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

Péter Ferdinandy1,2*, István Baczkó3, Péter Bencsik2, Zoltán Giricz1,2, Anikó Görbe1,2, Pál Pacher4, Zoltán V. Varga1,4, András Varró3, and Rainer Schulz5*

1Department of Pharmacology and Pharmacotherapy, Semmelweis University, Nagyvárad tér 4, Budapest 1089, Hungary; 2Pharmahungary Group, Hajnoczy u. 6, Szeged 6722, Hungary; 3Department of Pharmacology and Pharmacotherapy, University of Szeged, Dóm tér 12, Szeged 6720, Hungary; 4Laboratory of Cardiovascular Physiology and Tissue Injury, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, 5625 Fishers Lane, Bethesda, MD 20892-9413, USA; and 5Institute of Physiology, Justus-Liebig University of Giessen, Aulweg 129, 35392 Giessen, Germany

Received 8 January 2018; revised 12 March 2018; editorial decision 29 April 2018; accepted 11 June 2018; online publish-ahead-of-print 2 July 2018

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 cardiotoxicity 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 cardiotoxicity, 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 analogesics, controversy still surrounds the use of some approved analogesics 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 comorbidity/cotreatments since they affect ion channel expression.
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,^9^ 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.7). 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 afterdepolarizations 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, 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.
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 function42 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 T48). 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 (extrinsic 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...
testing these pathways. However, current pre-clinical safety testing does not require conditions of comorbidities may potentially show hidden cardiotoxicity. Therefore, the autophagy should be determined as dynamic process, by assessing autophagic flux. 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. 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 and suppression of COX2 activity increases leukocyte adherence to endothelial cells of normo- and hypertensive rats and increases smooth muscle cell calcification in mice with impaired kidney function. Impairment of endothelial cell prostaglandin synthesis by COX2 inhibition elevates blood pressure and diminishing COX2 expression or activity in hematopoietic cells can result in a predisposition to salt-sensitive hypertension. 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. 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. The reduction of the dose of NSAIDs may mitigate, but not avoid, the risk of cardiovascular adverse effects.

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 (Nexavar)], the antiretroviral compound azidothymidine (AZT, Zidovudine), and several oral antidiabetics [e.g. rosiglitazone (Avandia)], the antiretroviral compounds (AZT, Zidovudine), and several oral antidiabetics [e.g. rosiglitazone (Avandia), sunitinib (Sutent), and sorafenib (Nexavar)], the antiretroviral compound azidothymidine (AZT, Zidovudine), and several oral antidiabetics (Figure 2).

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

| Drug class          | Compound                  | Possible arrhythmic mechanism(s)                          |
|---------------------|---------------------------|-----------------------------------------------------------|
| Antibiotics         | Erythromycin, clarithromycin | hERG inhibition                                           |
|                     | Grepafloxacin, sparflaxine | hERG inhibition                                           |
| Antidepressants     | Imipramine                | hERG, hERG inhibition                                      |
|                     | Fluoxetine                | hERG current and trafficking block                        |
|                     | Citalopram                | hERG current and trafficking inhibition                   |
| Antiepileptics      | Retigabine                | hERG, hERG inhibition                                      |
|                     | Lacosamide                | hERG inhibition                                           |
| Antifungal agents   | Fluconazole               | hERG current and trafficking inhibition                   |
| Antihistamines      | Astemizole                | hERG inhibition                                           |
|                     | Terfenadine               | hERG inhibition                                           |
| Antimuscarinics     | Terodiline                | hERG inhibition                                           |
| Antipsychotics      | Haloperidol               | hERG inhibition                                           |
|                     | Risperidone               | hERG inhibition                                           |
|                     | Clozapine                 | hERG inhibition                                           |
| ß2-agonists         | Salbutamol                | hERG inhibition                                           |
| NSAIDs              | Diclofenac                | hERG, hERG inhibition                                      |
|                     | Celecoxib                 | hERG, hERG inhibition                                      |
| Opioid analgesics   | Methadone                 | hERG inhibition                                           |
| PDE inhibitors      | Milrinone (PDE3 inhibitor) | cAMP dependent SR Ca ++ release, I 
|                     | Vardenafil (PDE5 inhibitor)| hERG inhibition                                           |
| Prokinetics         | Cisapride                 | hERG inhibition                                           |
| Vasodilators        | Bepridil                  | hERG, hERG inhibition                                      |

hERG, human ether-a-go-go-related gene potassium current; I f, hyperpolarization-activated cyclic nucleotide gated pacemaker ‘funny’ current; I k, 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.
Introduction

