Journal of Pharmacological and Toxicological Methods 82 (2016) 74–82

Contents lists available at ScienceDirect

Journal of Pharmacological and Toxicological Methods

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

Research article

Influence of field potential duration on spontaneous beating rate of human induced pluripotent stem cell-derived cardiomyocytes: Implications for data analysis and test system selection

Georg Rast [a,*], Udo Kraushaar [b], Sandra Buckenmaier [b], Carina Ittrich [c], Brian D. Guth [a]

[a] Boehringer Ingelheim Pharma GmbH & Co. KG, Drug Discovery Support, Birkendorfer Straße 65, 88397 Biberach, Germany
[b] Natural and Medical Sciences Institute at the University of Tübingen, Electrophysiology, Markwiesenstraße 55, 72770 Reutlingen, Germany
[c] Boehringer Ingelheim Pharma GmbH & Co. KG, Clinical Statistics, Birkendorfer Straße 65, 88397 Biberach, Germany

ARTICLE INFO

Article history:
Received 9 June 2016
Received in revised form 19 July 2016
Accepted 4 August 2016
Available online 9 August 2016

Keywords:
Cardiomyocyte
Delayed repolarization
Drug safety
Induced pluripotent stem cell
Methods
Proarrhythmic risk
Rate correction algorithm

ABSTRACT

Introduction: Field potential duration in human pluripotent stem cell (hiPSC)-derived cardiomyocytes is discussed as parameter for the assessment of drug-induced delayed repolarization. In spontaneously beating hiPSC-derived cardiomyocytes field potential duration varies depending on beating rate but beating rate can also be influenced by field potential duration. This interdependence is not fully understood and therefore mandates careful data analysis and cautious interpretation of the results.
Methods: We analysed data from several types of hiPSC-derived cardiomyocytes and, for comparison, primary embryonic chick cardiomyocytes using reference compounds to study the relationship between spontaneous rate and field potential duration. Based on such data we developed a method based on a regression model of drug-induced changes in the inter-beat interval versus changes in the field potential duration to distinguish primary rate from repolarisation effects.
Results: We demonstrate the application of this approach with reference and research compounds. Cells from different sources differed with regard to the direct or indirect effects of reference compounds on spontaneous beating. All cell types showed an adaptation of field potential duration upon rate changes induced by reference compounds, however, the adaptation of the spontaneous rate after compound-induced changes in field potential duration varied considerably between cell types.
Discussion: As shown by comparison with data from guinea pig papillary muscle, an ex vivo model with a fixed stimulation rate, this approach is more appropriate than the application of correction algorithms routinely used for in vivo data since such algorithms do not account for a dependence of rate on field potential duration.

Definitions

To account for the fact that the parameters defined for a field potential recorded in a two-dimensional cell culture differ fundamentally from those observed in an ECG in vivo, the convenient abbreviations borrowed from traditional ECG endpoints are printed in lower case:

rr: Δt between two consecutive threshold events (threshold is set to detect the fast and large voltage deflection relating to the depolarisation phase). rr may be interpreted as well as "inter-beat interval" or "inter-spike interval", however, it must be appreciated that synchronized contractions of a two-dimensional cell culture neither resemble "beats" as they are known from intact hearts, nor do regular action potentials from cardiomyocyte-like cells resemble "spikes" as they are known from neurons. The inverse of the rr can be interpreted as an instantaneous "rate".

qt_{max}: Δt between threshold event and maximum of small and slow voltage deflection (usually within a window between 300 ms and 1000 ms after the threshold event). The qt_{max} may also be interpreted as a measure for field potential duration; the same restrictions apply as for "rr".

rr/qt model: The model of the relation between rr and qt is defined by the actual combination of rr and qt at any given point in time or at any concentration of a specific pharmacologically active compound

Abbreviations: ECG, electro cardiogram; hiPSC, human induced pluripotent stem cells; MEA, multi-electrode array; QT, time interval between Q and T waveform in the ECG; QTc, rate corrected QT.

* Corresponding author at: Boehringer Ingelheim Pharma GmbH & Co. KG, Dept. of Drug Discovery Support, Birkendorfer Strasse 65, 88397 Biberach, Germany.
E-mail address: Georg.Rast@boehringer-ingelheim.com (G. Rast).

http://dx.doi.org/10.1016/j.vascn.2016.08.002
1056-8719/

under the assumption that a steady state for both rr and qt is reached. Whether rr or qt is used as independent variable is arbitrary, however, qt was chosen as the independent variable in all cases, as the square polynomial used for fitting is easier to handle than its inverse function.

