A Role for In Vitro Disease Models in The Landscape of Preclinical Cardiotoxicity and Safety Testing

Vijayalakshmi Varma 1,*
1Biomarkers and Alternative Models Branch, Division of Systems Biology, National Center for Toxicological Studies, Jefferson, AR

Abstract:
Drug-induced cardiotoxicity is one of the predominant reasons for drug attrition and withdrawals. This is of critical concern when potentially cardiotoxic drugs are administered to individuals with inherited arrhythmogenic cardiac diseases or with metabolic diseases such as obesity and diabetes, which are key risk factors for cardiovascular diseases. Pathophysiological alteration prevalent under such conditions can alter or exacerbate cardiotoxic responses. The growing incidence of obesity, diabetes and metabolic syndrome subject a significant percentage of the population to drug treatments, thereby augmenting their risk for drug-induced cardiovascular toxicity. Hence, screening for drug-induced cardiotoxicity early in the preclinical stages of drug development, by using appropriate human disease models, can be effective in ensuring safety in clinical trials and preventing late stage and post-marketing drug withdrawals owing to cardiotoxicity. The advent of human pluripotent stem cells (hPSC) and induced pluripotent stem cell (iPSC)-derived cardiomyocytes are revolutionizing safety/toxicity screening in human cells by providing relevant human-specific, renewable model systems to explore human drug toxicity. The ability to generate patient-specific iPSCs that can model cardiac diseases, now offers a valuable option that can further improve drug safety assessments and enable a more accurate prediction of toxicity that occurs in the representative population that are prescribed the drugs. Use of appropriate disease models will not only provide cost savings by decreasing potential drug attrition and withdrawals, seen with many drugs, but will also be a promising option to advance precision medicine.

Corresponding author: Vijayalakshmi Varma, Division of Systems Biology, Biomarkers and Alternative Models Branch, National Center for Toxicological Research, U.S. Food and Drug Administration, 3900, NCTR Road, Building 54, Rm#126, Jefferson AR-72079. E-Mail: Vijayalakshmi.varma@fda.hhs.gov; Tel.: +1-870-543-7115;

Running Title: In vitro disease models in preclinical cardiotoxicity and safety testing

Key Words: Preclinical cardiotoxicity, drug-induced toxicity, drug safety, embryonic stem cells, induced pluripotent stem cells, in vitro disease models, cardiovascular diseases, obesity and type II diabetes

Received: July 13, 2017 Accepted: July 21, 2017 Published: July 29, 2017
Introduction

The drug development pipeline is an arduous, labor-intensive and expensive process that entails stringent regulations by the FDA, in order to ensure the final marketed drug will be safe and efficacious for use in all patients that are prescribed the drugs [1-3]. Although rigorous screening and testing of drugs occur as part of preclinical studies and clinical trials, safety and toxicity continue to be leading causes for the withdrawal of drugs from the markets [1, 4, 5]. Amongst specific organ toxicities, cardiac toxicity is one of the predominant reasons for late-stage attrition of drugs [6-8]. A potential reason for late-stage drug attrition and post-marketing withdrawals may be attributed to drug-induce cardiotoxicity augmented by cardiovascular risk factors that are prevalent in many patients with metabolic disorders such as obesity (36% of the population [9] type II diabetes (9.3% of the population [10], and metabolic syndrome (~35% of the population) [11]. Such patients are usually not part of clinical trials, unless the clinical trial is specifically for anti-diabetic or anti-obesity drugs. Thus, drugs prescribed to a significant percentage of the population displaying cardiovascular risk factors put them at a higher risk for drug-induced cardiovascular toxicity.

Cardiovascular adverse events can result when drugs impact either the structural and functional aspects of the different components of the cardiovascular system including cardiac myocytes, fibroblasts, vascular smooth muscle cells and endothelial cells lining the vasculature, or affect the electrical conductivity of the heart or a combination of both, resulting in cardiac dysfunction [12]. Conditions that mediate drug-induced cardiotoxicity include 1) Cardiac arrhythmias: which results from the altered electrical conductivity of the heart causing tachycardia, brachycardia, ventricular fibrillation or asystole. [13] Torsade de pointes, is a dangerous form of ventricular arrhythmia, that is commonly triggered by cardiotoxic drugs [14]. It is characterized by tachycardia and associated with a prolonged QT interval in an electrocardiogram [12]. 2) Cardiac hypertrophy: This results from the increased size of the cardiomyocytes consequent to decreased or compromised cardiac function [15]. 3) Cardiomyopathy: This is a condition that manifests due to structural and functional alterations in the heart resulting in decreased cardiac output [16]. 4) Congestive heart failure: Is a condition which occurs due to dysfunctional systoly, diastoly and myocardial contractility resulting in an inability to maintain cardiac output [17] and 5) Vascular toxicity: Is a condition which results from structural and functional alterations of vascular endothelial cells induced by oxidative stress or accumulation of toxins in these cells [18]. Other notable factors that lead to compromise in function of cardiomyocytes include lipotoxicity, oxidative stress, mitochondrial dysfunction and ischemic reperfusion etc. [19].