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 cycling71–74 or iron-dependent75–77 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 dismutase85, catalase86, metallothionein,87 thioredoxin-188; and glutaredoxin289; and glutathione levels90) and to neutralize the mitochondrial ROS/RNS by mitochondrially targeted antioxidants91 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 eventually 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 mice92,93 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 isozyme 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.\textsuperscript{95} Accordingly, this enzyme is critically involved in cardiomyocyte-specific toxicity of doxorubicin.\textsuperscript{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\textsuperscript{97} and models\textsuperscript{98,99} both in the clinical\textsuperscript{100,101} as well as in the pre-clinical settings. These efforts should also focus on identification of toxicity biomarkers,\textsuperscript{102} patient at risk,\textsuperscript{103} development of more efficient targeted drug delivery systems\textsuperscript{104–106} allowing reduction of the dose, and use of personalized prophylactic cardioprotective therapies.\textsuperscript{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 (RNAs)] including transcripts of ion channels, enzymes involved in mitochondrial energy metabolism, transcription factors, etc. (for extensive reviews, see refs\textsuperscript{108–112}). Therefore, drugs may exert ‘hidden’ cardiotoxic actions on the diseased heart via interfering with cell death and cardioprotective signalling.\textsuperscript{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.\textsuperscript{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\textsuperscript{109–111}). High-dose glyceryl trinitrate-induced nitrate tolerance blocked both pre- and post-conditioning\textsuperscript{114,115} and long-term use of statins antagonized the cardioprotective effect of ischaemic post-conditioning.\textsuperscript{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).\textsuperscript{117} Angiotensin converting enzyme (ACE) inhibitors reduce irreversible ischaemia/reperfusion injury, delay heart failure progression and are additive to or restore endogenous cardioprotection.\textsuperscript{118,119} Angiotensin converting enzyme transforms angiotensin I to angiotensin II, and also pro- motes 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.\textsuperscript{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 more guidelines recommend avoiding their use in such patients.\textsuperscript{121}

Apart from its direct cardiotoxic effects (as outlined above), doxorubicin depletes GATA-4, which in turn causes cardiomyocyte apoptosis.\textsuperscript{122} Endogenous cardioprotection increased GATA-4 expression and activity in the heart, thereby increasing affecting cardiomyocyte survival.\textsuperscript{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\textsuperscript{124} (for review, see ref\textsuperscript{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\textsuperscript{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.\textsuperscript{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.\textsuperscript{102} Thus, early changes in microRNA expression might also allow to predict cardiotoxicity in patients.\textsuperscript{130–132}

However, all of these detection assays fail to address the issue of the importance of comorbidities and co-treatments 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 co-treatments. 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 cardiotoxicity in a disease condition;
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.

Funding

P.F. is the vice chair and R.S. is a working group leader of the European Co-operation in Science and Technology [COST action CA16225, EU-Cardioprotection]. P.F. holds grants from the Hungarian National Research, Development, and Innovation Office [OTKA K 109737, OTKA KH_17 125570, NVKP 16-1-2016-0017, and VEKOP-2.3.2-16-2016-00002]. R.S. holds grants from the German Research Foundation [CRC 1213, B05]. P.F. was supported by the Higher Education Institutional Excellence Programme of the Ministry of Human Capacities in Hungary, within the framework of the Therapeutic Development thematic programme of the Semmelweis University. Z.V.V. was supported by the Premium Postdoctoral Fellowship Program of the Hungarian Academy of Sciences.

Conflict of interest: P.F. is the founder and CEO of Pharmahungary Group, a group of R&D companies. R.S. received honoraria for lecturing from Sanofi.

References

1. Onakpoya IJ, Heneghan CJ, Aronson JK. Post-marketing withdrawal of 462 medicinal products because of adverse drug reactions: a systematic review of the world literature. BMC Med 2016;14:10.
2. Onakpoya IJ, Heneghan CJ, Aronson JK. Worldwide withdrawal of medicinal products because of adverse drug reactions: a systematic review and analysis. Crit Rev Toxicol 2016;46:477–489.
3. Onakpoya IJ, Heneghan CJ, Aronson JK. Post-marketing withdrawal of analgesic medications because of adverse drug reactions: a systematic review. Expert Opin Drug Saf 2018;17:63–72.
24. Vos MA, de Groot SH, Verduyn SC, van der Zande J, Leunissen HD, Cleutjens JG. The role of the delayed rectifier component IKs in dog ventricular muscle repolarization reserve in mammalian heart. J Physiol 2000; 528:121–131.

