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CLINICAL REVIEW
Clinical update

Definition of hidden drug cardiotoxicity: paradigm change in cardiac safety testing and its clinical implications

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Unexpected cardiac adverse effects are the leading causes of discontinuation of clinical trials and withdrawal of drugs from the market. Since the original observations in the mid-90s, it has been well established that cardiovascular risk factors and comorbidities (such as ageing, hyperlipidaemia, and diabetes) and their medications (e.g. nitrate tolerance, adenosine triphosphate-dependent potassium inhibitor antidiabetic drugs, statins, etc.) may interfere with cardiac ischaemic tolerance and endogenous cardioprotective signalling pathways. Indeed drugs may exert unwanted effects on the diseased and treated heart that is hidden in the healthy myocardium. Hidden cardiotoxic effects may be due to (i) drug-induced enhancement of deleterious signalling due to ischaemia/reperfusion injury and/or the presence of risk factors and/or (ii) inhibition of cardioprotective survival signalling pathways, both of which may lead to ischaemia-related cell death and/or pro-arrhythmic effects. This led to a novel concept of 'hidden cardiotoxicity', defined as cardiotoxity of a drug that manifests only in the diseased heart with e.g. ischaemia/reperfusion injury and/or in the presence of its major comorbidities. Little is known on the mechanism of hidden cardiotoxocity, moreover, hidden cardiotoxicity cannot be revealed by the routinely used non-clinical cardiac safety testing methods on healthy animals or tissues. Therefore, here, we emphasize the need for development of novel cardiac safety testing platform involving combined experimental models of cardiac diseases (especially myocardial ischaemia/reperfusion and ischaemic conditioning) in the presence and absence of major cardiovascular comorbidities and/or cotreatments.

Keywords Toxicity • Safety • Cardiac • Heart • Ischaemia • Conditioning • Pre-conditioning • Post-conditioning • Comorbidity • Comedication • Remote conditioning

'Hidden cardiotoxicity': definition of term

Over the last 60 years, 462 medicinal products were withdrawn from the market for toxicity reasons, either worldwide or in one country only.[1] Deaths, hepatic, cardiac, and nervous system toxicity accounted for most of the drug withdrawals.[2] While among the withdrawn drugs are many analgesics, controversy still surrounds the use of some approved analgesics for pain management,[3] since they might induce cardiotoxicity at higher concentrations.[4] Thus drug-induced cardiotoxicity is a major problem, even occurring after introduction of the drug on the market. One explanation for these unwanted drug actions relates to the fact that current cardiac safety testing platforms focus on investigations of the unwanted actions of drug candidates on cardiac electrophysiology including some ion channels only in healthy animals/tissue ('direct toxicity'), while the effects of drugs on the heart (tissue), however, may be altered in the presence of comorbidities/cotreatments since they affect ion channel expression

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and/or activity, mitochondrial function, electro-mechanical coupling, and modification of extracellular matrix composition favouring the induction of arrhythmias, contractile dysfunction, and potentially cardiomyocyte death. Thus, toxic drug effects can be 'hidden' when safety testing is only done in healthy heart (tissue) but may become obvious in the diseased state ('hidden toxicity'). Thus, we define 'hidden cardiotoxicity' as toxicity that manifests only in the diseased state, e.g. in the heart during ischaemia/reperfusion injury and/or in the presence of major comorbidities leading to cardiovascular disease(s).

The major clinical importance of the novel concept of hidden cardiotoxicity is that it may lead to development of safety testing platforms that can detect hidden cardiotoxicity at the early pre-clinical stage, thereby preventing clinical trials and marketing of potentially cardiotoxic drugs, decrease the overall cost of development via increasing the success rate of drug development.

Drug-induced arrhythmias

Anti-arrhythmic drugs have been associated with relatively frequent pro-arrhythmic adverse effects for a long time. They may prolong the duration of repolarization and induce Torsades de Pointes (TdP) ventricular tachycardia that can degenerate into ventricular fibrillation,[5] or they may impair impulse conduction. On the other hand, there has been growing concern regarding the very rare provocation of TdP and sudden cardiac death by several non-cardiovascular drugs,[6] although the prevalence of arrhythmias associated with these non-cardiac drugs is very low (0.01–0.001%).

