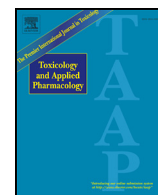
Toxicology and Applied Pharmacology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

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



High-throughput cardiac safety evaluation and multi-parameter arrhythmia profiling of cardiomyocytes using microelectrode arrays

Kristin H. Gilchrist *, Gregory F. Lewis, Elaine A. Gay, Katelyn L. Sellgren, Sonia Grego

RTI International, 3040 E. Cornwallis Road, Research Triangle Park, NC 27709, USA

ARTICLE INFO

Article history:
Received 11 May 2015
Revised 16 July 2015
Accepted 24 July 2015
Available online xxxx

Keywords:
Arrhythmia
Automated analysis
Human induced pluripotent stem cell-derived cardiomyocytes
QT prolongation
Microelectrode array
Field potential duration

ABSTRACT

Microelectrode arrays (MEAs) recording extracellular field potentials of human-induced pluripotent stem cell-derived cardiomyocytes (hiPS-CM) provide a rich data set for functional assessment of drug response. The aim of this work is the development of a method for a systematic analysis of arrhythmia using MEAs, with emphasis on the development of six parameters accounting for different types of cardiomyocyte signal irregularities. We describe a software approach to carry out such analysis automatically including generation of a heat map that enables quick visualization of arrhythmic liability of compounds. We also implemented signal processing techniques for reliable extraction of the repolarization peak for field potential duration (FPD) measurement even from recordings with low signal to noise ratios. We measured hiPS-CM's on a 48 well MEA system with 5 minute recordings at multiple time points (0.5, 1, 2 and 4 h) after drug exposure. We evaluated concentration responses for seven compounds with a combination of hERG, QT and clinical proarrhythmia properties: Verapamil, Ranolazine, Flecainide, Amiodarone, Ouabain, Cisapride, and Terfenadine. The predictive utility of MEA parameters as surrogates of these clinical effects were examined. The beat rate and FPD results exhibited good correlations with previous MEA studies in stem cell derived cardiomyocytes and clinical data. The six-parameter arrhythmia assessment exhibited excellent predictive agreement with the known arrhythmogenic potential of the tested compounds, and holds promise as a new method to predict arrhythmic liability.
© 2015 Elsevier Inc. All rights reserved.

Introduction

Drug attrition is a significant concern for the development of new pharmaceuticals, and adverse cardiovascular drug reactions have been a major cause of drug withdrawal from the market and termination of drug development candidates (Valentin and Hammond, 2008). The current drug cardiac safety paradigm aims at assessing the primary clinical endpoint of ventricular cardiac arrhythmias using drug-induced in vitro hERG channel blockade measured in non-cardiac cell lines and in vivo/clinical QT interval prolongation (Sager et al., 2014). This approach in recent years has prevented new drugs from entering the market with unanticipated potential for proarrhythmia (e.g. torsade de pointes, TdP); however, it may also have stopped the development of potentially valuable therapeutics because of false positives such as hERG positive Verapamil and QT prolonger Ranolazine (Lawrence et al., 2008; Chi, 2013; Sager et al., 2014).

Given the limited predictive utility of preclinical hERG inhibition as a marker of hERG-mediated QT prolongation, there is a need for a more efficient and mechanistic assessment for the cardiac risks of compounds at early stages of drug development. For this purpose, an intense research focus on novel assays has been enabled by the recent advent of human induced pluripotent stem cell-derived cardiomyocytes (hiPS-CMs). These cells are available on a large scale and exhibit molecular and electrophysiological properties (ionic currents and channel gating) similar to human cardiomyocytes (Ma et al., 2011). Drug induced electrophysiological alteration of hiPS-CMs have been investigated with multiple approaches offering adequate throughput for screening applications including automated patch-clamp (Kramer et al., 2013), impedance (Guo et al., 2011a), intracellular calcium concentration (Cerignoli et al., 2012; Sirenko et al., 2013), and microelectrode array (MEA) (Guo et al., 2011a,b; Harris et al., 2013; Clements and Thomas, 2014; Nozaki et al., 2014).

