Journal of Pharmacological and Toxicological Methods 85 (2017) 73–81

Contents lists available at ScienceDirect

Journal of Pharmacological and Toxicological Methods

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

Research article

CSAHi study: Detection of drug-induced ion channel/receptor responses, QT prolongation, and arrhythmia using multi-electrode arrays in combination with human induced pluripotent stem cell-derived cardiomyocytes

Takashi Kitaguchi [a,b,c,*], Yuta Moriyama [b,c], Tomohiko Taniguchi [b,d], Sanae Maeda [b,d], Hiroyuki Ando [b,e], Takaaki Uda [b,e], Koji Otabe [b,f], Masao Oguchi [b,f], Shigekazu Shimizu [b,g], Hiroyuki Saito [b,g], Atsushi Toratani [b,h], Mahoko Asayama [b,h], Wataru Yamamoto [b,i], Emi Matsumoto [b,i], Daisuke Saji [b,j], Hiroki Ohnaka [b,j], Norimasa Miyamoto [a,b,d]

[a] Non-Clinical Evaluation Expert Committee, Drug Evaluation Committee, Japan Pharmaceutical Manufacturers Association (JPMA), 2-3-11 Nihonbashi-Honcho, Chuo-ku, Tokyo 103-0023, Japan
[b] Consortium for Safety Assessment using Human iPS Cells (CSAHi), Japan [1]
[c] Discovery Research, Mochida Pharmaceutical Co., Ltd., 722, Uenohara, Jimba, Gotemba, Shizuoka 412-8524, Japan
[d] Biopharmaceutical Assessment Core Function Unit, Medicine Development Center, Eisai Co., Ltd., 5-1-3 Tokodai, Tsukuba, Ibaraki 300-2635, Japan
[e] Safety Research Laboratories, Ono Pharmaceutical Co., Ltd., 50-10, Yamagishi, Mikuni-cho, Sakai-shi, Fukui 913-8538, Japan
[f] Research Administration Department, Ina Research Inc., 2148-188, Nishiminowa, Ina-shi, Nagano 399-4501, Japan
[g] Bioresearch Center, CMIC Pharma Science Co., Ltd., 10221, Kobuchisawa-cho, Hokuto-shi, Yamanashi 408-0044, Japan
[h] Sohyaku, Innovative Research Division, Mitsubishi Tanabe Pharma Corporation, 2-2-50, Kawagishi, Toda, Saitama 335-8505, Japan
[i] Pharmaceutical Development Research Laboratories, Toxicology Research Department, Teijin Pharma Ltd., 4-3-2, Asahigaoka, Hino-shi, Tokyo 191-8512, Japan
[j] Shiga Laboratory, Nisseibilis Co., Ltd., 555, Ukawa, Minakuchi-cho, Koka, Shiga 528-0052, Japan

ARTICLE INFO

Article history:
Received 7 September 2016
Received in revised form 5 December 2016
Accepted 1 February 2017
Available online 3 February 2017

Chemical compounds studied in this article:
(±)-Bay K8644 (PubChem CID: 2303)
Mibefradil dihydrochloride (PubChem CID: 60662)
NS1643 (PubChem CID: 10177784)
Levcromakalim (PubChem CID: 93504)
Ouabain octahydrate (PubChem CID: 122130920)
$BaCl_2$ dihydrate (PubChem CID: 5284346)
ZD7288 (PubChem CID: 123983)
Isoproterenol hydrochloride (PubChem CID: 5807)

ABSTRACT

Introduction: The use of multi-electrode arrays (MEA) in combination with human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) provides a promising method to predict comprehensive cardiotoxicity, including drug-induced QT prolongation and arrhythmia. We previously demonstrated that MEA in combination with hiPSC-CMs could provide a generalizable platform by using 7 reference drugs at 10 testing facilities. Using this approach, we evaluated responses to reference drugs that modulate a range of cardiac ion currents and have a range of arrhythmogenic effects.
Methods: We used the MEA system (MED64) and commercially available hiPSC-CMs (iCell cardiomyocytes) to evaluate drug effects on the beat rate, field potential duration (FPD), FPD corrected by Fridericia's formula (FPDc), and the incidence of arrhythmia-like waveforms.
Results: This assay detected the repolarization effects of Bay K8644, mibefradil, NS1643, levcromakalim, and ouabain; and the chronotropic effects of isoproterenol, ZD7288, and $BaCl_2$. Chronotropy was also affected by K^+ and Ca^{2+} current modulation. This system detected repolarization delays and the arrhythmogenic effects of quinidine, cisapride, thioridazine, astemizole, bepridil, and pimozide more sensitively than the established guinea pig papillary muscle action potential assay. It also predicted clinical QT prolongation by drugs with multiple ion channel effects (fluoxetine, amiodarone, tolterodine, vanoxerine, alfuzosin, and ranolazine).
Discussion: MEA in combination with hiPSC-CMs may provide a powerful method to detect various cardiac electrophysiological effects, QT prolongation, and arrhythmia during drug discovery. However, the data require

