in the original survival plot that used all confirmed CV events) in this extended analysis is not clearly suggestive of a time-dependent effect. Furthermore, the statistical test for this hypothesis was not significant ($p = 0.80$). Apart from these, a new and interesting finding comes out of this analysis. Investigators were able to assess whether the increased cardiovascular risk observed among individuals exposed to rofecoxib persisted after discontinuation. Although the number of events was limited (23 in the rofecoxib arm and 12 in placebo) they did observe a non-significant increased risk of APTC endpoints in the first year after discontinuation (1.95, 0.97–3.93). However, after one year off treatment, the risk seemed to disappear (0.92, 0.35–2.40). If we were to extrapolate these findings to other NSAIDs this would mean that those patients exposed to NSAIDs for long periods of time (median length of treatment approached 3 years in the APPROVe study) would not return to their background risk until at least one year off treatment.

In order to test this hypothesis we have been able to reanalyze data from a previously mentioned nested case-control study performed by our group in THIN. Thanks to the large sample size of this study we were considerably more powered than APPROVe to detect a persisting CV toxicity after NSAID withdrawal. To this end we identified those individuals who discontinued NSAID use between 7 and 365 days before the study index date. A total of 1478 cases and 2917 controls met this criterion. Overall we found that this group experienced a borderline significant slightly elevated risk of non-fatal MI (1.11, 1.03–1.20) compared to non-users of NSAIDs . However, when among those discontinuing NSAID recently we analyzed separately those who had used NSAIDs for one year or more we found that the risk was 1.58 (1.27–1.96). Furthermore, the main analysis of this study found a similar risk of

AMI among patients currently exposed to NSAIDs for a duration of more than one year (1.45, 1.27–1.65). These results suggest that traditional NSAIDs could also experience this persisting risk for a defined period of time after discontinuation. When analyzed in further detail, this risk seems to decay gradually with time after ceasing NSAID treatment. Thus the relative risk goes from a 70% increase in the first three months after discontinuation to less than 10% increase after more than 6 months [63].

Whether cardiotoxicity increases with duration of NSAID treatment remains uncertain. In fact some authors have speculated that differences in background risk and ASA use could explain the seemingly contradictory results of studies that found a delayed NSAID cardiotoxicity (or did not find it after all) and those who detected increased cardiotoxicity shortly after NSAID initial exposure [64]. Thus, patients with a low cardiovascular background risk may require a longer duration of use in order to experience similar deleterious effects that are observed in the first weeks of treatment of patients at high risk of CV disease. Exclusion criteria in the APPROVe study included uncontrolled hypertension, angina or congestive heart failure, and history of AMI, cerebrovascular disease, or coronary intervention in the last two years. Other studies that found a delayed cardiotoxic effect include the APC study, also with patients at low risk of CV events, that showed a gradual emergence of CV risk for celecoxib users and a population based observational study that found higher risks of MI with longer duration use of diclofenac [65]. In contrast, studies that found an immediate NSAID-related cardiotoxity include the VIGOR trial, in which rofecoxib and naproxen were compared among patients with rheumatoid arthritis (a condition known to be linked to an increased risk of MI), and two studies in individuals undergoing coronary artery grafting (CABG) [66,67]. These two studies are paradigmatic to this hypothesis since they were able to detect an increased risk with a very limited follow-up period (10 and 14 days, respectively) in relatively small groups of patients with an extremely high background risk.

Other RCTs, like TARGET, that included low risk population were unable to detect differences in CV risk between lumiracoxib, naproxen and ibuprofen after treatment for one year [10].

In essence, this hypothesis of differential background risk can be used to explain to a certain extent the sometimes conflicting results between RCTs and observational studies. Two mechanisms could be behind the increased CV risk with NSAIDs. A short-term risk can appear soon after NSAID introduction in populations that carry an inherent elevated risk of MI. When the background CV risk is low, then extended exposure to

these agents would be required to translate into a long term elevated risk in some individuals. In some ways it all boils down to a matter of the statistical power of the studies, which is largely determined by the background risk.

9.5 INTERACTION

Even though non-ASA NSAIDs do not achieve a permanent blockade of COX-1 enzyme, some tNSAIDs do compete with ASA for the same binding site. Thus it is plausible that permanent inhibition exerted by ASA is not attained if a tNSAID is administered prior to aspirin. This would translate into a loss of ASA cardioprotection properties when co-administered with other NSAID. The existence of such a pharmacodynamic interaction was initially suggested more than 25 years ago [68,69]. Later, several groups, including Catella-Lawson *et al.*, confirmed this hypothesis [70]. They conducted a crossover experiment in which a low dose ASA was administered followed by a single dose of ibuprofen 400 mg two hours later for six consecutive days. The cross-over phase shared the same scheme but reversed the order in which the drugs were taken. They found that irreversible platelet TXA2 inhibition occurred when ASA was administered first, whereas inhibition of TXA2 and platelet aggregation by ASA was blocked when ibuprofen was given first. Another experiment was conducted with multiple doses of ibuprofen and a single dose of aspirin administered two hours before the morning dose of ibuprofen. In this case they found that ibuprofen interfered with the irreversible inhibition of platelet COX-1 by aspirin even if it was administered after aspirin. However, when instead of ibuprofen, acetaminophen, rofecoxib or diclofenac (i.e. drugs which have low affinity for platelet COX-1) were administered the pharmacodynamic properties of ASA were preserved. Besides ibuprofen, some authors have suggested that other NSAIDs like indomethacin, naproxen, and tiaprofenic acid may also antagonize the ASA effect [71].