21. Li GR, Lau CP, Leung TK, Nattel S. Ionic current abnormalities associated with repolarization reserve in mammalian heart: heart failure, myocardial infarction, and atrial fibrillation. Circulation 2004; 109:2437–2443.

18. Waldo AL, Camm AJ, deRuyter H, Friedman PL, MacNeil DJ, Pauls JF, Pitt B, Pratt CM, Schwartz PJ, Veltri EP. Effect of d-sotalol on mortality in patients with left ventricular dysfunction after recent and remote myocardial infarction. The SWORD Investigators. Survival With Oral d-Sotalol. Circulation 1996; 94:1517–1523.

10. Benito B, Gay-Jordi G, Serrano-Mollar A, Guasch E, Shi Y, Tardif JC, Brugada J, Nattel S. Persistent long-QT syndrome caused by a de novo missense mutation in human KCNQ1. Circulation 1999; 99:2284–2289.

7. Cardiac Arrhythmia Suppression Trial (CAST) Investigators. Preliminary report: effect of encainide and flecainide on mortality in a randomized trial of arrhythmia suppression after myocardial infarction. N Engl J Med 1989; 321:406–412.

4. Faria J, Barbosa J, Leal S, Afonso LP, Lobo J, Moreira R, Queiros O, Carvalho F, Dinis-Oliveira RJ. Effective analgesic doses of tramadol or tapentadol induce QTc prolongation and pro-arrhythmia by non-anti-arrhythmic drugs: clinical and regulatory implications. Report on a Policy Conference of the European Society of Cardiology. Circulation 2000; 102:219–233.

2. Haverkamp W, Breshardt G, Camm AJ, Janse MJ, Rosen MR, Antzelevitch C, Escande D, Franz M, Malik M, Moss A, Shah R. The potential for QT prolongation and pro-arrhythmia by non-anti-arrhythmic drugs: clinical and regulatory implications. Report on a Policy Conference of the European Society of Cardiology. Circulation 2000; 102:219–233.

27. Studer R, Reinecke H, Bilger J, Eschenhagen T, Bohn M, Hasenfuss G, Just H, Holz J, Drexlter H. Gene expression of the cardiac Na(+)–Ca(2+) exchanger in end-stage human heart failure. Circ Res 1994; 75:443–453.

28. Cerbai E, Pino R, Porciatti F, Sani G, Toscano M, Maccherini M, Guinti G, Mugelli A. Characterization of the hyperpolarization-activated current, I(f), in ventricular myocytes from human failing heart. Circulation 1997; 95:548–571.

29. Vennervald JT, McGuirk SA, Stroobants S, de Belder M, Copping C, Janse MJ. Triggered activity and automaticity in ventricular trabeculae of failing human and rabbit hearts. Cardiovasc Res 1994; 28:1547–1554.

30. Shannon TR, Pogwizd SM, Ber DS. Elevated sarcoplasmic reticulum Ca2+-leak in intact ventricular myocytes from rabbits in heart failure. Circ Res 2003; 93:592–598.

31. Priori SG, Napolitano C, Menin M, Colombo D, Brigo F, Gasparini M, DeSimone L, Coltorti F, Bisleo R, Keegan R, Cruz Filho FE, Vignati G, Benatar A, Delugio C. Clinical and molecular characterization of patients with catecholaminergic polymorphic ventricular tachycardia. Circulation 2002; 106:69–74.

32. Elder M, Pras E, Lahtah A. A nonsense mutation in the CASQ2 gene is associated with autosomal-recessive catecholamine-induced polymorphic ventricular tachycardia. Trends Cardiovasc Med 2003; 13:148–151.

33. Atchley AE Jr, Douglas PG. Left ventricular hypertrophy in athletes: morphologic features and clinical correlates. Cardiol Clin 2007; 25:371–382, v.

