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Multi-parametric assessment of cardiomyocyte excitation-contraction coupling using impedance and field potential recording: A tool for cardiac safety assessment

Xiaoyu Zhang [a], Liang Guo [b], Haoyu Zeng [c], Stephen L. White [d], Michael Furniss [b], Bharathi Balasubramanian [c], Edward Lis [c], Armando Lagrutta [c], Frederick Sannajust [c], Li Leyna Zhao [a], Biao Xi [a], Xiaobo Wang [a], Myrtle Davis [e], Yama A. Abassi [a,*]

[a] ACEA Biosciences, Inc., San Diego, CA 92121, USA
[b] Laboratory of Investigative Toxicology, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD 21702, USA
[c] SALAR, Safety & Exploratory Pharmacology Department, Merck Research Laboratories, West Point, PA 19486, USA
[d] Drug Synthesis & Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA
[e] Toxicology and Pharmacology Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA

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ABSTRACT

Introduction: The ICH S7B guidelines recommend that all new chemical entities should be subjected to hERG repolarization screening due to its association with life-threatening "Torsades de Pointes" (TdP) arrhythmia. However, it has become evident that not all hERG channel inhibitors result in TdP and not all compounds that induce QT prolongation and TdP necessarily inhibit hERG. In order to address the limitations of the S7B/E14 guidelines, the FDA through a public/private partnership initiated the Comprehensive in vitro Proarrhythmia Assay (CiPA) initiative to examine the possible modification and refinement of the ICH E14/S7B guidelines. One of the main components of the CiPA initiative is to utilize a predictive assay system together with human cardiomyocytes for risk assessment of arrhythmia.
Method: In this manuscript we utilize the xCELLigence® CardioECR system which simultaneously measures excitation-contraction coupling together with human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) to assess the effect of 8 reference compounds across 3 different independent sites. These 8 compounds were part of Phase I CiPA validation study.
Results: Our data demonstrate that hERG channel blockers, such as E4031 and moxifloxacin, prolonged field potential duration (FPD) at low concentration and induced arrhythmic beating activity as measured by field potential (FP) recording and impedance (IMP) recordings at higher concentrations. On the contrary, nifedipine, an inhibitor of calcium channel, didn't disrupt the periodicity of cell beating and weakened cell contractile activity and shortened FPD. Multichannel inhibitors, such as flecainide, quinidine and mexiletine, not only increased FPD and induced arrhythmia but also significantly reduced the amplitude of FP spike. JNJ303, an IK_s inhibitor, only affected FPD. Comparison of the compound effect on FPD across the 3 different sites is consistent in terms of trend of the effect with observed 3–10 fold differences in minimal effective concentration at which a minimum of 10% response is detected. In addition, pentamidine, a hERG trafficking inhibitor which induced irregular beating activity over a more prolonged duration of time was readily flagged in this assay system. Taken together, this multi-parameter assay using hiPSC-CMs in conjunction with simultaneous measurement of ion channel activity and contractility can be a reliable approach for risk assessment of proarrhythmic compounds.
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1. Introduction

Drug toxicity is a pervasive issue in pharmaceutical drug development that is difficult to avoid but needs to be identified and well mitigated. Failure to recognize such toxicities during the course of drug development can pose great risk not only to the target population but

* Corresponding author.
E-mail address: yabassi@aceabio.com (Y.A. Abassi).

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can also be of tremendous financial burden to drug developers due to drug withdrawals from the market and legal ramifications. The potential for clinical manifestation of ventricular arrhythmias, known commonly as "Torsade de Pointes" (TdP), by pharmaceutical drugs continue to be a major concern for both pharmaceutical industry and regulatory agencies (Redfern et al., 2003). In order to address this concern, the International Conference for Harmonization (ICH) released both non-clinical and clinical guidelines for assessment of cardiac risk of new chemical entities (Cavero & Crumb, 2005). The S7B guideline provides recommendations for non-clinical evaluation of new chemical entities and focuses on ventricular repolarization while the E14 guideline addresses the clinical evaluation of such compounds in a thorough QT (TQT) study (Sager, Gintant, Turner, Pettit, & Stockbridge, 2014; Wallis, 2010). Since the adoption and implementation of S7B and E14 guidelines in 2005, the number of drugs withdrawn from the market due to risk of TdP has been substantially reduced (Sager et al., 2014). While this has been a great achievement, it has had other unintended consequences justifying a reexamination of and possible modification of the existing guidelines.

One of the major drawbacks of the existing guidelines has been its narrow focus on the surrogates for TdP arrhythmias, rather than directly on the risk itself, namely arrhythmias (Fermini et al., 2016; Gintant, Sager, & Stockbridge, 2016; Mirams et al., 2014; Sager et al., 2014). For example, as part of the ICH S7B guidelines the emphasis of compound effect has been primarily focused on the repolarization current as manifested by the IK_r through the hERG channel. While a number of the withdrawn drugs had been shown to directly interfere with the hERG channel, it has been since shown that multiple ionic currents can influence hERG current block and furthermore hERG block alone sometimes does not provide a meaningful indication of QT prolongation or for that matter TdP. Furthermore, measurement of QT prolongation itself was shown to be highly sensitive for TdP, but not specific (Guo et al., 2013; Hanton, 2007; Hoffmann & Warner, 2006). The emphasis on hERG in the non-clinical part of the guideline has had the unintended effect of substantially increasing the attrition rate of potentially promising compounds in development. According to De Ponti at least 60% of compounds in development can interfere with hERG and as a result, a number of promising compounds were not pursued due to hERG liability profiles (De Ponti, 2008). Furthermore, QT prolongation itself can be susceptible to many different factors including age, sex, pathophysiological conditions (sympathetic and parasympathetic tone balance), glucose and insulin levels, and diet (Kallergis, Goudis, Simantirakis, Kochiadakis, & Vardas, 2012; Kannankeril, Roden, & Darbar, 2010).