Unexpected pro-arrhythmic events associated with drug administration following myocardial infarction are best illustrated by the historical CAST and SWORD clinical trials that studied the effects of sodium and potassium channel inhibitor anti-arrhythmic drugs in post-myocardial infarction patients with impaired left ventricular function.[7,8] Both trials were discontinued before completion due to increased all-cause mortality in patients assigned to treatment. In addition to the well-known acute ventricular arrhythmias occurring within a few minutes to hours following myocardial infarction, arrhythmogenic structural, and electric remodelling of the heart develops in the course of days to weeks favouring arrhythmogenesis (for review, see ref.[9]) The remodelling process in the surviving border zone tissue causes slowed impulse conduction, abnormal cell-to-cell coupling, and generation of early after-depolarizations [due to fibrosis, reduced connexin expression, ion channel (sodium, calcium, potassium) down-regulation], all promoting the induction and maintenance of re-entry type arrhythmias.[10] It is conceivable, therefore, that cardiovascular and non-cardiovascular drugs with sodium channel blocking properties will further exacerbate these abnormalities (i.e. they induce unidirectional conduction block in tissue previously exhibiting slowed conduction) and can precipitate arrhythmias during ischaemia and following myocardial infarction. In this regard, some non-steroidal anti-inflammatory drugs (NSAIDs) and selective cyclooxygenase 2 (COX2) inhibitors were found to block cardiac ionic currents.[11,12] A meta-analysis by Trelle *et al.*[13] showed that most NSAIDs administered chronically increased morbidity and mortality in patients with cardiovascular disease. Clinically relevant cardiotoxicity is associated with the anti-emetics domperidone and metoclopramide due to their rather potent and local anaesthetic-like inhibition of cardiac sodium channels, leading to cardiovascular side effects such as malignant arrhythmias.[14] Inhibition of the hERG (human Ether-a-go-go Related Gene) channel by clozapine also results in clinically overt cardiotoxicity.[15,16]

'Hidden' cardiac electrophysiological toxic effects of drugs can be also based on impairment of the repolarization process, which contributes to the weakening of repolarization reserve and enhancement of the arrhythmia substrate. The concept of repolarization reserve suggests that myocardial repolarization is redundant, and congenital or acquired loss of function of a repolarizing current and/or gain of function of a depolarizing current may not manifest as marked QT-interval prolongation on the electrocardiogram because other repolarizing currents can compensate.[17,18] The repolarizing I_{Ks} potassium current was found to play a key role in repolarization reserve.[19,20] Importantly, as part of electrical remodelling in myocardial infarction, chronic heart failure, cardiac hypertrophy, diabetes mellitus, the down-regulation of various potassium currents was observed.[21–23] The possible combination of down-regulation, acute pharmacological block, or congenital loss of function of potassium channels—as multiple hits on repolarization—leads to impaired repolarization reserve and a consequent increase in susceptibility to ventricular arrhythmias.[18,24–26] In the presence of proper triggers, otherwise harmless non-cardiovascular drugs even with mild potassium channel blocking effects can provoke unexpected but serious ventricular arrhythmias and sudden cardiac death, as illustrated on *Figure 1*.

Diseases such as heart failure, hypertrophic cardiomyopathy, and ion channelopathies can provide arrhythmia trigger mechanisms as well. The expressions of sodium-calcium exchanger and the funny channel are enhanced in the failing myocardium.[27,28] Delayed after-depolarizations can develop and cause triggered activity in congestive heart failure due to spontaneous calcium leak from the sarcoplasmic reticulum.[29,30] Catecholaminergic polymorphic ventricular tachycardia triggers arrhythmias by abnormally increasing calcium release from the sarcoplasmic reticulum following beta-adrenergic stimulation as a consequence of mutations in the ryanodine receptor or calsequestrin.[31,32] Athlete's heart may represent a special example, where increased physical demand leads to compensatory electrical and structural remodelling manifested by cardiac hypertrophy,[33] interstitial myocardial fibrosis,[34] bradycardia,[35,36] and increased repolarization heterogeneity making these hearts more susceptible to arrhythmias following additional challenges such as non-cardiovascular drugs, dietary ingredients, or certain doping agents.[37]

Thus, the reliable assessment of pro-arrhythmic potential during drug development is essential. Current pre-clinical and clinical guidelines on cardiac electrophysiological safety testing advocate pro-arrhythmic potential studies in cell lines, healthy tissues, isolated hearts, animals, and healthy human volunteers, and mainly concentrate on hERG channel inhibition and repolarization prolonging effects of drug candidates,[38,39] not representing patients who exhibit increased arrhythmia susceptibility. There is an unmet need for more reliable models representing vulnerable patients for arrhythmias, with structural heart disease,[24] reduced repolarization reserve,[18] and/or other comorbidities. In addition, species dependent cardiac electrophysiological differences in pro-arrhythmia studies need to be considered when extrapolating results to humans.[40,41]

A selection of drugs found to cause unexpected serious ventricular arrhythmias and/or sudden cardiac death as 'hidden cardiotoxicity' is presented in *Table 1*.