MEA's provide a data rich method for characterizing cardiomyocyte drug response by recording extracellular field potentials from cell monolayers or clusters and accessing multiple parameters including field potential duration (FPD), an in vitro analogue for QT. The utility of hiPS-CM MEA assays for preclinical cardiovascular risk assessment was investigated by Harris and colleagues with a set of drugs including hERG, sodium, and calcium blockers (Harris et al., 2013). That work found that MEA-acquired parameters (amplitude, beat rate and full potential duration) were in concentration-effect comparisons in good correlation with traditional preclinical assays using ex vivo preparations such as the rabbit ventricular wedge, and concluded that MEA assays offer a reliable, cost effective surrogate to preclinical assay without the

* Corresponding author at: RTI International, 3040 E. Cornwallis Road, Research Triangle Park, North Carolina 27709-2194, USA.
E-mail address: kgilchrist@rti.org (K.H. Gilchrist).

http://dx.doi.org/10.1016/j.taap.2015.07.024
0041-008X/© 2015 Elsevier Inc. All rights reserved.

ARTICLE IN PRESS

use of animal studies. Nozaki et al. (Nozaki et al., 2014) demonstrated FPD from MEA with hiPS-CMs can detect QT prolongation induced by multichannel blockers such as Terfenadine and Amiodarone. However, drugs that prolong QT are not necessarily proarrhythmic, such as Ranolazine.

There is a growing consensus that full and efficient assessment of cardiac liability for a new compound is not restricted to QT prolongation as a surrogate of arrhythmia but must aim at determination of proarrhythmia risk by using functional responses of cardiomyocytes. In the pursuit of a novel method for quantifying drug-induced proarrhythmia risk, Guo and colleagues (Guo et al., 2013) proposed an arrhythmia parameter based on irregular beat ratio. Clements and Thomas (Clements and Thomas, 2014) proposed cardiac liability assessment based on clustering analysis of FPD in combination with other MEA parameters. Harris and colleagues (Harris et al., 2013) reported irregular beats for Cisapride, however did not systematically quantify such irregularity. Arrhythmia from MEA recording has also been described as ventricular tachycardia-like and ventricular-fibrillation-like for the cardiac glycoside Ouabain (Guo et al., 2011b) and reported as fraction of the number of experiments in which it was observed; the response of hiPS-CM was in agreement with the response from guinea pig isolated heart preparation.

The aim of this work is the development of a method for a systematic analysis of arrhythmia in MEA, with emphasis on the development of a multiplicity of parameters accounting for different types of cardiomyocyte signal irregularities. Furthermore, we describe a software approach to carry out such analysis automatically. Traditionally MEA data analysis requires significant manual intervention that presents a bottleneck for screening for early drug cardiac liability at the level of 48 and 96 well plates. An open source code addressing automation has been reported for MEA analysis (Pradhapan et al., 2013), however it is primarily focused on automation of FPD calculation and lacks functionality for efficiently navigating data from a large number of wells.

We have developed software for a 48 well system recording from 768 electrodes with high temporal resolution and generating overall a large volume of data (approximately 1 GB/min of recording). Our approach accounts for missed beat detection and outliers that can significantly alter results and for temporal variations such as arrhythmia that can be overlooked with averaging. It includes a method for reliable extraction of the repolarization peak for FPD measurement even from recordings with low signal to noise ratios. The approach carries out automatic extraction of six parameters describing arrhythmia. The limitation of manual data analysis is exacerbated in the analysis of arrhythmia since a large volume of data needs to be screened to seek a relatively rare event. Furthermore, we propose a convenient visualization of arrhythmia parameters in a heat map that enables quick assessment of arrhythmic liability of compounds.

This manuscript describes our analysis methods and reports the results of the screening of seven standard hERG and multichannel blocking compounds selected because of their different effects in terms of beat rate, QT and clinical arrhythmia. The predictive utility of MEA parameters as surrogates of these clinical effects was examined.

