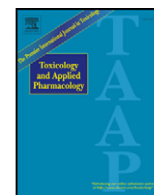
Toxicology and Applied Pharmacology 322 (2017) 60–74

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/taap



In vitro cardiotoxicity assessment of environmental chemicals using an organotypic human induced pluripotent stem cell-derived model

Oksana Sirenko [a,*,1], Fabian A. Grimm [b,1], Kristen R. Ryan [c], Yasuhiro Iwata [b], Weihsueh A. Chiu [b], Frederick Parham [c], Jessica A. Wignall [d], Blake Anson [e], Evan F. Cromwell [f], Mamta Behl [c], Ivan Rusyn [b], Raymond R. Tice [c]

[a] *Molecular Devices, LLC, Sunnyvale, CA, USA*
[b] *Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, TX, USA*
[c] *Division of the National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA*
[d] *ICF, Fairfax, VA, USA*
[e] *Cellular Dynamics International, Madison, WI, USA*
[f] *Protein Fluidics, Inc., Burlingame, CA, USA*

A R T I C L E I N F O

Article history:
Received 4 November 2016
Revised 24 January 2017
Accepted 27 February 2017
Available online 1 March 2017

Keywords:
Environmental toxicology
Cardiotoxicity
Cardiomyocytes
High-content screening
Induced pluripotent stem cells
Alternative methods

A B S T R A C T

An important target area for addressing data gaps through *in vitro* screening is the detection of potential cardiotoxicants. Despite the fact that current conservative estimates relate at least 23% of all cardiovascular disease cases to environmental exposures, the identities of the causative agents remain largely uncharacterized. Here, we evaluate the feasibility of a combinatorial *in vitro*/*in silico* screening approach for functional and mechanistic cardiotoxicity profiling of environmental hazards using a library of 69 representative environmental chemicals and drugs. Human induced pluripotent stem cell-derived cardiomyocytes were exposed in concentration-response for 30 min or 24 h and effects on cardiomyocyte beating and cellular and mitochondrial toxicity were assessed by kinetic measurements of intracellular Ca^{2+} flux and high-content imaging using the nuclear dye Hoechst 33342, the cell viability marker Calcein AM, and the mitochondrial depolarization probe JC-10. More than half of the tested chemicals exhibited effects on cardiomyocyte beating after 30 min of exposure. In contrast, after 24 h, effects on cell beating without concomitant cytotoxicity were observed in about one third of the compounds. Concentration-response data for *in vitro* bioactivity phenotypes visualized using the Toxicological Prioritization Index (ToxPi) showed chemical class-specific clustering of environmental chemicals, including pesticides, flame retardants, and polycyclic aromatic hydrocarbons. For environmental chemicals with human exposure predictions, the activity-to-exposure ratios between modeled blood concentrations and *in vitro* bioactivity were between one and five orders of magnitude. These findings not only demonstrate that some ubiquitous environmental pollutants might have the potential at high exposure levels to alter cardiomyocyte function, but also indicate similarities in the mechanism of these effects both within and among chemicals and classes.

© 2017 Published by Elsevier Inc.

1. Introduction

A large number of environmental agents remain inadequately tested for their potential toxicological effects (Judson et al., 2009). In the United States, the National Toxicology Program at the National Institute of Environmental Health Sciences, the Environmental Protection Agency, the National Center for Advancing Translational Science, and the Food and Drug Administration are addressing toxicity data needs through the Tox21 collaboration, an initiative to implement novel *in vitro* screening for thousands of chemicals (Collins et al., 2008; Tice et al., 2013).

Important target areas for addressing data gaps through *in vitro* screening include the detection of potential cardiotoxic effects. Despite the fact that current conservative estimates relate at least 23% of all cardiovascular disease cases to environmental exposures, the identities of the causative environmental agents remain largely uncharacterized (Pruss-Ustun and Corvalan, 2006). Moreover, cardiotoxicity remains

Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenyl hydrazine; CDK, Chemistry Development Kit; %CV, percent of the coefficient of variation; DDT, dichlorodiphenyltrichloroethane; DMSO, dimethyl sulfoxide; iPSC, induced pluripotent stem cell; POD, Point-of-Departure; PAH, polycyclic aromatic hydrocarbon; PCA, principal components analysis; ToxPi, Toxicological Priority Index.