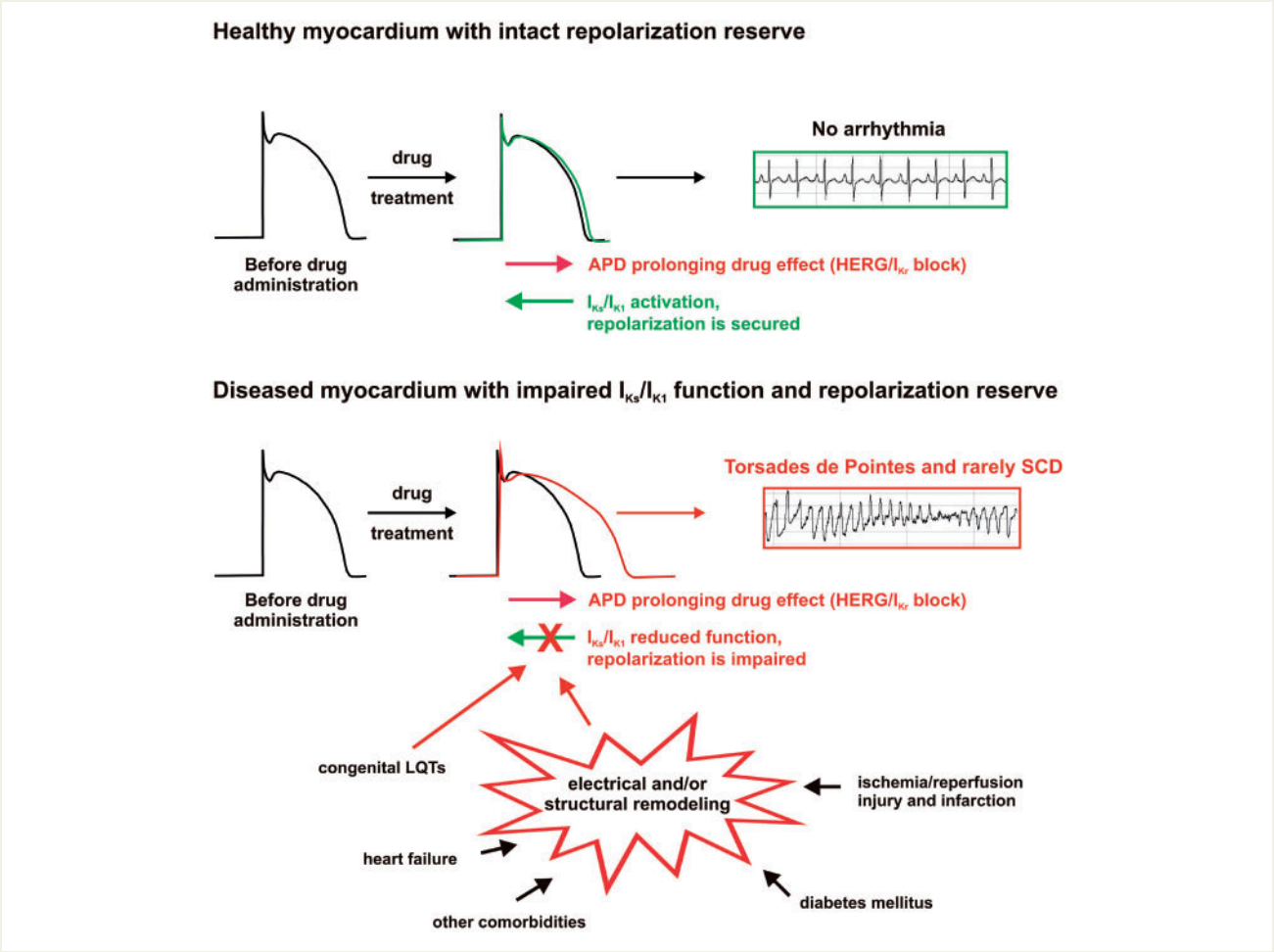

Figure 1 Schematic illustration of the role of impaired repolarization reserve in drug-induced arrhythmias in healthy and diseased cardiac tissue (hidden cardiotoxicity). In healthy myocardium (upper panel), the slow delayed rectifier (I_{Ks}), and the inward rectifier (I_{K1}) potassium currents, key components of repolarization reserve, counteract the mild repolarization prolonging (mostly due to hERG/I_{Kr} blocking) effect of drugs. Therefore, repolarization (action potential duration) is only slightly prolonged and no arrhythmias occur. The proarrhythmic side effect of the drug remains hidden in normal conditions. However, in the diseased heart (lower panel), a number of congenital, and acquired pathological conditions lead to electrical and/or structural remodelling featuring impaired function and/or down-regulation of repolarizing currents, consequently, leading to reduced repolarization reserve and increased arrhythmia susceptibility. Without the compensating effect of I_{Ks}/I_{K1} activation, drug administration can lead to lethal ventricular arrhythmias. The hidden cardiotoxicity of the drug is revealed.