Abbreviations: CSAHi, Consortium for Safety Assessment using Human iPS Cells; DMSO, dimethyl sulfoxide; EAD, early after depolarization; FP, field potential; FPD, field potential duration; FPDc, field potential duration corrected by Fridericia's formula; $FPDc_{10}$, concentrations inducing FPDc prolongation by 10%; gpAPD, action potential duration measurement using guinea pig papillary muscles; hC_{max}, maximum plasma concentration in human; hC_{QT}, reported plasma concentrations which prolonged QT interval in human; hC_{TdP}, reported plasma concentrations which caused torsade de pointes in human; hERG, human *ether-à-go-go*-related gene; hESC-CMs, human embryonic stem cell-derived cardiomyocytes; hiPSC-CMs, human induced pluripotent stem cell-derived cardiomyocytes; ICH, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; I_{K1}, inward rectifier K^+-current; I_{KATP}, ATP-sensitive inward rectifier K^+-current; I_{Kr}, rapid component of the delayed-rectifier K^+-current; I_{Ks}, slow component of the delayed-rectifier K^+-current; I_{Na}, Na^+-current; IS, internal standard; I_{to}, transient outward K^+-current; $MC_{EAD/TA}$, minimum concentration inducing early after depolarization and/or triggered activity-like waveforms; MEA, multi-electrode arrays; TA, triggered activity; TdP, torsade de pointes.

* Corresponding author at: Discovery Research, Mochida Pharmaceutical Co., Ltd., 722, Uenohara, Jimba, Gotemba, Shizuoka 412-8524, Japan.
E-mail address: takashi.kitaguchi@mochida.co.jp (T. Kitaguchi).

[1] http://csahi.org/en/.

http://dx.doi.org/10.1016/j.vascn.2017.02.001
1056-8719/

Keywords:
CSAHi
Field potential
Human induced pluripotent stem cell-derived cardiomyocytes
Multi-electrode array
Proarrhythmia
QT prolongation
Torsade de pointes
Methods

careful interpretation to predict chronotropic effects and arrhythmogenic effects of candidate drugs with multiple ion channel effects.

1. Introduction

Drug-induced arrhythmia is recognized as a major concern for drug development, and may result in market withdrawal. Drugs that cause delayed ventricular repolarization (QT interval prolongation) followed by polymorphic ventricular tachyarrhythmia, known as torsade de pointes (TdP), require the inclusion of a boxed warning (Kannankeril, Roden, & Darbar, 2010). The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) S7B (ICH, 2005) recommended *in vitro* inhibition assays of the rapid component of the delayed-rectifier K^+-current (I_{Kr}); this is determined by the alpha subunit of the K^+ channel, which is encoded by the human *ether-à-go-go*-related gene (hERG). In addition, the ICH recommended *in vivo* QT interval assessment and *ex vivo* or *in vivo* follow-up studies in animal models in order to estimate the risk of QT prolongation and related arrhythmia in humans (ICH, 2005).