Thus, Capone *et al.* performed a study in healthy subjects co-administered with low-dose aspirin and naproxen 500 mg BID. In this case the administration of naproxen did not allow detection of a significant change in platelet COX-1 inhibition and function when the naproxen was given 2 h before aspirin or in the reverse order. However, when the two drugs were given at the same time a pharmacodynamic interaction was detected. These data suggest that many variables are operative in

the possible interference of nonaspirin NSAIDs and aspirin, such as the dose, half-life and affinity for COX-1 of the nonaspirin NSAID, their scheme of coadministration and the extent of inhibition of platelet COX-1 by aspirin. In fact, it is plausible that the pharmacodynamic interaction is minimized in the presence of saturation of COX-1 by aspirin [72].

While it seems clear that the pharmacodynamic interaction exists, the implications of this interaction for clinical practice are still very much open to debate. In order to evaluate this we would need to measure the impact of this interaction in terms of a clinically relevant endpoint, such as incidence of CV events, survival, and so on. Conducting a RCT designed specifically to address this hypothesis among patients receiving ASA treatment does not seem a possibility due to ethical concerns. The only feasible alternative would be an observational study. The problem with this approach is that an observational study is not an experiment and researchers cannot control when and how the drugs are being taken. We have learned from pharmacodynamic studies that the order in which drugs are taken is crucial to observe the interaction. In observational studies we may ascertain patients using ASA and ibuprofen simultaneously or just ASA and we can compare CV risk among these two groups of individuals. However, if we fail to detect an increased risk of CV associated with concomitant use this result could be due either to a lack of interaction or to our inability to precisely identify the subgroup of individuals taking ibuprofen right before ASA (i.e. misclassification in the exposure ascertainment) or a combination of these two factors.

Several observational studies have explored this issue with inconsistent results. McDonald *et al.* found that CV mortality was higher in patients using ASA plus ibuprofen compared to those receiving ASA alone (1.73 (1.05–2.84). This increased risk was not observed when ASA was combined with diclofenac (0.80 (0.49–1.31) or other NSAIDs (1.03 (0.77–1.37) [51]. These results contrast with another study by Patel *et al.* comparing incidence of MI in a cohort of ASA users and a cohort of users of ASA plus ibuprofen that found a lower risk in the second group (0.70 (0.59–0.83) [73].

Kurth *et al.* reanalyzing data from the PHS detected a significant interaction between ASA and NSAID and observed that the combined use was associated with a 1.57 (0.70–3.56) risk compared to placebo, whereas the corresponding estimate for ASA alone showed a decreased risk [27]. These results are based, however, on a single event in the ASA group and 7 events in the combination group (that included all NSAIDs not just ibuprofen).

Two additional studies by Curtis [50] and Hudson [74] studied the risk of a recurrent MI and reported no increased risk with the combination of ASA plus ibuprofen (0.84 (0.70–1.01)) and non-significant increased risk (1.83 (0.76–4.42)), respectively. Finally, two studies performed on British Primary Care Automated Databases found little support for the presence of a clinical interaction [7,38].

If we take into perspective the conflicting results and the limitations inherent to the difficulties of evaluating this interaction, we can resolve that we do not have (and probably never will have) enough information in order to draw definitive conclusions about the clinical implications of this pharmacodynamic interaction in the general population.

9.6 CONCLUSIONS

Available evidence suggests that cardiotoxicity is a class effect common to all non-aspirin NSAIDs. We found elevated risk in many NSAIDs, ranging from coxibs to naproxen (in just a couple of studies for the latter). However, it is likely that the magnitude of the risk will vary between different individual NSAIDs as a result of the extent of inhibition of COX-2 inhibition (which is defined as drug potency/exposure) at the doses administered in the general population in the absence of a complete suppression of thromboxane-mediated platelet function. Results from RCTs and observational studies are remarkably consistent and show a gradient in risk that is partly a function of the degree of reduction of COX-2-dependent prostacyclin. Following this line of reasoning, we can conclude that CV risk is maximum for NSAIDs with high COX-2 potency not accompanied by lasting and profound suppression of platelet COX-1, such as diclofenac. The risk seems to be moderate for other NSAIDs, such as ibuprofen, which is for the most part used at low dose, and it is lower for NSAIDs that exert a functional platelet COX-1 inhibition ($\geq$ 95%), such as naproxen.

However, observed heterogeneity does not stem solely from the degree of COX-2 inhibition. In fact we observed heterogeneity between studies evaluating the same NSAID. Other factors like the daily dose that is being used and, especially, the nature of the study population (as a result of inclusion and exclusion criteria) can be helpful in explaining apparent discrepancies.

Furthermore, this information should be transferred into clinical practice. Thus, clinicians should consider in their therapeutic decisions

evidence that indicates that individuals with cardiovascular comorbidity seem to carry a greater risk of developing NSAID-related cardiotoxicity and this effect tends to appear earlier (i.e. does not require extended duration). Also of relevance is the recent observation that the risk can persist for a number of months after NSAID treatment discontinuation among individuals who have been exposed to NSAIDs for a long period of time.