Drug-induced cardiac dysfunction and/or irreversible myocardial injury

Cardiac dysfunction might occur either by (i) directly affecting cardiomyocyte function through modification of excitation-contraction coupling and/or intracellular calcium homeostasis and/or mitochondrial function[42] or (ii) alterations of loading conditions (preload reserve/afterload mismatch)[43] or heart rate (force-frequency relation)[44] or (iii) alterations of the extracellular matrix composition.[45]

Irreversible myocardial injury may develop via different types of cell death mechanisms such as necrosis, apoptosis, necroptosis, and possibly altered autophagy. Necrosis is an energy-independent process that results in the disintegration of cells in living tissue, which could be exacerbated in the presence of compounds with 'hidden cardiotoxicity'. The point of no return in necrosis is when the sufficient amount of energy for the maintenance of membrane potential and integrity is no longer available. The extent of necrotic tissue can be described either by histology,[46] magnetic resonance imaging,[47] or by measuring release of cellular components (e.g. lactate dehydrogenase, troponin I or T[48]). Apoptosis is an adenosine triphosphate-dependent, regulated process in which activation of effector caspases occur due to loss of mitochondrial membrane potential (intrinsic pathway) or activation of tumour necrosis factor receptors (exstrinsic pathway).[49,50] Apoptosis can be characterized by e.g. caspase 3 activation,[51] annexin-V externalization,[52] or the TUNEL assay.[53] Necroptosis, is a recently described form of caspase-independent programmed cell death,[54] which could also be assessed to further explore details of cell death mechanisms.[49,54] Autophagy is a pro-survival mechanism, which provides energy for cells via

Table 1 Selected examples of drugs associated with possible hidden cardiotoxicity based on adverse electrophysiological actions

Drug class	Compound	Possible arrhythmogenic mechanism(s)
Antibiotics	Erythromycin, clarithromycin	hERG inhibition
	Grepafloxacine, sparfloxacine	hERG inhibition
Antidepressants	Imipramine	I_{Na}, hERG inhibition
	Fluoxetine	I_{Na}, $I_{Ca,\ L}$, hERG current and trafficking block
	Citalopram	hERG current and trafficking inhibition
Antiepileptics	Retigabine	hERG, I_{Na} inhibition
	Lacosamide	I_{Na} inhibition
Antifungal agents	Fluconazole	hERG current and trafficking inhibition
Antihistamines	Astemizole	hERG inhibition
	Terfenadine	I_{Na}, hERG inhibition
Antimuscarinics	Terodiline	hERG inhibition
Antipsychotics	Haloperidol	hERG inhibition
	Risperidone	hERG inhibition
	Clozapine	hERG inhibition
$ß_2$-agonists	Salbutamol	hERG inhibition
NSAIDs	Diclofenac	I_{Na}, hERG, I_{Ks} inhibition
	Celecoxib	I_{Na}, hERG, I_{Ks} inhibition
Opioid analgesics	Methadone	I_{Na}, hERG inhibition
PDE inhibitors	Milrinone (PDE3 inhibitor)	cAMP dependent SR Ca^{2+} release, I_f activation
	Vardenafil (PDE5 inhibitor)	hERG inhibition
Prokinetics	Cisapride	hERG inhibition
Vasodilators	Bepridil	hERG, I_{Na} inhibition

hERG, human ether-a-go-go-related gene potassium current; I_f, hyperpolarization-activated cyclic nucleotide gated pacemaker 'funny' current; I_{Ks}, slow component of the delayed rectifier potassium current; I_{Na}, voltage-gated sodium current; NSAIDs, non-steroidal anti-inflammatory drugs; PDE, phosphodiesterase; SR, sarcoplasmic reticulum.

consuming their own components.[55] However, in a number of studies, excessive activation of autophagic processes resulted in apoptotic- or necrotic cell death.[56] Therefore, the autophagy should be determined as dynamic process, by assessing autophagic flux.[57] Drugs that may exacerbate these cell death signalling pathways in different conditions of comorbidities may potentially show hidden cardiotoxic effects, however, current pre-clinical safety testing does not require testing these pathways.

Direct vascular and/or cardiotoxicity

Apart from their arrhythmogenic potential, analysis of various pre-clinical data, meta-analysis and observational studies showed that COX2 inhibitors and NSAIDs increase the risk of vascular and cardiotoxicity.