34. Benito B, Gay-Jordi G, Serrano-Mollar A, Guasch E, Shi Y, Tardif JC, Brugada J, Nattel S, Mont L. Cardiac arrhythmia remodeling in a rat model of long-term intensive exercise training. Circulation 2011; 123:12–22.

35. D’Souza A, Buch CL, McCray TT, Cordova ME, Scholfield DJ, Schaper D, Sweeney DL, Wu X, Bennett H, Robertson A. A missense mutation in the KCNJ2 gene is associated with acquired torsade de pointes arrhythmias in the dog with left ventricular dysfunction. Circulation 2003; 108:1125–1135.

36. D’Souza A, Pearman CM, Wang Y, Nalcao S, Loganaga SJR, Cox C, Bennett H, Zhang Y, Johnson AB, Linscheid NP, Poulsen PC, Elliott J, Coulson M, McPhee J, Robertson A, de Costa Martins PA, Kitimoto A, Wislaff U, Cartwright EJ, Monfredi O, Lundby A, Dobryanski H, Oceandy D, Morris GM, Boyett MR. Exercise training reduces resting heart rate via downregulation of the funny channel HCN4. Nat Commun 2014; 5:3775.

37. D’Souza A, Pearman CM, Wang Y, Nalcao S, Loganaga SJR, Cox C, Bennett H, Zhang Y, Johnson AB, Linscheid NP, Poulsen PC, Elliott J, Coulson M, McPhee J, Robertson A, de Costa Martins PA, Kitimoto A, Wislaff U, Cartwright EJ, Monfredi O, Lundby A, Dobryanski H, Oceandy D, Morris GM, Boyett MR. Targeting mIR-423-3p reverses exercise training-induced HCN4 channel remodeling and sinus bradycardia. Circ Res 2017; 121:1058–1068.

38. Varro A, Baczko I. Possible mechanisms of sudden cardiac death in top athletes: a basic cardiac electrophysiological point of view. Pfugers Arch 2010; 460:31–40.

39. Food and Drug Administration, HHS. International Conference on Harmonisation: guidance on S7B Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals: availability. Notice. Fed Regist 2005; 70:61133–61134.

40. Food and Drug Administration, HHS. International Conference on Harmonisation: guidance on E14 Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarhythmic Drugs: availability. Notice. Fed Regist 2005; 70:61134–61135.

41. Jost N, Virag L, Comtos P, Ordog B, Szuts V, Bitter V, Horvath T, Janz J, Nattel S. Persistent long-QT syndrome caused by a de novo missense mutation in human KCNQ1. Circulation 1999; 100:1392–1397.

42. Zicha S, Moss I, Allen B, Varro A, Papp J, Dumaine R, Antzelevich C, Nattel S. Mechanisms of cardiac contraction. What roles for preload, afterload and inotropic state in heart failure? Eur Heart J 1983; 14:19–28.

43. Endoh M. Force-frequency relationship in intact mammalian ventricular myocytes in vivo: an in vivo study with magnetic resonance imaging. Circulation 1993; 87:2455–2461.

44. Endoh M. Force-frequency relationship in intact mammalian ventricular myocytes in vivo: heart failure, myocardial infarction, and atrial fibrillation. Circulation 2004; 109:2437–2443.

45. Villars PS, Hamlin SK, Shaw AD, Kanusky JT. Role of diastole in left ventricular function, I: biochemical and biomechanical events. Am J Physiol 2004; 286:H1641–H1649.
Definition of hidden drug cardiotoxicity

49. Giricz Z, Koncso G, Rajtki T, Varga ZV, Baranyai T, Csonka S, Szob A, Adameova A, Gottlieb RA, Ferdinandy P. Hypercholesterolemia downregulates autophagy in the rat heart. Lipids Health Dis 2017;16:60.

50. Bartekova M, Smovickov P, Fagarassyova M, Ivanova M, Okruhuilova L, Tribulova N, Dovinova I, Barancik M. Quercetin restores postischemic recovery of heart function in doxorubicin-treated rats and prevents doxorubicin-induced matrix metalloproteinase-2 activation and apoptosis induction. Int J Mol Sci 2015;16:8168–8185.

51. Barlak E, Gorbe A, Gaspar R, Paloczi J, Ferdinandy P, Lazov A. Activation of PPARβ/δ protects cardiac myocytes from oxidative stress-induced apoptosis by suppressing generation of reactive oxygen/nitrogen species and expression of matrix metalloproteinases. Pharmacol Res 2015;95:102–110.