It is not clear whether concomitant use of NSAID and ASA can suppress the cardioprotection afforded by the latter. Although the pharmacodynamic interaction has been shown with some NSAIDs, the implications for real life concomitant use remain unclear. Unfortunately, we are not likely to obtain unequivocal evidence on this issue but observational studies should further investigate this possibility in order to exclude some important effect.

Even though the impact of NSAID-related cardiotoxicity needs to be seriously considered when using these drugs, it is the accumulated knowledge of the peculiarities of cardiovascular and gastrointestinal adverse effects that enables the customized selection of a specific NSAID among the vast number of members of this therapeutic class. The same NSAID may not be suitable for everyone, depending on the different personal characteristics and the regimen of use. This is probably true for most commonly used drugs and should not be seen as a problem but as an opportunity for rational drug use that is possible thanks to the vast experience of use and research with these drugs.

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10

Cardiovascular Toxicities of Life-Saving Drugs: Antiviral Therapy

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10.1 INTRODUCTION AND OVERVIEW

Antivirals encompass the group of drugs that inhibit replication of pathogenic viruses. Human immunodeficiency virus (HIV) and hepatitis B virus (HBV) are among the targets for antiviral therapy. In the case of anti-HIV drugs, different classes of anti-retroviral agents are approved and are classified functionally into: (i) agents that inhibit HIV reverse transcriptase (nucleoside/nucleotide reverse transcriptase inhibitors, NRTIs/NtRTIs; and non-nucleoside reverse transcriptase inhibitors, NNRTIs); (ii) agents that inhibit HIV-specific protease (protease inhibitors, PIs), and (iii) agents that inhibit HIV-entry (fusion inhibitors, FIs). Guidelines for anti-retroviral therapy recommend using combinations of agents. These therapeutic regimens, called highly active anti-retroviral therapy (HAART) are the cornerstones for inhibiting HIV (and in some cases HBV) replication, and have brought HIV disease to be considered a manageable chronic illness.

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The impact of HIV on world health is implicit. HIV/AIDS is the leading cause of death worldwide for all people aged 15 to 59 [1]. Untreated, AIDS was rapidly fatal early in the epidemic. HAART blocks HIV viral replication and disease progression, providing prolonged, quality life for patients with HIV/AIDS [2]. Current World Health Organization (WHO) guidelines recommend two NRTIs along with a NNRTI or PI as the treatment of choice for first-line HIV anti-retroviral therapy [3].

An estimated 400 million people are chronically infected with HBV worldwide, and 1 million deaths occur annually as a result of HBV infection [4]. Co-infection of HIV and HBV is frequently observed; approximately 5–10% of HIV-infected patients in the United States are co-infected [5]. In the absence of HIV infection, acquisition of HBV in adulthood results in chronic infection in fewer than 5% of cases, however, patients infected with HIV have a higher likelihood of chronic HBV diseases (~21%) [6]. Since a vaccine against HBV exists, the increased incidence of co-infection may relate to lower immune responses to HBV vaccination.

Despite the initial positive impact of HAART, side effects have been reported that include subcellular organelle toxicity of NRTIs (i.e. mitochondria) and organ (i.e. heart and blood vessel) dysfunction [7–12]. It may be reasonable to consider that as survival with HIV, HBV or co-infections is improved by treatment, long-term side effects of HAART (particularly NRTIs) will be observed more frequently [13]. This may be explained by the fact that despite a decline in overall death rates from HIV/AIDS, an increased proportion of deaths attributable to non-AIDS diseases has been found [14]. This chapter examines cardiac side effects associated with HAART (focused on the NRTI backbone) and possible mechanisms of toxicity at a biological, pathological and pharmacological level.

10.2 ANTIRETROVIRAL DRUG CLASSES (NRTIs, NNRTIs, PIs)

Twenty-two antiretroviral agents (and co-formulated combinations) are available for the treatment of HIV infection [15] (Table 10.1) and new drugs are in development [16]. The majority fit into three major classes (NRTIs/NtNRTIs, NNRTIs, and PIs). One fusion inhibitor, an entry inhibitor and an integrase inhibitor are recent additions. HAART typically includes three or more drugs in combinations from the three major classes.

Table 10.1 Current antiretrovirals available for treatment of HIV infection[a]

Class	Brand Name	Generic Name(s)	Abbreviation
NRTI	Combivir	lamivudine and zidovudine	3TC/AZT
	Emtriva	emtricitabine	FTC
	Epivir	lamivudine	3TC
	Epzicom	abacavir and lamivudine	ABC/3TC
	Hivid	zalcitabine/ dideoxycytidine	ddC
	Retro vir	zidovudine/ azidothymidine	AZT, ZDV
	Trizivir	abacavir, zidovudine, and lamivudine	ABC/AZT/ 3TC
	Truvada	tenofovir disoproxil fumarate and emtricitabine	TDF/FTC
	Videx EC	enteric coated didanosine	ddI EC
	Videx	didanosine, dideoxyinosine	ddl
	Viread	tenofovir disoproxil fumarate	TDF
	Zerit	stavudine	d4T
	Ziagen	abacavir sulfate	ABC
NNRTI	Intelence	etravirine	
	Rescriptor	delavirdine	DLV
	Sustiva	efavirenz	EFV
	Viramune	nevi rapine	NVP
PI	Agenerase	amprenavir	APV
	Aptivus	tipranavir	TPV
	Crixivan	indinavir	IDV
	Fortovase	saquinavir (no longer marketed)	
	Invirase	saquinavir mesylate	SQV
	Kaletra	lopinavir and ritonavir	LPV/RTV
	Lexiva	Fosamprenavir calcium	FOS-APV
	Norvir	ritonavir	RTV
	Prezista	darunavir	
	Reyataz	atazanavir sulfate	ATV
	Viracept	nelfinavir mesylate	NFV
Fusion Inhibitor	Fuzeon	enfuvirtide	T-20
Entry Inhibitor	Seize ntry	maraviroc	
Integrase Inhibitor	Isentress	raltegravir	
Multi-class Combination	Atripla	efavirenz, emtricitabine and tenofovir disoproxil fumarate	EFV/FTC/ TDF