Although COX2 is regarded an inducible enzyme, experimental and clinical studies suggest that COX2 is constitutively expressed in some tissues, among them in the vascular endothelium, where it contributes to the maintenance of vascular homeostasis and integrity.[58] Selective depletion of COX2 in vascular smooth muscle cells and endothelial cells depresses biosynthesis of prostaglandins and accelerates atherogenesis in low-density lipoprotein receptor knockout mice[59] and suppression of COX2 activity increases leucocyte adherence to endothelial cells of normo- and hypertensive rats[60] and increases smooth muscle cell calcification in mice with impaired kidney function.[61] Impairment of endothelial cell prostaglandin synthesis by COX2 inhibition elevates blood pressure[59,60] and diminishing COX2 expression or activity in hematopoietic cells can result in a predisposition to salt-sensitive hypertension.[62] Together with increased platelet reactivity following COX2 inhibition (for review, see ref.[63]) these effects might lead to an increase in vascular toxicity and cardiovascular risk (for review, see ref.[64])

The vascular and/or cardiotoxic risk depends on the dose, duration, and frequency of NSAID administration.[65] For example, the NSAID diclofenac induces proteasome and mitochondrial dysfunction in murine cardiomyocytes and hearts leading to an increase in reactive oxygen species (ROS) formation and altered protein turnover.[66] The reduction of the dose of NSAIDs may mitigate, but not avoid, the risk of cardiovascular adverse effects.[67]

Numerous commonly used drugs such as certain anticancer medications [anthracyclines—(Doxorubicin/Adriamycin), cisplatin (Platinol), trastuzumab (Herceptin), imatinib (Gleevec), mitoxantrone (Novantrone), arsenic trioxide (Trisenox), bevacizumab (Avastin), sunitinib (Sutent), and sorafenib (Nevaxar)], the antiretroviral compound azidothymidine (AZT, Zidovudine), and several oral antidiabetics [e.g. rosiglitazone (Avandia)], likewise various substances of abuse [e.g. alcohol, methamphetamine, ecstasy, cocaine, and synthetic cannabinoids (K2, spice)] may induce direct cardiotoxicity.[68] This cardiotoxicity is sometimes dose- and time-dependent, but may also develop unpredictably years after the initial drug exposure, more frequently in patients with cardiovascular comorbidities (*Figure 2*).

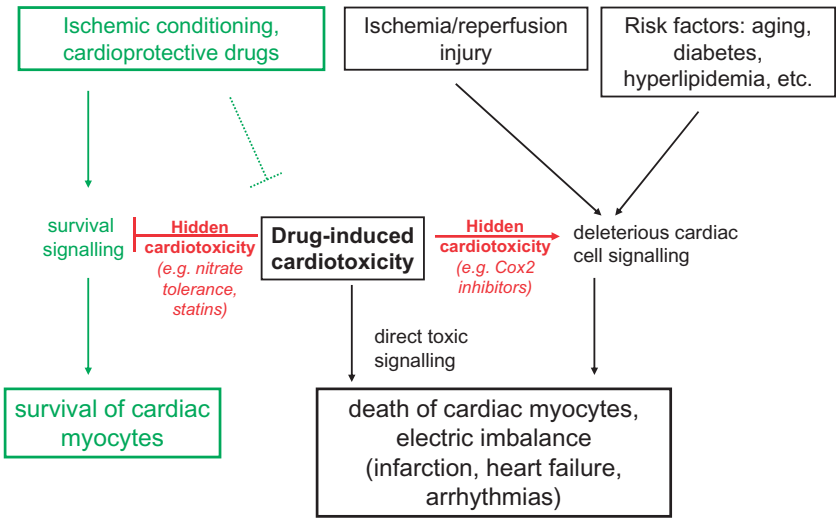

Figure 2 Influence of ischemia/reperfusion injury and cardiovascular risk factors on cardiotoxic effects of drugs. Hidden cardiotoxicity of a drug is revealed if the drug inhibits cell survival signalling or activates deleterious cell signalling induced by cardiac diseases especially ischemia/reperfusion injury and/or its major risk factors including their comedications. APD, action potential duration; HERG, human ether-a-go-go-related gene potassium channel; LQTs, long QT syndromes; SCD, sudden cardiac death.