Methods

Cell culture

Human induced pluripotent stem cell-derived cardiomyocytes (iCell Cardiomyocytes, Cellular Dynamics, Madison, WI) were used for all experiments. The direct plating protocol from Cellular Dynamics was used with 48-well MEA plates from Axion Biosystems. The MEA plates were coated with 50 μg/mL fibronectin and stored in the incubator for 1 h prior to plating. The iCells were thawed for 4 min at 37 °C and transferred to a 50 mL conical tube and gradually mixed with 9 mL of plating media. The number of viable cells was counted using trypan blue. Cells were resuspended in plating media to achieve 20,000 cells in 10 μL. Fibronectin was removed and the 10 μL drop of cell suspension was placed on the center of each electrode array. The plates were returned to the incubator for one hour prior to the addition of 300 μL of maintenance media to each well. Cells were fed with maintenance media every 2 days.

Compound selection and preparation

Seven compounds were selected for analysis based on a combination of hERG and clinical proarrhythmia properties. Cisapride is a serotonin receptor agonist that blocks hERG channels and was removed from the US market. Terfenadine is an antihistamine that blocks multiple ion channels and was also removed from the US market. Verapamil, used clinically for treatment of hypertension and angina, blocks L-type calcium and potassium channels. Flecainide is an antiarrhythmic drug that blocks sodium and potassium channels. Ranolazine is a sodium channel blocker used to treat angina. Amiodarone is a class III antiarrhythmic that blocks multiple ion channels. Ouabain is a cardiac glycoside that selectively inhibits Na+/K+-ATPase and increases cardiac contractility.

Cisapride, Flecainide, Ranolazine, and Terfenadine were purchased from Tocris Bioscience. Amiodarone, Ouabain, and Verapamil were purchased from Sigma Aldrich. All compounds were dissolved in dimethyl sulfoxide (DMSO) and serially diluted in DMSO. Compounds were then diluted in maintenance media to 10× the final desired concentration, so that the final DMSO concentration in the well was 0.1% for all compound concentrations. All compounds were tested at half-log concentration increments (e.g. 1 uM, 3.16 uM, 10 uM). For convenience, the half-log values are rounded to one significant digit in all presented data.

MEA experimental protocol

MEA recordings were performed with a commercial system (Maestro, Axion Biosystems, Atlanta, GA) that accommodates 12–96 well plates with 768 electrodes sampled at 12.5 kHz. The system consists of temperature controlled plate reader and an amplifier unit. The plate reader unit was enclosed in a custom enclosure flowing 5% CO_2 to ensure appropriate cell culture conditions for multi-hour experiments. Cardiomyocyte cell cultures were used for experiments between days 7–11. Prior to the experiment, all media was aspirated as 450 μL of maintenance media was added to each well to ensure a known volume of media. Cells were returned to the incubator for one hour. MEA plates were placed on the Maestro system and given 15 min to equilibrate after a stable reading of temperature 35 °C was obtained on the system. Baseline activity was recorded for 5 min. Compounds were administered by adding 50 μL of the prepared 10× samples along the edge of the well. A separate well was used for each concentration to avoid the ambiguity between time and concentration effects that can result from sequential dosing. Three compounds were tested per 48 well plates. Each compound was administered at 7 different concentrations in duplicate and 4 wells with 0.1% DMSO were used as controls. Compounds were tested on at least two different plates. Additional 5 min recordings were obtained at 30 min, 1 h, 2 h, and 4 h after the completion of the compound addition. All data was stored in the raw format for subsequent analysis.

Data analysis

All data analysis was performed in MATLAB (The MathWorks, Inc., Natick, MA) with a high degree of automation. The raw Axion format files were first parsed into different wells with 16 electrodes each and saved as MATLAB files. Each of these files was automatically run through a single well processing module where beats were detected and parameters extracted. For a typical experiment using a 48-well plate with data collected at 5 different times, this operation was performed 5 × 48, or 240 times. Beats were detected from each electrode using an adaptive

Please cite this article as: Gilchrist, K.H., et al., High-throughput cardiac safety evaluation and multi-parameter arrhythmia profiling of cardiomyocytes using microelectrode arrays, Toxicol. Appl. Pharmacol. (2015), http://dx.doi.org/10.1016/j.taap.2015.07.024

Download English Version:

https://daneshyari.com/en/article/5845923

Download Persian Version:

https://daneshyari.com/article/5845923

Daneshyari.com