[a]Listed and approved by U.S. Food and Drug Administration (FDA)

NRTIs (which include seven NRTIs and one NtRTI) are structural analogs of the native DNA nucleotides essential for DNA synthesis (adenosine, gaunosine, cytidine, or thymidine). As such, NRTIs can serve as substrates for viral reverse transcriptase (RT) in the synthesis of

cDNA from viral RNA in the initial step of the viral replication cycle. NRTIs are administered as pro-drugs that require phosphorylation by cellular kinases to form the active triphosphate metabolite [17]. The chemically modified 3'-OH group of the deoxyribose sugar (normally forming the 3'–5' phosphorester bond of DNA) is a key step. The lack of a 3'OH group inhibits the polymerase function of HIV reverse transcriptase and prematurely terminates the growing viral cDNA [12,18,19]. In contrast to the above, tenofovir disoproxyl fumarate (TDF) is a nucleo*tide* (Nt) (not a nucleo*side*), and thus is more accurately referred to as an NtRTI. Of note, TDF is a congener of cidofovir and adefovir (approved for HBV treatment), with activity against HIV, hepadnaviruses, and herpes viruses [20,21]. As DNA viruses (e.g. hepatitis B and the herpes viruses), these antivirals inhibit the viral polymerases by competing with the natural substrate deoxyadenosine 5'-triphosphate and rendering DNA chain termination [22].

Unlike nucleoside analogs, NNRTIs do not require phosphorylation to become active and are not incorporated into viral DNA. NNRTIs bind directly and noncompetitively to viral RT [23,24]. This binding site is distinct from the substrate (dNTP) binding site and NNRTIs block the DNA polymerase activity by causing a conformational change and disruption of the catalytic site of the enzyme [25].

Negative drug–drug interactions are important considerations with NNRTIs [26]. For example, nevirapine (NVP) and efavirenz (EFV) are inducers of the hepatic cytochrome CYP3A4, and are metabolized by it and CYP2B6 (members of the P450 enzyme system). As such, these NNRTIs may impact the pharmacological steady-state levels of co-administered drugs that are also metabolized by the cytochrome P450 system.

PIs target HIV protease that cleaves viral precursor polypeptides into smaller, functional proteins. Maturation of the HIV virion is thus inhibited by PI [27,28] and results in the release of structurally disorganized and noninfectious HIV particles. With PIs, again drug interactions are important considerations, since PIs are substrates for cytochrome P450 and inhibitors of the system. Protease inhibitors (except nelfinavir) are commonly boosted with ritonavir.

Despite the broad range of available drugs, many of these antivirals are toxic. Cohort studies indicate that approximately 20–30% of patients initiating therapy discontinue treatment in the first year, primarily due to drug toxicity [29]. Current IAS-USA guidelines recommend initial treatment of HIV infection with an antiretroviral drug regimen comprising two NRTIs given in combination with either an NNRTI or a

Table 10.2 DHHS Recommendation initial Antiretroviral Treatment of HIV Infection*

Recommendation	2 NRTIs	NNRTI or PI
Preferred choices	tenofovir/emtricitabine (co-formulated)	efavirenz (NNRTI) atazanavir + ritonavir (boosted PI) darunavir + ritonavir (boosted PI) fosamprenavir + ritonavir (boosted PI) lopinavir/ritonavir (co-formulated)
Alternative choices	abacavir/lamivudine (co-formulated) didanosine + (lamivudine or emtricitabine) zidovudine/lamivudine (co-formulated)	Nevirapine (NNRTI) atazanavir (unboosted) fosamprenavir (unboosted) saquinavir + ritonavir (boosted PI)

*Current guidelines by the DHHS Panel (updated Nov. 3, 2008)

ritonavir-boosted PI [30]. Specifically, fixed dose tenofovir/emtricitabine is the preferred combination choice of NRTIs in combination with either an NNRTI or a ritonavir-boosted PI (Table 10.2). These recommended first-line therapies have the greatest clinical success in the majority of patients with minimal variation in pharmacokinetics and little (if any) short-term toxicity and side effects.