This guideline has been successful, because since the implementation of ICH S7B and E14, no proarrhythmic risk-related market withdrawals have occurred. Currently, hERG inhibition-centered screening is conducted by many pharmaceutical companies. On the other hand, there are increasing concerns that this process could result in the failure of promising drug candidate development due to false positive results, especially at the early drug discovery stage. This exclusion of 'hERG positives' without any detailed preclinical and/or clinical assessment could limit patient access to potentially beneficial drugs (Sager, Gintant, Turner, Pettit, & Stockbridge, 2014). In accordance with these concerns, assessments of the effects on multiple ion channels were more predictive of arrhythmic effects than hERG inhibition or QT prolongation alone. These assessments include evaluations of inhibition of the hERG channel, the $Na_v1.5$ voltage-gated Na^+-channel, and the $Ca_v1.2$ voltage-gated Ca^{2+}-channel, or of the index of cardiac electrophysiological balance, which assesses the balance between QT interval and QRS duration (Kramer et al., 2013; Lu, Yan, & Gallacher, 2013). Under these circumstances, pharmaceutical companies have tried to apply various screening assays using isolated cardiac tissues from experimental animals to evaluate the net cardiac electrophysiological effects of compounds at the drug discovery stage (Lu et al., 2013; Morissette et al., 2013; Takasuna, Chiba, & Manabe, 2009). However, these assays require experimental animals and operator skill, and have a relatively low throughput. In addition, facility-specific differences in the experimental conditions including the choice of animal species, type of anesthetic, origin of tissues, and equipment could lead to discordant results. The measurement of action potential duration using guinea pig papillary muscles (gpAPD) is a well-validated model and its utility as a follow-up safety pharmacology study was confirmed in ICH S7B (ICH, 2005; Yamazaki et al., 2005); however, this assay is also known to have produced some false negative results in terms of its prediction of drug-induced QT prolongation. Therefore, more generalizable and more predictive assays are needed to assess clinical proarrhythmic potential.

Recently, large-scale production of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) has been achieved, and the expression of cardiac ion channels including I_{Na}, I_{CaL}, I_f, I_{to}, I_{K1}, I_{Kr}, and I_{Ks} by these cells has been confirmed (Ma et al., 2011). Therefore, an assay using hiPSC-CMs may be able to assess the net cardiac electrophysiological activity. However, the pharmacological responses of the cardiac ion channels and receptors expressed by these cells have not been fully established.

Accordingly, we established the Consortium for Safety Assessment using Human iPS cells (CSAHi; http://csahi.org/en/) in 2013, which is based on the Japan Pharmaceutical Manufacturers Association. The mission of the CSAHi is to promote technological advancements and policy recommendations that will facilitate the application of human iPS cell-derived cardiomyocytes, hepatocytes, and neurons in drug safety evaluation. The main goal of the CSAHi heart team is to propose comprehensive screening strategies that may be used to predict diverse cardiotoxic effects. These strategies may include monitoring the pleiotropic phenotypes of hiPSC-CMs using extracellular field potentials (FPs) determined by multi-electrode array (MEA), action potentials determined by patch-clamp recording, cellular contraction determined by impedance or cell deformation analysis, and Ca^{2+} transients and cytotoxicity determined by high content imaging. The combination of these methodologies has the potential to predict not only QT prolongation but also arrhythmia, contractile dysfunction, and structural toxicity. In particular, MEA has been used in many pharmacological studies of cardiac tissue preparations, including hiPSC-CMs. This approach requires less operator skill than required for traditional current or action potential assay methods and its noninvasive nature allows for easy and stable drug studies (Stett et al., 2003). MEA can sensitively detect electrophysiological parameters such as the FP duration (FPD), which reflects cellular repolarization and is suggested to provide an indication of the QT interval. Furthermore, MEA can also detect arrhythmia-like waveforms that may serve as direct biomarkers for TdP.

We previously demonstrated that MEA in combination with hiPSC-CMs could provide a generalizable platform by using 7 reference drugs at 10 testing facilities. The assay detected repolarization delay and/or arrhythmogenic effects of inhibitors of I_{Kr} and the slow component of the delayed-rectifier K^+-current (I_{Ks}) (Kitaguchi et al., 2016).

To investigate the utility of MEA in combination with hiPSC-CMs at the drug discovery stage, we assessed whether hiPSC-CMs could predict electrophysiological effects by evaluating their responses to drugs acting *via* a range of mechanisms. These included an L-type Ca^{2+} current (I_{CaL}) activator, a T-type Ca^{2+} current (I_{CaT}) and I_{CaL} inhibitor, an I_{Kr} activator, an ATP-sensitive inward rectifier K^+-current (I_{KATP}) activator, a Na^+/K^+-ATPase inhibitor, a β adrenergic agonist, a funny current (I_f) inhibitor, and an inward rectifier K^+-current (I_{K1}) inhibitor. Next, we assessed the sensitivity of MEA with hiPSC-CMs by evaluating 6 drugs that showed 'false negative' results in the gpAPD assay and 6 drugs with multiple ion channel effects to determine whether this assay system could be used to predict QT prolongation and arrhythmogenic liability in humans, and to detect the net cardiac repolarization effects.

2. Materials and methods

The experimental procedures described below were the same as those described previously, with the exception of the band-pass filter conditions (Kitaguchi et al., 2016). The work was conducted at 8 facilities.