Multiple lines of evidence suggest that direct or indirect mitochondria-related toxicity is an important common effector mechanism of drug-induced direct cardiotoxicity. Mitochondrial toxicity may develop as a consequence of interference with the mitochondrial respiratory chain (e.g. uncoupling) or due to inhibition of the important mitochondrial enzymes (oxidative phosphorylation, Szent-Györgyi–Krebs cycle, mitochondrial DNA replication) among others. All these may facilitate increased generation of mitochondrial ROS, calcium overload, depletion of cellular nicotinamide-adenine-dinucleotide (NAD+) and adenosine triphosphate, and opening of the mitochondrial permeability transition pore with consequent triggering of apoptotic and/or necrotic cell death pathways.[68]

Doxorubicin is still a commonly used effective and broad spectrum antineoplastic agent despite its dose limiting cumulative cardiotoxicity. Among all cardiotoxic agents the mechanisms of doxorubicin-induced cardiotoxicity are among the best characterized, yet very complex and not completely understood. These will be briefly discussed in the following paragraphs, while for the discussion of the mechanisms of other direct cardiotoxic drugs, we would like to refer readers to recent overviews on the subject.[68–70]

Cardiomyocytes and endothelial cells are particularly sensitive to the direct toxic effects of doxorubicin. In the mitochondria of these cells doxorubicin via non-enzymatic redox cycling[71–74] or iron-dependent[75–77] processes triggers increased generation of ROS (e.g. superoxide anion). Mitochondrial iron accumulation due to defective function of ABCB8, a mitochondrial protein that facilitates iron export, may also contribute to the deleterious effects of doxorubicin in cardiomyocytes.[77] Superoxide anion can be converted to hydrogen peroxide by mitochondrial superoxide dismutase or via diffusion-limited reaction it can rapidly react with nitric oxide to form peroxynitrite,[78] a potent oxidant and cytotoxic reactive nitrogen species (RNS) that promotes mitochondrial protein oxidation/nitration and initiation of cell death pathways.[79,80] Doxorubicin can also directly bind to mitochondrial abundant phospholipid, cardiolipin and can form adducts with mitochondrial DNA,[81] and activate matrix metalloproteinases.[82] Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 2 has also been proposed to contribute to doxorubicin-induced ROS generation in the heart.[79,83,84]

The doxorubicin-induced cardiotoxicity also involves disruption of key antioxidant mechanisms. Conversely, interventions aimed to enhance the key antioxidant defence systems (e.g. manganese superoxide dismutase[85]; catalase[86]; metallothionein,[87] thioredoxin-1[88]; glutaredoxin 2[89]; and glutathione levels[90]) and to neutralize the mitochondrial ROS/RNS by mitochondrialy targeted antioxidants[91] have demonstrated cardioprotective effects in rodent models of doxorubicin-induced cardiomyopathy, the latter without interference with its antitumour activity. The doxorubicin induced increased ROS/RNS generation coupled with impaired antioxidant defence eventualy leads to oxidative DNA injury and consequent activation of the nuclear enzyme poly(ADP)-ribose polymerase 1 (PARP-1) resulting in cellular depletion of NAD+ and adenosine triphosphate triggering cell death (both apoptotic or necrotic).[92] Poly(ADP)-ribose polymerase 1 genetic deletion and inhibition is protective against doxorubicin-induced cardiotoxicity in mice[92,93] Logically PARP inhibitors (e.g. the Federal Drug Administration approved anticancer drug olaparib to treat specific forms of ovarian cancer), could be combined with doxorubicin or cisplatin, due to potentially increased chemotherapeutic efficacy and decreased cardiotoxicity.[68,92–94]

Cardiomyocytes as non-dividing cells are considerably less sensitive to the topoisomerase inhibiting adverse effect of doxorubicin. However, the topoisomerase isoenzyme IIβ is essential in maintaining normal transcriptional activity in cardiomyocytes, and it has specific function in the maintenance of mitochondrial DNA, allowing

mitochondrial transcription and replication.[95] Accordingly, this enzyme is critically involved in cardiomyocyte-specific toxicity of doxorubicin.[96]

The above mentioned examples of doxorubicin-induced dose-dependent cumulative cardiotoxicity illustrate the complexity and the need for better understanding the common mechanisms of drug-induced direct cardiotoxicity to develop more effective screening strategies[97] and models[98,99] both in the clinical[100,101] as well as in the pre-clinical settings. These efforts should also focus on identification of toxicity biomarkers,[102] patient at risk,[103] development of more efficient targeted drug delivery systems[104–106] allowing reduction of the dose, and use of personalized prophylactic cardioprotective therapies.[107]

'Hidden toxicity'

Since the original observations in the mid-90s, it has been well established that cardiovascular risk factors and comorbidities and their medications may interfere with cardiac ischaemic tolerance and endogenous cardioprotective signalling pathways by several cellular mechanisms including robust changes in cardiac gene expression profile at the transcript level [coding and non-coding ribonucleic acid (RNA)s] including transcripts of ion channels, enzymes involved in mitochondrial energy metabolism, transcription factors, etc. (for extensive reviews, see refs[108–112]) Therefore, drugs may exert 'hidden' cardiotoxic actions on the diseased heart via interfering with cell death and cardioprotective signalling.[109,110] Some examples of drugs that show(ed) 'hidden cardiotoxic' effects that can be evidenced only in the comorbid, ischaemic heart are provided below.