10.3 MITOCHONDRIAL TOXICITY OF NRTIs

Mitochondrial toxicity is specifically attributed to NRTI treatment [31]. A major toxicity of HAART, which has been recognized for more than a decade, is related to their impact on mitochondrial DNA (mtDNA) (e.g. deletions, mutations, and depletions) [8,11,32]. Patients treated with one or more NRTI analogs experience a variety of side effects, including peripheral neuropathy, cardiac and skeletal muscle myopathy, pancreatitis, hepatic steatosis, lactic acidosis and bone marrow suppression [33–36]. The effects of NRTI-induced mitochondrial toxicity are known to be tissue specific [33]. This chapter focuses on cardiac and skeletal muscle mitochondrial toxicity.

A dose-dependent skeletal and cardiac myopathy has been reported following zidovudine treatment [37]. Ultrastructurally, mitochondria become enlarged and swollen and contain disrupted cristae, and occasional paracrystalline inclusions, an ultrastructural hallmark of

mitochondrial disease [38–40]. This toxicity is cumulative and becomes apparent with long-term therapy [41].

Some of the interrelated mechanisms by which NRTIs can decrease mtDNA abundance include: (i) inhibition of polymerase γ (pol-γ), (ii) nucleotide pool imbalance, and (iii) decreased number of mitochondria (reviewed in [7]). A subcellular pharmacological mechanism of mitochondrial toxicity is reasonably related (in part) to inhibition of pol-γ. Pol-γ is the only known DNA polymerase found in mitochondria and is responsible for the replication of mtDNA. The 'DNA pol-γ hypothesis' [11] offers a framework for experimental and clinical evaluation of mechanisms of mitochondrial toxicity from NRTIs. It postulates that inhibition of pol-γ leads to the depletion of mtDNA, subsequent depletion of mtRNA and of mitochondrial-encoded polypeptides involved in oxidative phosphorylation (OXPHOS), leading to mitochondrial dysfunction. Pol-γ is unique from other cellular replicative DNA polymerases in that it is sensitive to inhibition by NRTIs, dideoxynucleotides, and other antiviral nucleotide analogs [42–47]. The relative effectiveness of NRTIs as inhibitors of retroviral reverse transcriptase and eukaryotic DNA polymerases has been determined *in vitro* (HIV RT >> DNA pol-γ > DNA pol-β > DNA **pol**-α = DNA pol-ε) [12]. Inefficient excision of dideoxynucleotides, and some NRTIs (i.e. stavudine, zidovudine and carbovir) incorporated into DNA leads to the prediction of persistence *in vivo* [48]. NRTI-induced inhibition of pol-γ exonuclease activity also occurs in cells [49]. Inhibition of pol-γ exonuclease activity is likely to result in lower fidelity and an increase in mutations with mtDNA which was demonstrated in patients undergoing NRTI therapy [50]. Together, incorporation of NRTIs leading to chain termination and inhibition of pol-γ exonucleolytic proofreading likely contributes to mitochondrial toxicity.

An indirect mechanism by which NRTIs can deplete mtDNA is through perturbations of the endogenous mitochondrial nucleotide pool, from competitive inhibition of endogenous kinases or other metabolic disturbances [51]. The original DNA pol-γ hypothesis stated that NRTI triphosphates compete with the native moiety at the nucleotide-binding site of the enzyme to yield inhibition of mtDNA replication at the level of polymerization of nascent mtDNA. Stoichiometrically, a sufficient mass of phosphorylated NRTI must be available intra-mitochondrially for inhibition of mtDNA synthesis at the level of pol-γ inhibition. This leads to depletion of mtDNA, and development of toxic manifestations.

NRTI import, compartmentalization, and phosphorylation to active moieties may play a role in this mechanism of mitochondrial toxicity.

Cellular nucleoside kinases (both cytoplasmic and intramitochondrial) that are responsible for NRTI phosphorylation to active drugs can impact mitochondrial drug levels and subsequent mitochondrial toxicity. In murine models with cardiac-targeted transgenic over-expression of mitochondrial thymidine kinase 2 (TK2) or TK2 mutants, AZT treatment resulted in increased mitochondrial toxicity and cardiomyopathy [52–54]. The perturbing effect of NRTIs on the nucleotide pool is well supported by both *in vitro* and *in vivo* studies [55,56].

A third mechanism of mitochondrial toxicity can result in depletion of mtDNA. Depletion of mtDNA reduces intracellular energy production, which can result in organ dysfunction. Depletion of mtDNA is hypothesized to occur as a protective cellular response. Specifically, cellular self-degradation, a process called autophagy [57], plays an important role in cellular homeostasis. Degrading excessive, damaged and/or aged proteins and organelles (e.g. mitochondria) maintains quality control of essential cellular components. Overproduction of reactive oxygen species (ROS, e.g, superoxide, hydrogen peroxide, lipid peroxides, hydroxyl radical, and peroxynitrite) [58] can trigger autophagy [59]. NRTI-induced oxidative stress has been reported in lymphoid [60] and non-lymphoid tissues and in skeletal muscle in mice [61,62] and rats [63,64]. Myocyte autophagy, while intended to provide protection against ROS overproduction, may in fact lead to cardiac damage [65]. Mitochondrial suicide or 'mitoptosis' is an alternative outcome of ROS production that has been proposed [66]. In mitoptosis, self-induced selective elimination of dysfunctional mitochondria occurs if rendered harmful to the cell through inefficient oxidative phosphorylation or overproduction of ROS. In either scenario, NRTI-induced ROS appear to play a direct role in mitochondrial depletion, leading to cardiac dysfunction.