* Corresponding author at: Molecular Devices, LLC, 1311 Orleans Drive, Sunnyvale, CA 94089, USA.

E-mail address: oksana.sirenko@moldev.com (O. Sirenko).

[1] O. Sirenko and F. Grimm have equally contributed to the manuscript.

http://dx.doi.org/10.1016/j.taap.2017.02.020
0041-008X/© 2017 Published by Elsevier Inc.

among one of the most pronounced reasons, comparable to those associated with hepatotoxicity, for drug attrition during clinical trials and post-marketing (Berridge et al., 2013; Laverty et al., 2011; Nadanaciva and Will, 2011; Pierson et al., 2013). Current pre-clinical approaches for cardiophysiologic evaluations of chemicals rely almost exclusively on large animal models and this approach has significant limitations in terms of the cost and complexity of the studies (Herman and Ferrans, 1998). Hence, there is a pressing need for the development of comprehensive, multi-parametric screening strategies that provide improved predictability of cardiotoxic effects (Roberts et al., 2014). In order to capture the wide range of potential cardiotoxic effects, novel approaches need to rely on cardiomyocyte functional assays, not just traditional cytotoxicity measurements, because interference with normal cellular electrophysiology as well as sub-cytotoxic perturbation of biochemical pathways can impact on contractility and organ function (Chen et al., 2016).

As an alternative to primary cardiomyocytes and myocardial tissue preparations, human inducible pluripotent stem cell (iPSC)-derived *in vitro* model systems have more recently emerged as a physiologically-relevant and highly reproducible option for cardiotoxicity testing (Guo et al., 2013; Ma et al., 2011; Mercola et al., 2013; Suter-Dick et al., 2015). iPSC-derived cardiomyocytes are a particularly attractive *in vitro* model as they form a synchronously beating monolayer that can be used to reliably reproduce drug-associated cardiophysiologic phenotypes using a fast kinetic fluorescence assay that monitors changes in intracellular Ca^{2+} flux (Grimm et al., 2016; Sirenko et al., 2013a, 2013b). This approach is amenable to quantitative multi-parametric assessment of various phenotypes, *e.g.,* positive and negative inotropic effects and prolonged repolarization, in medium- to high-throughput screening formats (Grimm et al., 2015). Of particular interest is the ability to use iPSC-derived cardiomyocytes to test for the potential of chemicals to induce cardiac arrhythmias because interference with cardiomyocyte repolarization, *i.e.,* the *in vitro* similarity to clinical QT prolongation, has been a useful phenotype easily observable *in vitro* (Blinova et al., 2017). A study evaluating 131 drugs demonstrated that a comprehensive concentration-response assessment of multiple functional readouts yields predictive and mechanistically-interpretable data on cardiotoxicity, thereby indicating its potential applicability to evaluate and prioritize environmental chemicals for this important endpoint (Sirenko et al., 2013b).

In this study, we evaluated the utility of multiplexed functional *in vitro* assays in iPSC-derived cardiomyocytes for the high-throughput screening of several diverse classes of environmental chemicals as well as drugs; some of the environmental chemicals have been associated with suspected cardiotoxicity potential (Kaufman et al., 1992; Zafiropoulos et al., 2014; Zhang et al., 2013). We used Ca^{2+} flux measurements in combination with high-content imaging to evaluate concentration-dependent effects on cardiomyocyte physiology, mitochondrial membrane potential, and cell viability. Benchmark concentration Point-of-Departure (POD) values were utilized for bioactivity grouping and ranking of the chemicals in Toxicological Prioritization Index Graphical User Interface (ToxPi GUI) software (Reif et al., 2013).

2. Materials and methods

The overall experimental design is shown in Fig. 1.