The FDA Adverse Event Reporting System (FAERS) has been an invaluable resource in identifying occurrence and providing details related to drug-induced adverse cardiotoxic events thus offering potential insights to the possible mechanisms of toxicity [20]. The following link (https://www.fda.gov/Drugs/DrugSafety/ucm504617.htm) is an example of a safety announcement by the FDA based on adverse events reporting of the cardiac adverse events to loperimide (Imodium®), a drug indicated for control and symptomatic relief of acute nonspecific diarrhea and of chronic diarrhea associated with inflammatory bowel disease. Several prominent drugs, for non-life-threatening diseases, such as Terfenadine, Vioxx, and Avandia were withdrawn from the market due to drug-induced cardiotoxicity. Well-documented classes of drugs that exhibit cardiotoxicity include anti-cancer drugs particularly anthracyclines, antipsychotics, antidepressants, some antibiotics, anti-inflammatory agents, antiarrhythmics, anesthetics, and beta-blockers among others. [21].

Cardiovascular risk factors, obesity and diabetes, can increase the potential for drug-induced cardiotoxicity

Disease states such as obesity and diabetes confer increased susceptibility to cardiovascular diseases [22]. The insulin resistant state which arises as a consequence to obesity, as well as diabetes are notable predictors of cardiovascular morbidity and mortality and are also independent risk factors for death in patients with heart failure [23-25]. The insulin resistant state alters insulin signaling in the heart, which compromises the contribution from glucose oxidation and consequently increases fatty acid oxidation, in order to maintain a constant energy supply to the heart [23].
Obesity is also associated with other risk factors for developing cardiac failure, such as hypertension, hyperlipidemia [26] and inflammation [24]. The presence of excess circulating free fatty acids (FFAs) in obesity can increase the delivery of FFAs to the heart resulting in cardiomyocyte lipotoxicity, augment reactive oxygen species (ROS) production and increase oxidative stress. [27]. Studies have shown that the accumulation of ROS in the myocardium consequent to hyperglycemia in the diabetic state can trigger myocardial apoptosis leading to diabetic cardiomyopathy [28]. Excess circulating FFAs in obesity activate toll receptors stimulating the downstream activation of the NFκB pathway resulting in augmented production of pro-inflammatory cytokines including TNFα and IL-6 [29]. TNFα can further increase the production of IL-6 and macrophage chemoattractant protein, (MCP-1), which plays a role in macrophage recruitment [24, 30]. These pro-inflammatory cytokines are prominent in stimulating the formation of atherosclerotic plaques [31]. The consequent state of chronic low-grade inflammation in obesity further plays a key role in the development of insulin resistance [24, 32]. Obesity and inflammation are associated with the development of endothelial dysfunction [33]. Diabetes is strongly associated with cardiovascular diseases and symptoms including atrial fibrillation, atrial flutter, coronary artery disease and left ventricular hypertrophy [34] and can contribute to the development of diabetic cardiomyopathy [35]. Thus, the pathophysiological changes accompanying obesity and diabetes exert their effects both at the cellular and systemic levels resulting in cardiac dysfunction [23, 36], which over time can alter myocardial structure and function causing heart failure [22]. Hence pre-existence of these cardiovascular risk factors in patients can augment drug-induced cardiotoxicity.