10.4 NRTI-ASSOCIATED CARDIOMYOPATHY

Early estimates suggested that over 6% of HIV-infected patients have diseases related to the cardiovascular system [67] including endocardial diseases [68], myocardial diseases [69,70], pericardial diseases [71,72] and cardiac neoplasms (Kaposi's sarcoma and lymphoma) [73,74]. An increasing body of evidence suggests adverse effects of specific antiviral agents on the heart and vasculature. Toxicities may vary among drugs in a similar class. Analysis from the Data Collection on Adverse Events of Anti-HIV Drugs (D: A: D) study, controlling for exposure to NRTIs reduced the

association between exposure to PIs and the risk of myocardial infarction, suggesting that NRTIs could contribute to cardiovascular risk [75,76].

Cardiomyopathy (CM) related to NRTIs (e.g. zidovudine) has been reported [77–82]. Clinical features of CM from NRTIs resemble some of those described for CM of other etiologies. Ultrastructural changes of intramyocytic vacuoles, myofibrillar loss, dilated sarcoplasmic reticulum, and disruption of mitochondrial cristae [78], features consistent with mitochondrial CM. While CM is reported in AIDS, the entity remains controversial. Cases of CM related to zidovudine have been rarely reported, despite widespread use of zidovudine. Discontinuation of NRTIs resulted in improved left ventricular function [80], supporting the working hypothesis of NRTI-induced CM.

The role of NRTI in CM in pediatric AIDS patients similarly remains controversial. Initially, Lipshultz *et al.* reported that impaired cardiac function was not attributed to zidovudine [83]. Other reports suggest that zidovudine CM in pediatric patients may be more prevalent than recognized [84]. However, skeletal muscle myopathy in zidovudine -treated children with AIDS is also uncommon [85]. In addition, some data from humans who received NRTIs *in utero* support the concept of NRTI-induced heart changes [86], whereas other reports [87,88] suggest that antenatal NRTI treatment is safe.

From a pathological perspective, mitochondrial ultrastructural changes were associated with NRTI-CM including enlargement and swelling with disrupted cristae and occasional paracrystalline inclusions [11,13,38,39]. The experimental literature has documented mitochondrial changes in selected tissues from rats, mice, other rodent species, and primates with a variety of NRTIs and dosing schedules [38,40,61,89–94].

10.5 ATHEROSCLEROSIS ASSOCIATED WITH ANTI-RETROVIRALS

Increased cardiovascular morbidity in HIV-infected individuals appears to be related to HAART where hyperlipidemia and hyperglycemia have been described [95–99]. Despite some clinical reports that suggest a relationship between atherosclerosis and use of PI therapy [100–104], unambiguous etiological associations remain to be defined.

Controversy persists in the understanding of the mechanisms of atherosclerosis in HIV/AIDS. One prospective study which included over 6000 patients revealed no difference in the rate of atherosclerosis in AIDS

patients treated with PIs [105]. In contrast, severe coronary atherosclerosis was found in patients treated with PIs with reported stenotic atherosclerotic lesions and/or coronary heart disease [100,106,107]. Another study reported nearly 75% incidence of demonstrated conditions (including diabetes mellitus, and elevated plasma cholesterol and triglycerides [108]) associated with increased risk of premature atherosclerosis following PI treatment [109]. Recent data support the hypothesis that both HIV infection and anti-retroviral treatment promote atherosclerosis [110, 111] and its clinical manifestations through inflammatory mechanisms involving endothelial cells (either directly or indirectly) and by the lipid alterations they induce [112,113].

PIs are competitive inhibitors of HIV aspartyl endopeptidase [114], an enzyme required for normal processing of the gag and gag-pol HIV proteins [115]. Several of the cleavage sites recognized by this protease on the gag-pol polypeptide include an aromatic amino acid that precedes proline. Most PIs have an analog of the phenylalanine-proline of gag-pol polyprotein [116], which is the basis for their inhibitory action.

Side effects include metabolic and endocrine abnormalities in PI-treated patients [117]. Mammalian enzymes involved in protein degradation have been shown to be inhibited by PIs [118–121]. Based on this, cellular mechanisms proposed to explain PI-induced dyslipidemia include inhibition of proteasomal degradation of apolipoprotein B leading to increased secretion of lipoproteins, and hepatic lipid synthesis [122,123].

Endothelial dysfunction may relate to the pathogenesis of atherosclerosis with PIs as it is suggested in the absence of PIs. Ritonavir and indinavir are able to cause endothelial dysfunction directly, with mtDNA damage and cell death [124]. Vascular cells involved in the pathogenesis of atherosclerosis may be directly affected by PIs. Several PIs, including amprenavir, ritonavir and indinavir, induce increased expression of CD36 in macrophages, leading to cholesteryl ester accumulation and enhanced foam cells formation, even in the absence of a dyslipidemic environment [125]. Vascular smooth muscle cells (VSMC) *in vitro* demonstrated altered function [126]. Impaired migration, proliferation and apoptosis have all been implicated in the pathogenesis of atherosclerosis.

Increased extracellular or intracellular production of ROS has been proposed as an inducer of VSMC apoptosis in atherosclerosis [127,128]. Therapeutically, increasing cellular antioxidant capacity by co-treating cells with either *N*-acetyl-cystein or with the antioxidant enzyme catalase significantly decreased ROS production and prevented apoptosis [129].

