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Safety pharmacology studies using EFP and impedance

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ABSTRACT

Introduction: While extracellular field potential (EFP) recordings using multi-electrode arrays (MEAs) are a well-established technique for monitoring changes in cardiac and neuronal function, impedance is a relatively unexploited technology. The combination of EFP, impedance and human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) has important implications for safety pharmacology as functional information about contraction and field potentials can be gleaned from human cardiomyocytes in a beating monolayer. The main objectives of this study were to demonstrate, using a range of different compounds, that drug effects on contraction and electrophysiology can be detected using a beating monolayer of hiPSC-CMs on the CardioExcyte 96.

Methods: hiPSC-CMs were grown as a monolayer on NSP-96 plates for the CardioExcyte 96 (Nanion Technologies) and recordings were made in combined EFP and impedance mode at physiological temperature. The effect of the hERG blockers, E4031 and dofetilide, hERG trafficking inhibitor, pentamidine, β-adrenergic receptor agonist, isoproterenol, and calcium channel blocker, nifedipine, was tested on the EFP and impedance signals.

Results: Combined impedance and EFP measurements were made from hiPSC-CMs using the CardioExcyte 96 (Nanion Technologies). E4031 and dofetilide, known to cause arrhythmia and Torsades de Pointes (TdP) in humans, decreased beat rate in impedance and EFP modes. Early afterdepolarization (EAD)-like events, an in vitro marker of TdP, could also be detected using this system. Isoproterenol and nifedipine caused an increase in beat rate. A long-term study (over 30 h) of pentamidine, a hERG trafficking inhibitor, showed a concentration and time-dependent effect of pentamidine.

Discussion: In the light of the new Comprehensive in Vitro Proarrhythmia Assay (CiPA) initiative to improve guidelines and standardize assays and protocols, the use of EFP and impedance measurements from hiPSCs may become critical in determining the proarrhythmic risk of potential drug candidates. The combination of EFP offering information about cardiac electrophysiology, and impedance, providing information about contractility from the same area of a synchronously beating monolayer of human cardiomyocytes in a 96-well plate format has important implications for future cardiac safety testing.

1. Introduction

For over a decade the cardiac safety guidelines S7B and E14 from the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH; http://www.ich.org/) have been in place to assess a potential drug candidate's ability to cause long QT syndrome which can lead to potentially fatal Torsades de Pointes (TdP). These guidelines have primarily focused on the interaction with the delayed rectifier current (IKr) mediated by the hERG channel. Although this approach has been largely successful in preventing new drugs reaching the market with unexpected potential to cause TdP, it is also possible that potentially valuable therapeutics have failed due to this early screening. Indeed, there are cases in which compounds interact with hERG but don't necessarily cause TdP, e.g. verapamil (Zhang, Zhou, Gong, Makielski, & January, 1999), and vice versa.

In order to address this problem and allow more potentially useful and safe drugs to reach the market, the Comprehensive in Vitro Proarrhythmia Assay (CiPA) initiative, an international collaborative effort to re-evaluate safety assessment procedures, was formed in 2013 (Cavero & Holzgrefe, 2014; Fermini et al., 2016; Sager, Gintant, Turner, Pettit, & Stockbridge, 2014). Although patch clamp of hERG and other ion channels e.g. $Na_V1.5$ and $Ca_V1.2$ remain an important part of cardiac safety testing, techniques such as multi-electrode arrays (MEA) and impedance are also being considered as complementary assays for cardiac safety assessment using cardiac cells such as human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). hiPSC-CMs are an attractive alternative to acutely isolated cardiomyocytes from animals for a number of reasons including their recapitulation of native

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behavior, long-term stability in culture and standardized production. Additionally, they are suitable for use on high throughput screening (HTS) platforms and have been used successfully on automated patch clamp systems (Abassi et al., 2012; Becker et al., 2013; Friedrichs, Malan, Voss, & Sasse, 2015; Scheel et al., 2014; Obergrussberger, Brüggemann, et al., 2015; Obergrussberger, Stölzle-Feix, et al., 2015; Obergrussberger, Haarmann, et al., in press; Stoelzle, Haythornthwaite, et al., 2011; Stoelzle, Obergrussberger, et al., 2011), MEA platforms (Clements & Thomas, 2014; Clements, Millar, Williams, & Kalinka, 2015; Gilchrist, Lewis, Gay, Sellgren, & Grego, 2015; Guo, Abrams, et al., 2011; Navarrete et al., 2013) and impedance devices (Clements & Thomas, 2014; Doerr et al., 2015; Guo, Abrams, et al., 2011; Jonsson, Wang, & Becker, 2011; Kho et al., 2015; Lamore, Scott, & Peters, 2015; Nguemo, Šaric, Pfannkuche, Reppel, & Hescheler, 2012; Obergrussberger, Thomas, et al., in press; Peters, Lamore, Guo, Scott, & Kolaja, 2014; Xi et al., 2011), amongst others.