52. Belliard A, Sottejeau Y, Duan K, Karabin JL, Pierre SV. Modulation of cardiac Na⁺, K⁺-ATPase cell surface abundance by simulated ischemia-reperfusion and ouabain preconditioning. Am J Physiol Heart Circ Physiol 2013;304:H94–103.

53. Valen G. The basic biology of apoptosis and its implications for cardiac function and viability. Am Thorac Surg 2003;75:5656–5660.

54. Adameova A, Goncalvesova E, Szobi A, Dhalla NS. Necroptotic cell death in failing heart: relevance and proposed mechanisms. Heart Fail Rev 2016;21:233–249.

55. Huang C, Yitzhaki S, Perry CN, Liu W, Giricz Z, Mentzer RM Jr, Gottlieb RA. Autophagy induced by ischemic preconditioning is essential for cardioprotection. J Cardiovasc Transl Res 2013;6:365–371.

56. Biala AK, Kirshenbaum LA. The interplay between cell death signaling pathways. Autophagy 2015;11:445–451.

57. Adameova A, Goncalvesova E, Szobi A, Dhalla NS. Necroptotic cell death in failing heart: relevance and proposed mechanisms. Heart Fail Rev 2016;21:233–249.

58. Guerin E, Nicoloso A, Giannessi F, Consales G, Ferrando P, Macaya C, Gambardella P, Pertile S. COX-2 inhibition with celecoxib elevates blood pressure in patients on angiotensin-converting enzyme inhibitors. Expert Opin Drug Saf 2004;3:470–479.

59. Funk CD, FitzGerald GA. COX-2 inhibitors and cardiovascular risk. J Cardiovasc Pharmacol 2007;50:470–479.

60. Walker C, Basucai LM. Cardiovascular safety of non-steroidal anti-inflammatory drugs revisited. Postgrad Med 2018;130:55–75.

61. Singh BK, Haque SE, Piliai KK. Assessment of non-steroidal anti-inflammatory drug-induced cardiotoxicity. Expert Opin Drug Metab Toxicol 2018;14:143–156.

62. Zhang MZ, Yao B, Wang Y, Yang S, Wang S, Fan X, Harris RC. Inhibition of cyclooxygenase-2 in endothelial and vascular smooth muscle cells restrains atherogenesis in hyperlipidemic mice. Circulation 2014;129:1761–1769.

63. Muscara MN, Vergnolle N, Lovren F, Triggle CR, Elliott SN, Asfaha S, Wallace JL. Selective cyclo-oxygenase-2 inhibition with celecoxib elevates blood pressure and promotes leukocyte adhesion. Br J Pharmacol 2010;161:1423–1430.

64. Cav C, Pu Yi, Li Y, Zhang X, Zhang L, Yu F, Xu S, Xu Q, Zhi Y, Guan Y, Wang X, Kong W. Microsomal prostaglandin E synthase-1-derived PGE2 inhibits vascular smooth muscle cell calcification. Antioxid Redox Signal 2016;26:108–121.

65. Zhang MZ, Yao B, Wang Y, Yang S, Wang S, Fan X, Harris RC. Inhibition of cyclooxygenase-2 in hematopoietic cells results in salt-sensitive hypertension. J Clin Invest 2015;125:428–429.

66. Tan SY, Monslow J, Todd L, Lawson J, Pure E, FitzGerald GA. Cyclooxygenase-2 in endothelial and vascular smooth muscle cells restrains atherogenesis in hyperlipidemic mice. Circulation 2014;129:1761–1769.

67. Muscara MN, Vergnolle N, Lovren F, Triggle CR, Elliott SN, Asfaha S, Wallace JL. Selective cyclo-oxygenase-2 inhibition with celecoxib elevates blood pressure and promotes leukocyte adhesion. Br J Pharmacol 2010;161:1423–1430.

68. Martinez-Gonzalez J, Badimon L. Mechanisms underlying the cardiovascular effects of COX-inhibition: benefits and risks. Curr Pharm Des 2007;13:2215–2227.

69. Tang SY, Monslow J, Todd L, Lawson J, Pure E, FitzGerald GA. Cyclooxygenase-2 inhibition of CB1 cannabinoid receptor protects against doxorubicin-induced cardiotoxicity. J Pharmacol Exp Ther 2018;365:1423–1430.