**Current preclinical testing strategies and model systems used in in vitro drug testing**

The model systems frequently used in investigating cardiotoxicities, in particular, proarrhythmic risk are transgenic in vitro systems such as Human Embryonic Kidney (HEK) cells and Chinese Hamster Ovary (CHO) cells expressing heterologous ion channel systems [37] with a focus on identifying mainly the arrhythmogenic effects of drugs. Although these systems to a large extent have contributed to understanding ion channel defects and arrhythmogenic mechanisms, they have limitations in accurately predicting toxicities in humans, due to the inability of these cell systems to accurately reproduce the human cardiac physiology and the clinical manifestations of cardiac toxicities. The rodent cell line H9C2 from rat heart [38] is another model that has been used to examine drug-induced toxicities by chemotherapeutic agents [39, 40]. It is noted that the H9C2 cells exhibit features that are morphologically distinct from human cardiomyocytes and are also less mature than the human adult cardiomyocytes [37]. Primary adult human ventricular cardiomyocytes are appropriate model systems for toxicity testing to recapitulate human physiologically functional cardiomyocytes of the human heart [41]. However, these cells are difficult to obtain and cannot be maintained and propagated long term in culture [42]. Alternatively, human pluripotent stem cells, such as embryonic stem (ES) cells, which are obtained from the blastocyst embryonic stage [43] as well as the iPSCs, which are derived from reprogramming somatic cells [44] can be induced to differentiate into any somatic cell type including cardiomyocytes [45]. The particular advantage of the iPSCs over the ES cells is twofold i) they overcome the ethical concerns associated with ES cells ii) while both ES and iPSCs can serve as an infinite source of cells, iPSCs can also be generated from somatic cells from individuals with disease enabling the modeling of the disease phenotype in a dish [46]. *In vivo* model systems including rodent and more recently Zebrafish [37, 47] have also been used as models to test for cardiotoxicity. However, given the varied structure and morphology of the cardiomyocytes and the electrophysiological characteristics and profiles of the various repolarizing and depolarizing currents channels in these non-human model systems, they may not effectively predict cardiotoxicity in humans.

To enable safety pharmacology efforts, several guidelines for preclinical safety using *in vitro* and *in vivo* approaches have been put forth by the US Food and Drug Administration and International Coalition for Harmonization (ICH) including i) ICH S7(A) Safety Pharmacology Studies for Human Pharmaceuticals ii) ICH S7(B) Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals, iii) ICH S9 Nonclinical Evaluation for Anticancer Pharmaceuticals, in
order to minimize the risks that may be associated with cardiovascular toxicity during drug development. These guidelines mainly place emphasis on assessing proarrhythmic risk, which is the most common risk associated with drug-induced cardiotoxicity. The approaches currently used for cardiotoxicity determination are i) an in vitro assay for the Ether-a-go-go-Related gene (hERG), which examines the blockage of the repolarizing potassium channel current (hERG/I\textsubscript{kr}) by the drug, and ii) non-clinical in vivo assessment of QT prolongation. However, blockage of hERG constitutes only one out of several cardiac current channels which result in proarrhythmia, when blocked. Hence, the strategy of only examining the blockage of a single ion channel is increasingly being recognized as an imperfect measure ventricular repolarization [48, 49]. Also, this much relied on in vitro assay for hERG, although highly sensitive, has only low specificity [49]. Furthermore, nonclinical QT prolongation assays are not fully predictive of the QT prolongation in humans [48]. Hence, although these guidelines have proven useful in decreasing drug-induced cardiotoxicity by enabling the early detection of potentially torsadogenic drugs, incidences of false positive results have led to the incorrect/inappropriate assignment of some drugs as torsadogenic [48]. In the case of some drugs, these approaches have also resulted in false negative results, leading to drug attrition [50-52]. In order to address these issue, partnered efforts by multidisciplinary scientists, representing international regulatory groups, industry and academia are underway for the development of more newer approaches to assess proarrhythmic risk under the Comprehensive in vitro Proarrhythmia Assay (CiPA) initiatives [49, 53].

**Limitations of current models and strategies**

The move from animal and non-cardiac human cell lines to human human iPSC-derived cardiomyocytes are enabling the assessment of cardiotoxicity due to proarrhythmic cardiotoxic risk in more relevant human models. A significant limitation to the use of iPSC-derived cardiomyocytes is that they are recognized to exhibit a more neonatal phenotype as opposed to the preferred adult-like phenotype [54]. Many studies are addressing this issue to obtain a more adult-like phenotype in the iPSC-derived cardiomyocytes [54]. However, a caveat in the use of in vitro iPSC-derived cardiomyocytes used in safety pharmacology studies and early drug development is that these iPSC-derived cardiomyocytes are derived from healthy individuals and are less representative of the pathophysiological state seen in patients to whom drugs are prescribed. Individuals with cardiovascular risk factors who are vulnerable to heart diseases demonstrate pathophysiological modifications in the hearts due to either i) genetic causes, for example, SNPs in specific heart channels causing channelopathies and resulting in cardiac dysfunction or ii) presence of obesity and diabetes, which are prominent risk factors of heart disease, making these individuals more susceptible to cardiotoxicity. Hence, the use of appropriate disease models that can model cardiovascular disease states will vastly improve the safety/toxicity assessments of drugs and may further decrease drug attrition resulting from disparities in susceptibility to cardiotoxicity in cardiovascular disease states compared to healthy individuals from whom in vitro models are currently being derived.