In addition to lipid metabolism, insulin may regulate vascular function [130,131]. Therefore, impairments in insulin action on the vasculature

may contribute to the pathogenesis of atherosclerosis. If insulin resistance plays a significant role in PI-induced side-effects, pharmacological (thiazolidinediones) [132] and non-pharmacological approaches (exercise, diet) to improve insulin action would benefit HIV-infected individuals undergoing PI treatment regimens [133–137].

Combined antiretroviral therapy containing NRTIs may also contribute to atherogenesis. Stavudine (d4T) treatment is associated with lipodystrophy [138]. Mechanisms are yet to be fully defined, but may involve altered mitochondrial biogenesis and/or oxidative changes [11,139].

10.6 NRTI-ASSOCIATED MI AND CHF

Myocardial infarction (MI) has become a matter of concern for HIV-positive patients. These individuals may have increased risk of cardiovascular disease as a result of the side effects associated with antiretrovirals, most notably vascular inflammation and dyslipidemia [140]. As mentioned, dyslipidemia has an established association with increased cardiovascular risk, even in the general population. However, in HIV-positive individuals, the risk may increase cumulatively with prolonged antiretroviral therapy.

The DAD study reported the risk of MI increased by 26% for every year on antiretroviral therapy (age-adjusted) [75]. This increased risk occurred for the treated group in all three antiretroviral drug classes (NRTIs, NNRTIs, and PIs) [141]. Other studies have examined changes in risk associating MI with NRTI/NNRTIs or PI use. Both retrospective and prospective studies have shown that the incidence of MI in HIV-positive individuals treated with antiretroviral therapy tends to be higher than in the general population, particularly in those receiving a PI-based treatment [142]. Contrasting results have been reported, and, therefore, it is unclear which drug class is or is not associated with increased risk for cardiovascular disease.

Some studies concluded that PI use resulted in increased risk of MI [95,143,144]. In contradiction to these results, one study with randomized clinical trials of four different PIs found that the stratified relative risk of MI was similar for patients receiving PIs versus those receiving NNRTIs alone [145]. Two other contrasting studies found no significant differences in the rates of hospitalization for MI among HIV-infected individuals before or after PI use [146] or any specific class of antiretrovirals [96].

A more recent DAD study found no significant associations between the development of MI and exposure to zidovudine, stavudine, or lamivudine, but an increased risk of MI in HIV-positive individuals exposed to abacavir and didanosine [147]. While the rate of MI in those exposed to abacavir and didanosine was evident while individuals were actually receiving the drugs as well as shortly after stopping them, it seemed to decrease within a few months of their cessation. This finding that the effect could be reversed on cessation of the drug supports a more rapidly acting underlying mechanism, possibly involving vascular inflammation, rather than a metabolic mechanism.

Abacavir does not cause clinically important changes in total or low-density lipoprotein (LDL) cholesterol or triglycerides and, therefore, is a favored treatment replacement for HIV-infected patients with pre-existing idopathic or PI-associated dyslipidemia (reviewed in [148]). However, despite abacavir being well tolerated in clinical trials, hypersensitivity reaction (HSR) to abacavir has been observed in approximately 5% of adult patients [149]. In the majority of the cases, the abacavir-related HSR occurred within the first six weeks of drug initiation and typically resolved without sequelae once abacavir was discontinued. Nevertheless, because an abacavir-related HSR may be life threatening, abacavir must be discontinued in suspected cases and patients should never be rechallenged. Several study groups have cited a strong association between abacavir-related HSR and HLA-B*5701 allele, especially among Caucasians [149–152]. Therefore, HLA-B*5701 testing is strongly recommended prior to starting abacavir treatment, to determine potential risk of individuals for abacavir-related HSR [153,154].

10.7 CONCLUSIONS

HAART has afforded long-term survival for infected individuals. Unfortunately, a consequence is increased prevalence of therapeutic side effects related to prolonged antiretroviral treatment. NRTIs in particular have reported side effects that include toxicity to mitochondria. Disrupted mtDNA replication, deletions or depletions can lead to mitochondrial dysfunction and ultimately to organ dysfunction. Cardiac myocytes are particularly susceptible to mitochondrial dysfunction, resulting in cardiomyopathy. In addition, PIs are associated with dyslipidemia and hyperglycemia which can contribute greater risk for atherogenesis in

HIV-positive individuals undergoing HAART. Clinical findings also demonstrate a growing increase in the reported incidence of MIs and cardiovascular disease related to vascular inflammation in patients treated with HAART.

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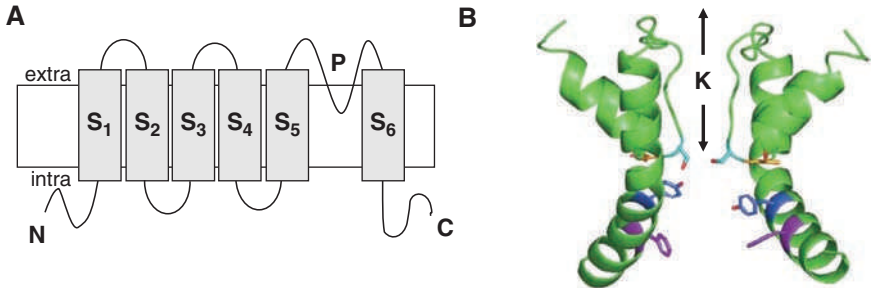