70. Mohamed HE, El-Swefy SE, Hagar HH. The protective effect of glutathione allothionein in the heart of transgenic mice suppresses doxorubicin cardiotoxicity. Cell Biol Toxicol 2015;31:95–105.

71. Davies KJ, Doroshow JH. Redox cycling of anthracyclines by cardiac mitochondria. II. Formation of superoxide anion, hydrogen peroxide, and hydroxyl radical. J Biol Chem 1986;261:3060–3067.

72. Doroshov JH, Davies KJ. Redox cycling of anthracyclines by cardiac mitochondria. I. Anthracycline radical formation by NADH dehydrogenase. J Biol Chem 1986;261:3060–3067.

73. Achterhuis JM, Wallace KB, Adameova A. Adriamycin-induced oxidative mitochondrial cardiotoxicity. Cell Biol Toxicol 2007;23:15–25.
Mukhopadhyay P, Rajesh M, Batkai S, Kashiyawa Y, Hasko G, Lusdat L, Szabo C, Pacher P. Role of superoxide, nitric oxide, and peroxynitrite in doxorubicin-induced cell death in vivo and in vitro. Am J Physiol Heart Circ Physiol 2009; 296: H1466–H1483.

Mallett A, Tan K, Chai X, Sadananda SN, Mehta A, Ooi J, Hayden MR, Paulad MA, Ghosh S, Shin W, Brunham LR. Modeling doxorubicin-induced cardiotoxicity in human pluripotent stem cell-derived cardiomyocytes. Sci Rep 2016; 6:33333.

Marwick TH. Cancer therapy-related cardiac dysfunction: unresolved issues. Con J Cardiol 2016;32:842–846.

Cardinale D, Colombo A, Bacchiani G, Tedeschi I, Meroni CA, Veglia F, Civelli M, Marwick TH. Cancer therapy-related cardiac dysfunction: unresolved issues. Con J Cardiol 2016;32:842–846.

Chaudhari U, Nemade H, Gaspar JA, Hescheler J, Hengstler JG, Sachinidis A. Chaudhari U. Nemade H. Gaspar JA. Hescheler J. Hengstler JG. Sachinidis A. Adaptation to myocardial stress in disease models of doxorubicin using the FDA adverse event reporting system. PloS One 2017;12:e0185654.

Kanwal U, Irfan Bukhari N, Ovais M, Abas N, Hussain K, Raza A. Advances in nano-delivery systems for doxorubicin: an updated insight. J Drug Target 2018; 26:296–310.

Qu C, Li J, Zhou Y, Yang S, Chen W, Li F, You B, Liu Y, Zhang X. Targeted delivery of doxorubicin via CD147-mediated ROSiPhD-Si-two-sensitive nanocarriers for the efficient therapy of hepaticcellular carcinoma. AAPS J 2018;20:34.

Abdel-Qadir H, Nolton MT, Thavendranathan P. Routine prophylactic cardioprotective therapy should be given to all recipients at risk of cardiotoxicity from cancer chemotherapy. Con J Cardiol 2016;32:921–925.

Ferdinandy P, Salzvasszy Z, Baxter GF. Adaptation to myocardial stress in disease states: is preconditioning a healthy heart phenomenon? Trends Pharmacol Sci 1998;19:223–229.

Ferdinandy P, Schulz R, Baxter GF. Interaction of cardiovascular risk factors with myocardial ischemia/reperfusion injury, preconditioning, and postconditioning. Pharmacov Rev 2007;59:418–458.

Ferdinandy P, Hausenloy DJ, Heusch G, Baxter GF, Schulz R. Interaction of risk factors, comorbidities, and comorbidities with ischemia/reperfusion injury and cardioprotection by preconditioning, postconditioning, and remote conditioning. Pharmacov Rev 2014;66:1142–1174.

Hausenloy DJ, Garcia-Dorado D, Batker HE, Davidson SM, Downey J, Engel FB, Jennings R, Le Cour S, Leor J, Madalena R, Ovize M, Perrino C, Prunier F, Schulz R, Sliujter JRG, Van Laake LW, Yellon DM. van Laake LW. Yellon DM. The second window of preconditioning (SWOP) figure. 2012. https://www.ifpma.org/wp-content/uploads/2016/01/IFPMA_-_Facts_and_Figures_2012_LowResSinglePage.pdf (accessed 1 April 2018).