2.1. Chemical library

The library used in these studies is comprised of 69 unique chemicals (Table 1) with mostly limited or unknown evidence for cardiotoxicity. These chemicals were provided by the National Toxicology Program at the National Institute of Environmental Health Sciences and information on their purity and supplier is provided in Supplementary Table 1. This set was selected to broadly reflect diverse chemical classes and included drugs ($n = 18$), pesticides ($n = 15$), flame retardants ($n = 10$), polycyclic aromatic hydrocarbons (PAHs; $n = 14$), and 12 chemicals classified as “other”. All tests described in the article were performed as duplicates. Four of these chemicals (deltamethrin, triphenyl phosphate, methyl mercuric (II) chloride, saccharin) were also tested in additional replicates on each microplate to assess assay-specific inter-plate replicability. In addition, assay-specific cardiotoxicity positive (valinomycin, berberine chloride, phenobarbital) and negative (biotin, sorbitol, adipic acid) control chemicals (all from Sigma, St. Louis, MO, USA) were included in the screening (Table 1) on every plate. Additional controls for mitochondria potential assay (antimycin A, digoxin, carbonyl cyanide *m*-chlorophenyl hydrazine (CCCP)) were included into the mitochondria assay plates. Stock solutions (20 mM) of all chemicals were prepared in cell-culture grade dimethyl sulfoxide (DMSO, Sigma) and stored at −20 °C. Vehicle ($n = 30$–38, DMSO concentration of 0.15% v/v) and untreated (media only) wells were included on each assay plate and used for normalization of plate-specific readouts and quality control. No differences in all phenotypes between cells treated with either DMSO or media were observed (data not shown).

2.2. In silico assessment of chemical diversity

Diversity among chemicals included in the screening library, as compared to a curated dataset of 32,464 unique environmental chemicals and drugs described in Mansouri et al. (2016), was assessed by principal components analysis (PCA) using *in silico*-derived physico-chemical descriptors. PCA was conducted using the *gplots* package in R studio. Supplementary Table 2 contains a list of the 153 chemical descriptors obtained using the Chemistry Development Kit (CDK) (Steinbeck et al., 2003).

2.3. Cell culture

Human iPSC-derived cardiomyocytes (iCell® cardiomyocytes), plating medium, and maintenance medium were provided by Cellular Dynamics International (Madison, WI, USA). Cells were plated and maintained according to the manufacturer's recommendations as described previously (Sirenko et al., 2013a). Cells were plated into gelatin coated wells at 8000 cells/well in 384 multi-well plates. Synchronous beating of cells was evident after 7 days of culture and experiments were conducted 12–14 days post-plating on plates when all wells demonstrated regular synchronous beating.

2.4. Calcium flux assay

The intracellular Ca^{2+} flux in cardiomyocytes was assessed at 30 min and 24 h using the EarlyTox Cardiotoxicity kit (Molecular Devices, Sunnyvale, CA, USA) as described in detail elsewhere (Grimm et al., 2015; Sirenko et al., 2013a). For both time points, chemicals were tested using a single exposure in duplicate at concentrations ranging from 0.3 to 100 μM with semi-log dilutions. Fluorescent measurements of intracellular Ca^{2+}-flux were accomplished using the FLIPR® Tetra high-throughput cellular screening system combined with a TetraCycler Microplate Handler (Molecular Devices). Final DMSO concentrations were 0.15% (v/v) with the exception of 100 μM concentration data point where DMSO concentration was 0.5%. Our previous studies showed that DMSO (up to 1% v/v) has no effect on *in vitro* iPSC cardiomyocyte phenotypes (Grimm et al., 2015; Grimm et al., 2016; Sirenko et al., 2013b).

Prior to treatment with test chemicals, basal kinetics of intracellular Ca^{2+}-flux was determined in each well. Peak frequency (beats per minute), peak width (at 10% amplitude), peak spacing, peak amplitude, peak rise time (from 10% to 90% amplitude), and peak decay time (from 90% to 10% amplitude) were derived using the ScreenWorks® Peak Pro® software (Molecular Devices). Based on previous data

Download English Version:

https://daneshyari.com/en/article/5558322

Download Persian Version:

https://daneshyari.com/article/5558322

Daneshyari.com