Plate 1 hERG subunit structure. A. Topological representation of a single hERG subunit; S_1 to S_6 cylinders indicate transmembrane regions of the channel; P indicates the loop within the pore region. B. Enlarged view of the pore region, which highlights important drug-binding residues, including Tyr652 (blue) and Phe656 (purple) in the S_6 domain, and Thr623 (orange) and Ser624 (cyan) near the selectivity filter. C. Sequence alignment of part of the S_6 domain of hERG and other K_v channels. Tyr652 and Phe 656 of the hERG channel are shown in blue and violet, respectively, and the Pro-Val-Pro motif of other K_V channels is shown in orange. (B and C are from Sanguinetti and Mitcheson, 2005, adapted with permission from Elsevier, Copyright 2005) (see Figure 3.3)

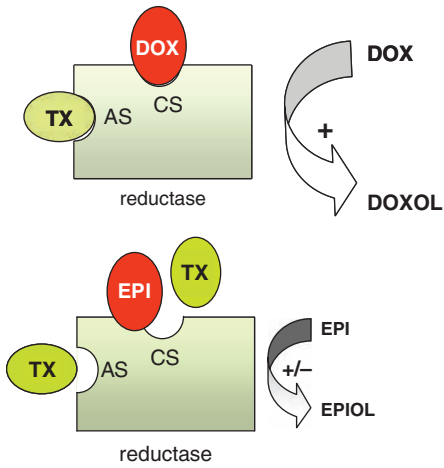

Plate 2 Kinetic simulation of active or defective taxane modulation of anthracycline metabolism in human myocardial strips. Upper panel shows that taxanes caused an accelerated conversion of DOX to DOXOL by binding with high affinity to the allosteric site of cytoplasmic aldehyde reductases. Bottom panel shows that taxanes failed to stimulate EPIOL formation because of the low affinity of EPI for the catalytic site and the possible competition of taxanes for such a site. Based on reference [19]. TX, taxane; AS, allosteric site; CS, catalytic site (see Figure 7.4)

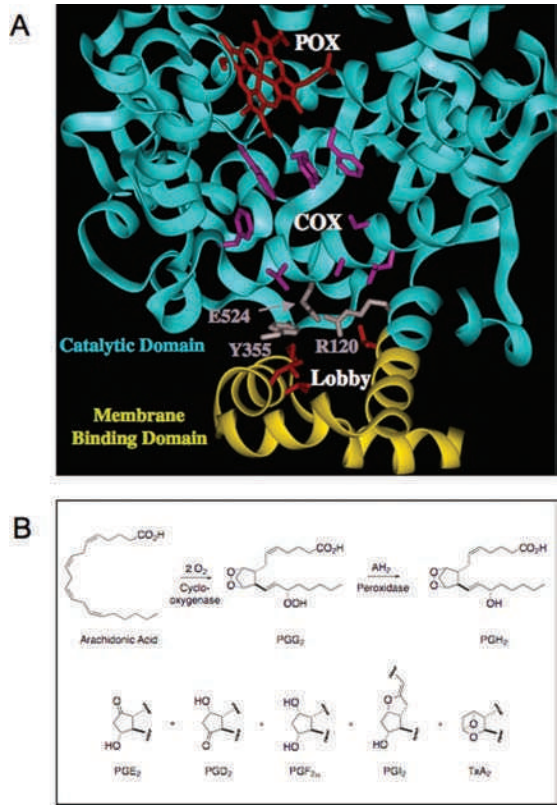

Plate 3 COX structure and reaction mechanisms. (A) The cyclooxygenase monomer is depicted with the membrane-binding domain in yellow and the catalytic domain in green. Residues that line the cyclooxygenase (COX) active site are shown in purple with the constriction site residues that separate the active site from the lobby shown in gray with labels. Residues in the lobby are shown in red. The peroxidase active site (POX) is identified by the presence of the heme. (B) The two sequential reactions of the cyclooxygenase enzymes are shown with the ultimate products of PGH_2 metabolism (see Figure 8.2)

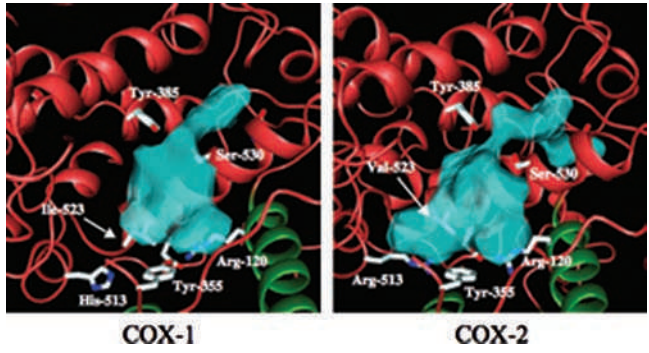

Plate 4 The solvent accessible space in the active sites of COX-1 and COX-2. A single amino acid substitution in the primary shell (Val-523 in COX-2 and Ile-523 in COX-1) along with several changes in the secondary shell allow for a larger active site in COX-2 and the existence of a "side pocket" where the diarylheterocycle COX-2 selective inhibitors bind [6]. Reprinted with permission, copyright 2007 The American Chemical Society (see Figure 8.5)