MEAs generate information-rich high throughput data about cardiomyocyte electrophysiology by measuring field potential waveforms. A number of MEA systems are commercially available and have been used to record hiPSC-CMs including the Axion Maestro (Axion Biosciences) (Clements & Thomas, 2014; Clements et al., 2015; Gilchrist et al., 2015), and MEA systems from Multi Channel Systems (Asakura et al., 2015; Guo, Abrams, et al., 2011; Guo, Qian, et al., 2011; Harris et al., 2013). Although impedance measurements of cells as a cell-based label-free assay have been reported previously, this has been with low sampling rates allowing only a broad characterization of cytotoxicity and cell morphology/motility (Giaever & Keese, 1993; Xi, Yu, Wang, Xu, & Abassi, 2008). The introduction of the xCelligence RTCA Cardio system (ACEA Biosciences and Roche Applied Biosciences) (Guo, Abrams, et al., 2011; Jonsson et al., 2011; Kho et al., 2015; Lamore et al., 2015; McGowan, Hoyt, Li, Lyons, & Abbadie, 2009; Nguemo et al., 2012; Peters et al., 2014; Peters, Scott, Ochalski, & Dragan, 2012) and CardioExcyte 96 (Clements & Thomas, 2014; Doerr et al., 2015; Obergrussberger, Thomas, et al., in press), with a temporal resolution of 12.9 ms and 1 ms, respectively, allows more detailed information about contractility to be obtained. The CardioExcyte 96 has the advantage that MEA-like EFP measurements and impedance recordings can be made using electrodes which are in the same place in the well. The CardioExcyte 96 is not an MEA system as there are not multiple electrodes per well, but one sensing and one circular reference electrode per well where both impedance and EFP recordings can be made from the same area. This is important as the position of the electrodes appears to have an influence on the signal recorded. The CardioExcyte 96, therefore, has an advantage over MEA devices which do not give any insight into contraction and an advantage over optical methods which give no information about electrophysiology. Indeed, compounds such as blebbistatin have been shown to disrupt contraction while having no effect on EFP (Abassi et al., 2012; Doerr et al., 2015; Guo, Abrams, et al., 2011; Lamore et al., 2015; Peters et al., 2014). In a system such as the CardioExcyte 96, such an effect can be readily detected, a distinct advantage over EFP or contractility measurements alone. Importantly the measurements are made from intact cells beating synchronously in a monolayer. The aim of this article is to show the potential of combined impedance and EFP recordings for cardiac safety testing using standard compounds targeting hERG and other cardiac channels.

2. Methods

The methods involved in sensor plate preparation, cell seeding, monitoring of monolayer formation, pharmacology and data analysis for the CardioExcyte 96 (Nanion Technologies GmbH) have been described in detail in Doerr et al. (2015) and Obergrussberger, Thomas, et al. (in press) and the steps are given here in brief.

2.1. System components

The CardioExcyte 96 (Nanion Technologies GmbH) is a hybrid system for recording in EFP and impedance modes (Fig. 1). The instrument can be used in an incubator or in combination with the incubation system to maintain physiological conditions (Fig. 1). The incubation system is a miniature incubation system for desktop operation. It ensures full control over temperature, humidity and CO_2, within, and beyond, physiological ranges. It is controlled by software and avoids temperature artifacts during liquid handling.

The data was acquired in EFP, impedance or combined mode using the CardioExcyte Control software (Nanion Technologies GmbH). Data was analyzed using DataControl 96 (Nanion Technologies GmbH).

2.2. Sensor plate preparation and cell seeding

Sensor plates (NSP-96) were coated using fibronectin. The wells to be used were incubated in 10 μg/ml fibronectin (Sigma Aldrich, St. Louis, MO, Product: FIBRP-RO 1 mg dissolved in 1 ml PBS) in PBS (including Ca^{2+} and Mg^{2+}; Thermofisher/Gibco 14040-083 DPBS) for 2 h at 37 °C or overnight at 4 °C. The fibronectin was removed shortly before cell seeding. Cells to be seeded were either frozen stocks which were thawed as per the manufacturer's instructions (see also Obergrussberger, Thomas, et al., in press for an outline of the most common steps involved). Trypan Blue staining was used to check viability and count cells. Cells were centrifuged 100 *g* for 2 min, the media were removed and the cell pellet was resuspended in manufacturer-supplied media to yield a cell density of 20,000–40,000 viable cells per well to ensure the formation of a synchronously beating monolayer when seeded. To seed the cells, after removal of the fibronectin coating solution from the wells, 100 μl prewarmed 37 °C medium and then the cell suspension were added to each well. After the cells were seeded, the NSP-96 was left under the cell culture hood for 30 min without moving it to allow the cells to settle evenly.

2.3. Monitoring formation of monolayer and synchronous beating

The CardioExcyte 96 (Nanion Technologies GmbH) was used in combined, impedance and EFP, mode. Once the cells were seeded on the NSP-96, the sensor plate was mounted on the CardioExcyte 96 using the lever (Fig. 1) with an incubation chamber or inside an incubator. The temperature was maintained at 37 °C. Online parameters in EFP and impedance modes, e.g. amplitude, beat rate, were monitored every 30–60 min for at least 4 days to allow the formation of the monolayer

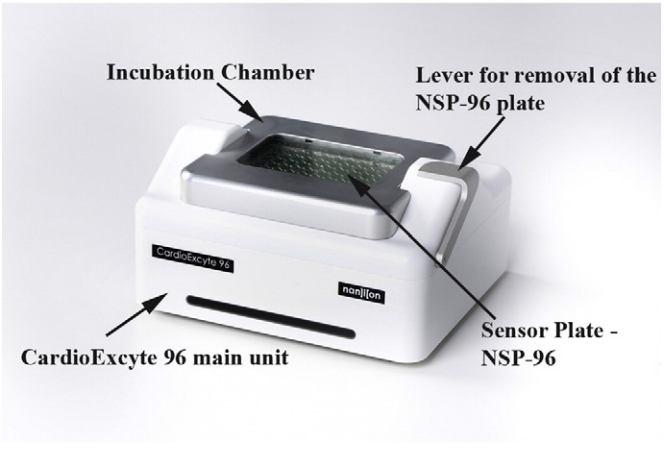

Fig. 1. The CardioExcyte 96 with incubation chamber, NSP-96 in recording position and hand lever for removal of the NSP-96. By using the incubation chamber, a constant temperature and humidity can be maintained without the need for a separate cell culture incubator. The use of the incubation chamber minimizes temperature changes when exchanging solutions, e.g. by media changes or addition of compounds.