**Cardiovascular disease models and its applications**

Many recent studies have successfully demonstrated the development of in vitro disease models of cardiovascular diseases that can be used in cardiac safety/toxicity testing to more accurately predict drug-induced clinical cardiotoxicity such as, congenital monogenic cardiac arrhythmic syndromes also known as cardiac channelopathies [55, 56] due to mutations in specific cardiac ion channels. Examples include mutations in KCNQ1, KCNH2, causing Long QT syndrome; mutations in Ryanodine receptor (RyR) leading to Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) as well as other conditions such as Brugada Syndrome [55-57].

Other types of hereditary cardiovascular disease conditions that have been modeled using iPSC-derived cardiomyocytes include dilated cardiomyopathy derived from a patient carrying a point mutation in the gene TNNT2, which encodes the sarcomeric protein troponin T [58]. These patient-specific iPSC-derived cardiomyocytes from patients with this condition demonstrated altered Ca\textsuperscript{2+} currents, abnormal contractility and abnormal sarcomeric organization compared to iPSC-derived cardiomyocytes from healthy individuals. The phenotype observed was corrected by treatment with the β blocker, metoprolol demonstrating the precise
capture of the phenotype in this model. Similarly, Lan et al. [59] modeled hypertrophic cardiomyopathy, which is a common form of congenital cardiac dysfunction resulting from the missense mutation of the MYH7 gene. These iPSC-derived cardiomyocytes demonstrated hypertrophic cardiomyocytes with abnormal calcium handling and electrophysiological function. Wang et al [60] have successfully modeled Barth syndrome which is a form of mitochondrial myopathy that can cause cardiac dysfunction.

While monogenic cardiac disorders are prevalent, the more common cause of cardiovascular disorders is of the polygenic form, which arises from a number of complex conditions such as dysfunctional metabolic disorders including diabetes and obesity. Drawnel et al. [58] were able to successfully induce and model the phenotypic state of diabetic cardiomyopathy by exposure of human iPSCs from normal healthy individuals exposed to a diabetic-milieu in culture, which demonstrated characteristic disease features including cellular hypertrophy, disorganized sarcomeres, altered calcium transients, intracellular lipid accumulation and oxidative stress. In another study Burridge et al. [61] modeled the phenotypic characteristics of doxorubicin-induced cardiotoxicity in iPSC-derived cardiomyocytes from patients who experienced increased doxorubicin-induced cardiotoxicity compared to iPSC-derived cardiomyocytes from those who did not experience doxorubicin-induced cardiotoxicity. The iPSC-derived cardiomyocytes from the doxorubicin-sensitive patients consistently demonstrated increased sensitivity in culture, similar to the response by patients from whom the iPSCs were derived, and was accompanied by decreased cell viability, impaired mitochondrial and metabolic function, impaired calcium handling, decreased antioxidant pathway activity, and increased ROS production [61]. These studies clearly demonstrate the possibilities of effectively capturing and modeling the defective phenotype in a dish, which can be used for drug safety/toxicity testing. The use of in vitro iPSC-derived cardiac disease models from subjects with genetic and/or environmental causes of metabolic and cardiovascular diseases resulting in increased cardiovascular risk, can improve the identification of toxicity early in preclinical studies. Ultimately, clinical trials can be made safer by identifying risks in phenotypically-relevant iPSC models in nonclinical assessments.

**Conclusions**

Drug-induced cardiovascular toxicity has been documented with several classes of therapeutic drugs, necessitating focus on cardiac safety studies early in drug development. While the field has come a long way in the detection and identification of drug-induced cardiotoxicity particularly arrhythmia using various in vitro model systems, including animal, non-cardiac human cell lines, primary human ventricular cardiomyocytes and more recently human iPSC-derived cardiomyocyte models, drug attrition, withdrawals and non-approvals due to cardiac side effects in patients continue to be of concern. While the development of newer tests and methodologies as part of the CiPA initiative will continue to improve safety pharmacology testing, the use of in vitro human iPSC-derived cells that model cardiovascular disease due to both genetic and non-genetic causes can be beneficial in detecting safety and/or increased susceptibility to toxicity. Human in vitro disease models employed early in preclinical stages will, therefore, be a valuable addition in making the clinical trials safer for patients, enhancing safety testing approaches and in further reducing and preventing late stage drug attrition and post market withdrawals. 

**Disclaimer:**

This document has been reviewed in accordance with the United States Food and Drug Administration (FDA) policy and approved for publication. The views presented in this article do not necessarily represent the views of the Food and Drug Administration

**References**


31. Ramji, D.P. and T.S. Davies, Cytokines in atherosclerosis: Key players in all stages of disease and
55. Tang, S., et al., Patient-Specific Induced Pluripotent Stem Cells for Disease Modeling and Phenotypic