The hidden cardiotoxic effect of a compound has been proven for the first time by an elegant study by Golomb *et al.*[113] They showed that that subtoxic dosage of a known 'direct' cardiotoxic agent bis(2-chloroethoxy)methane may cause 'hidden' cardiotoxicity as revealed by impaired mitochondrial function only under ischaemic conditions.

Nitrate tolerance developing due to long-term use of nitrates, long-term use of statins, ATP-dependent potassium channel blocker anti-diabetic drugs, and COX2 inhibitors have been shown to interfere with ischaemia/reperfusion injury and the effect of endogenous cardioprotection (for reviews, see refs[109–111]) High-dose glyceryl trinitrate-induced nitrate tolerance blocked both pre- and post-conditioning[114,115] and long-term use of statins antagonized the cardioprotective effect of ischaemic post-conditioning.[116] Several studies demonstrated that ATP-dependent potassium channel blockers increase ischaemia/reperfusion injury and block the cardioprotective effect of ischaemic conditioning. Thus, it might not be surprising that ATP-dependent potassium channel blockers increase the risk of major adverse cardiac events and cardiovascular death in diabetic patients (especially with concomitant heart disease).[117] Angiotensin converting enzyme (ACE) inhibitors reduce irreversible ischaemia/reperfusion injury, delay heart failure progression and are additive to or restore endogenous cardioprotection.[118,119] Angiotensin converting enzyme transforms angiotensin I to angiotensin II, and also promotes the degradation of bradykinin into inactive metabolites. Bradykinin stimulates nitric oxide synthesis and synthesis of vasodilator prostaglandin via a COX pathway. Moreover, COX2 activation is also involved in endogenous cardioprotective signalling.[120] COX inhibitors may therefore be deleterious in cardiovascular disease by counteracting part of ACE inhibitor efficacy. This has been clearly demonstrated with NSAIDs in hypertension, coronary artery disease, and chronic heart failure and most guidelines recommend avoiding their use in such patients.[121]

Apart from its direct cardiotoxic effects (as outlined above), doxorubicin depletes GATA-4, which in turn causes cardiomyocyte apoptosis.[122] Endogenous cardioprotection increased GATA-4 expression and activity in the heart, thereby increasing affecting cardiomyocyte survival.[123] Thus, depletion of GATA-4 by doxorubicin might interfere with endogenous cardioprotection and thus add a component of 'hidden toxicity' to the well-established direct toxicity of doxorubicin.

Need for novel assays to predict cardiotoxicity thereby increasing drug safety

Novel assays for early pre-clinical detection of cardiotoxicity of drugs are of great importance to increase success rate of drug development and patient safety. Using three-dimensional cardiac tissues derived from human-induced pluripotent stem cells (3D-hiPSC-CT) a doxorubicin-sensitive cytotoxicity and hERG channel blocker-sensitive change in electrical activity was detected, indicating its potential usefulness as drug screening system for drug discovery[124] (for review, see ref.[125]) Similarly, using hiPSC-cardiomyocytes, drug effects on ROS production, intracellular calcium concentration, formation of DNA double strand breaks, gene or micro RNA expression, and electrophysiological properties can be quantified[102,126,127] and together with parallel assessment of motion field imaging-derived contractile properties thus allow a better risk estimation of cardiotoxic drug effects.[128,129] In hiPSC-cardiomyocytes exposed to doxorubicin changes in microRNA expression occurred before the occurrence of cytotoxicity markers such as lactate dehydrogenase, and the affected microRNAs also demonstrated a significant involvement in heart failure in patients and animal models.[102] Thus, early changes in microRNA expression might also allow to predict cardiotoxicity in patients.[130–132]

However, all of these detection assays fail to address the issue of the importance of comorbidities and cotreatments and thus do not detect 'hidden' cardiotoxicity of drugs.