Consequently, in order to address the limitations of the ICH-S7B/E14 guidelines, the FDA through a public/private partnership initiated the Comprehensive in vitro Proarrhythmia Assay (CiPA) initiative (Gintant et al., 2016) to examine the possible modification of the ICH E14/S7B guidelines. On the clinical side, the CiPA initiative proposes to eliminate the dedicated but costly TQT studies in Phase III and, to substitute with a detailed ECG study in standardized Phase I as recently defined by the E14 Working Group Q&As (R3) from December 10, 2015 (Bloomfield, 2015) to reduce time, number of subjects and therefore costs of ECG protocol. On the non-clinical side, CiPA proposes to utilize three complementary approaches which include: (i) additional ion channel screening above and beyond hERG; (ii) an in silico cardiac modeling approach which can be used to integrate and model the data from the ion channel screening and (iii) a relevant human cardiomyocyte model system such as induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) to assess the effect of pharmaceutical compounds and also confirm the isolated ion channel screening and in silico prediction of arrhythmia/proarrhythmia (Gintant et al., 2016; Sager et al., 2014).

In this paper, we report our results utilizing a platform technology (ACEA Biosciences, xCELLigence RTCA CardioECR™, San Diego, CA, USA) which has multi-readout capabilities to simultaneously monitor viability, cardiomyocyte integrated electrophysiology and impedance amplitude (IMP amp; or contractility) together with hiPSC-CMs to screen 8 reference compounds which were part of Phase I evaluation of the CiPA initiative for the cardiomyocyte group. The 8 compounds were initially blinded and used across 3 different sites to assess the consistency of the data across the sites. We have demonstrated the CardioECR assay system can indeed predict cardio-modulating activities of the reference compounds and that the results were compared across 3 different evaluation sites.

2. Methods

2.1. Cell culture

hiPSC-CMs from Cellular Dynamics International (CDI/iCell Cardiomyocytes: CMC-100-010-001, Madison, WI, USA, Lot #1093711) were kept in liquid nitrogen until thawed and cultured according to manufacturer instructions. Briefly, each well of the E-Plate CardioECR 48 (ACEA Biosciences, Inc., San Diego, CA, USA) was coated with 50 μl of a 1:100 diluted fibronectin (FN) solution at 10 μg/ml (F1114, Sigma-Aldrich, St. Louis, MO, USA) and incubated at 37 °C for 1 h, which was followed by replacing fibronectin solution with 50 μl of pre-warmed iCell CM plating medium. Cells were thawed and diluted in pre-warmed plating medium at 400,000 platable cells/ml. 50 μl of the cell suspension was transferred using multichannel pipette and seeded directly onto pre-coated E-Plate CardioECR 48 (20,000 platable cells/well) in laminar hood. The plates containing iCell cardiomyocytes (iCell CMs) were kept in the hood at room temperature for 30 min and then placed and cultivated in a humidified incubator with 5% CO_2 at 37 °C. The cardiomyocytes were washed to remove cell debris and the plating medium was replaced with iCell cardiomyocyte maintenance medium 48 h post-seeding. Medium change was performed every other day afterwards. Cells were then, incubated with test compounds on day 17.

2.2. Chemical reagents

All the chemical reagents were purchased from Tocris (Minneapolis, MN, USA), Sigma-Aldrich (St. Louis, MO, USA) or provided by the Chemotherapeutic Agents Repository of the National Cancer Institute. 1000-fold chemical stock solutions were prepared in DMSO and stored at −20 °C. The serial diluted chemicals (1000-fold) were further prepared in DMSO immediately prior to compound addition. The 10-fold final dilution of the chemicals was prepared with culture medium for single time use only. The final concentration of DMSO in the treated well was 0.1%.

2.3. xCELLigence® RTCA CardioECR monitoring of cardiomyocyte attachment, viability, field potential and contractility

Normally, hiPSC-CMs can generate very robust and consistent beating rate and FP signals on day 17 (beating rate > 25 beats/min; beating amplitude > 0.1 and amplitude of field potential spike > 0.5 mV).

Media were refreshed with 90 μl of pre-warmed hiPSC-CMs maintenance medium at least 4 h prior to compound addition. 10 μl of compound solutions were added to the wells in a single dose per well mode. xCELLigence® RTCA CardioECR system (ACEA Biosciences, Inc., San Diego, CA, USA) was used to monitor cardiomyocyte attachment, viability, contraction and FP from spontaneously beating hiPSC-CMs. IMP data were sampled at 2 ms (500 Hz), while FP data were collected at 0.1 ms (10 K Hz) with a bandwidth of 1 Hz to 3 KHz. Data acquisition is controlled by xCELLigence® RTCA CardioECR Data Acquisition software which operates the hardware and allows the user to define interval between each recording and the duration within each recording. In a typical experiment, a 30-min baseline of IMP and FP signals was taken by sampling hiPSC-CMs every 5 min for 20 or 60 s. Cells were then treated with vehicle control and studied compounds. The cell responses to

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