Chaudhari U, Nemade H, Gaspar JA, Hescheler J, Hengstler JG, Sachinidis A. Identification of genomic biomarkers for anthracycline-induced cardiotoxicity in human iPSC-derived cardiomyocytes: an in vitro repeated exposure toxicity approach for safety assessment. Arch Toxicol 2016;90:2763–2777.

Kopjar I, De Bondt A, Vinken P, Tesman A, Damiano B, Goemmine N, Van den Wyngaert J, Gallacher DJ, Lu HR. Chronic drug-induced effects on contractile motion properties and cardiac biomarkers in human induced pluripotent stem cell-derived cardiomyocytes. Br J Pharmacol 2017;174:3766–3779.

Zhang L, Xu MX, Yin QS, Zhu CY, Cheng XL, Ren YR, Zhang PW, Zhang YJ. Screening, verification, and analysis of biomarkers for drug-induced cardiotoxicity in vitro based on RTCA coupled with PCR Array technology. Toxicol Lett 2017;268:17–22.

Nishimura Y, Kondo C, Morikawa Y, Tonomura Y, Torii M, Yamate J, Uehara T. Plasma miR-208 as a useful biomarker for drug-induced cardiotoxicity in rats. J Appl Toxicol 2015;35:173–180.

Sandhu H, Maddock H. Molecular basis of cancer-therapy-induced cardiotoxicity in vitro based on RTCA coupled with PCR Array technology. Toxicol Lett 2017;268:17–22.

Kopjar I, De Bondt A, Vinken P, Tesman A, Damiano B, Goemmine N, Van den Wyngaert J, Gallacher DJ, Lu HR. Chronic drug-induced effects on contractile motion properties and cardiac biomarkers in human induced pluripotent stem cell-derived cardiomyocytes. Br J Pharmacol 2017;174:3766–3779.

Nishimura Y, Kondo C, Morikawa Y, Tonomura Y, Torii M, Yamate J, Uehara T. Plasma miR-208 as a useful biomarker for drug-induced cardiotoxicity in rats. J Appl Toxicol 2015;35:173–180.

Sandhu H, Maddock H. Molecular basis of cancer-therapy-induced cardiotoxicity in vitro based on RTCA coupled with PCR Array technology. Toxicol Lett 2017;268:17–22.

Kopjar I, De Bondt A, Vinken P, Tesman A, Damiano B, Goemmine N, Van den Wyngaert J, Gallacher DJ, Lu HR. Chronic drug-induced effects on contractile motion properties and cardiac biomarkers in human induced pluripotent stem cell-derived cardiomyocytes. Br J Pharmacol 2017;174:3766–3779.

Zhang L, Xu MX, Yin QS, Zhu CY, Cheng XL, Ren YR, Zhang PW, Zhang YJ. Screening, verification, and analysis of biomarkers for drug-induced cardiotoxicity in vitro based on RTCA coupled with PCR Array technology. Toxicol Lett 2017;268:17–22.

Nishimura Y, Kondo C, Morikawa Y, Tonomura Y, Torii M, Yamate J, Uehara T. Plasma miR-208 as a useful biomarker for drug-induced cardiotoxicity in rats. J Appl Toxicol 2015;35:173–180.

Sandhu H, Maddock H. Molecular basis of cancer-therapy-induced cardiotoxicity in vitro based on RTCA coupled with PCR Array technology. Toxicol Lett 2017;268:17–22.

Kopjar I, De Bondt A, Vinken P, Tesman A, Damiano B, Goemmine N, Van den Wyngaert J, Gallacher DJ, Lu HR. Chronic drug-induced effects on contractile motion properties and cardiac biomarkers in human induced pluripotent stem cell-derived cardiomyocytes. Br J Pharmacol 2017;174:3766–3779.

Zhang L, Xu MX, Yin QS, Zhu CY, Cheng XL, Ren YR, Zhang PW, Zhang YJ. Screening, verification, and analysis of biomarkers for drug-induced cardiotoxicity in vitro based on RTCA coupled with PCR Array technology. Toxicol Lett 2017;268:17–22.

Nishimura Y, Kondo C, Morikawa Y, Tonomura Y, Torii M, Yamate J, Uehara T. Plasma miR-208 as a useful biomarker for drug-induced cardiotoxicity in rats. J Appl Toxicol 2015;35:173–180.