Therefore, we urge the need for development of novel cardiac safety testing platforms involving combined experimental models of various cardiac diseases, especially myocardial ischaemia/reperfusion and ischaemic conditioning in the presence and absence of major cardiovascular risk factors and comorbidities such as e.g. ageing, hyperlipidaemia, and diabetes and their major cotreatments. Although these additional tests will definitely increase the time and cost for pre-clinical safety testing, via the early detection of hidden cardiotoxicity of drugs it will ultimately lead to (*Figure 3*):

- overall saving of time and cost of drug development for the pharmaceutical industry by early pre-clinical termination of the development of potentially cardiotoxic compounds;
- increasing success rate of clinical drug development by more rational design of clinical trials to enroll patients that are not prone to manifest certain cardiotoxic side effects of a drug with potential hidden cardiotoxity in a disease condition;

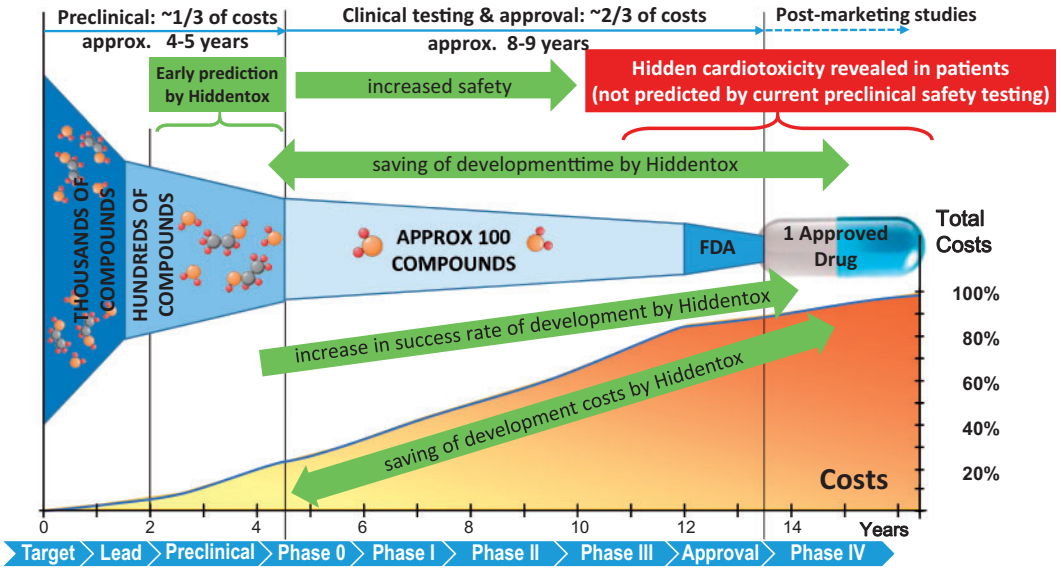

Figure 3 Benefits of pre-clinical prediction of hidden cardiotoxicity by pre-clinical testing platforms (Hiddentox) in drug development. Hiddentox may lead to potential savings of drug development cost and development time by timely pre-clinical termination of compounds that show hidden cardiotoxic properties in diseased experimental models. Moreover, Hiddentox may increase success rate of drug development by pre-clinical determination of certain comorbidities in the presence of which hidden cardiotoxicity may manifest, thereby, improving knowledge for rational design of clinical trials on targeted patient populations. Finally, Hiddentox will increase patient safety during clinical trials and clinical use of drugs in the market by preventing potentially cardiotoxic drugs entering into clinical trials or to market.

- increased patient safety by preventing the clinical testing and clinical use of potentially cardiotoxic drugs in patient populations that are prone to manifest hidden cardiotoxicity.

As an example, in case the potential cardiotoxic effect of rofecoxib (Vioxx) were detected by assays for hidden cardiotoxicity in the early pre-clinical phase of its development, the manufacturer company could have saved significant amount of resources burnt for the development of rofecoxib and for the still ongoing legal issues related to its withdrawal from the market in 2004.[133,134] Early prediction of hidden cardiotoxicity of rofecoxib could have prevented the unexpected manifestation of myocardial infarction of some patients taking Vioxx. However, more than a decade after its withdrawal, the mechanism of hidden cardiotoxicity of rofecoxib is still a question of debate. However, to increase the productivity of drug development, we definitely need to increase knowledge on mechanisms and early prediction of drug toxicity (*Figure 3*).[135,136]

Conclusion and outline

Cardiotoxicity seen only in the diseased heart with e.g. ischaemia/reperfusion injury and/or in the presence of its major comorbidities is termed as 'hidden cardiotoxicity'. Little is known on the mechanism of hidden cardiotoxicity and 'hidden cardiotoxicity' cannot be revealed by the routinely used cardiac safety testing methods on healthy animals or tissues. Therefore, here, we emphasize the need for development of novel cardiac safety testing platforms involving combined experimental models of cardiac diseases, especially myocardial ischaemia/reperfusion and ischaemic conditioning in the presence and absence of major cardiovascular risk factors and comorbidities such as e.g. ageing, hyperlipidaemia, and diabetes and their cotreatments.

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