1. Introduction

The availability of human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes and the desire to replace animal studies have spurred attempts to integrate cellular systems into cardiac safety testing, particularly in the assessment of proarrhythmic risk based on delayed repolarisation (Guo et al., 2011; Harris et al., 2013; Navarrete et al., 2013; Tanaka et al., 2009). An initiative lead by the Health and Environmental Sciences Institute (HESI) is working on a consolidated consensus framework (Comprehensive in vitro Proarrhythmia Assay (CiPA); Sager, Gintant, Turner, Pettit, & Stockbridge, 2014) giving guidance on validation, standardization, interpretation, and strategic positioning of assays utilizing hiPSC-derived cardiomyocytes. This initiative is based on the assumption that cardiomyocytes of human origin will have a higher level of predictivity with regard to drug-induced effects on the myocardial action potential than animal-based models. However, many open questions need to be resolved before the assay systems can be considered to be validated sufficiently for a successful implementation (Cavero & Holzgrefe, 2015).

For example, cultures of hiPSC-derived cardiomyocytes display a spontaneous, stable and regular beating pattern (Guo et al., 2011; Harris et al., 2013; Nakamura et al., 2014; Navarrete et al., 2013; Qu & Vargas, 2015; Rast et al., 2015). The beating rate or its inverse, rr (see Definitions), may be analysed as a secondary read-out parameter in addition to qt_{max}, but it becomes a confounding factor as soon as it varies concomitantly with field or action potential duration. The magnitude of this issue is unknown, since a systematic comparative study of the spontaneous beating rate in hiPSC-derived cardiomyocytes from different sources and under different culture conditions is lacking. Nevertheless, under the influence of reference compounds rr may often depend on qt_{max} in addition to a rate-dependency of qt_{max}, as it is known from in vivo systems. Although such effects are evident in several reports (Guo et al., 2011; Harris et al., 2013; Nakamura et al., 2014; Navarrete et al., 2013; Qu & Vargas, 2015; Rast et al., 2015), the findings were rarely discussed, their implications remain unclear, or their occurrence is found to be inconsistent (Qu & Vargas, 2015). This situation prompted us to undertake a systematic study approach with hiPSC-derived cardiomyocytes from various sources, to include primary embryonic chick cardiomyocytes as a reference point for the judgment of heterogeneity, and to propose a careful interpretation of the findings.

When there is an interdependence of rr and qt_{max}, for any drug-induced change of both parameters it would have to be decided which of the changes was cause and which was effect and whether a change in one parameter was sufficient to explain the change in the other.

For in vivo data rate correction algorithms are routinely used to compensate for potential drug-induced rate changes. This approach has also been applied to hiPSC-derived cardiomyocyte data (Asakura et al., 2015; Harris et al., 2013; Nakamura et al., 2014; Navarrete et al., 2013) but without a clear rationale as this is based on the assumption that there is only a dependence of qt_{max} on rr but not vice versa. Therefore, as an alternative, novel approach, we suggest modelling the drug-induced changes in the inter-beat interval versus changes in the field potential duration, which we refer to as the "rr/qt model" (see Definitions). We have applied this approach using data from reference compounds with known pharmacology that produce a selective, primary change of either rate or field potential duration. Based on these models we propose to assess the discriminative power of a specific cellular test system to separate rate effects from repolarisation effects. A comparison with data from guinea pig papillary muscle, an ex vivo model stimulated at a constant rate, demonstrates that our approach detects prolonged field potential duration more reliably than a conventional rate correction approach.

2. Methods

2.1. Compounds

Test compounds were purchased as dry powder from Sigma Aldrich, Fluka, and Tocris. Research compounds were synthesized in the medicinal chemistry department of Boehringer Ingelheim. All compounds were dissolved in DMSO as master stock solutions having a concentration 1000-fold the highest final concentration. Serial dilutions were then made from the master stock in DMSO being 1000-fold as concentrated as the remaining final concentrations to be tested. In studies employing a perfusion system (iCells, Pluricytes, papillary muscle studies) final concentrations were generated by a single 1:1000 dilution step into a baseline solution, in studies using a fixed incubation volume (Cor.4U, Cor.V4U, iCells^2, primary embryonic chick cardiomyocytes) by exchanging 10% of the recording solution by a 10-fold concentrated compound solution, followed by careful mixing using a pipette.

2.2. Field potential studies

Commercially available cells were purchased from the respective vendors as frozen stocks and processed as follows:

iCells and iCells^2 (Cellular Dynamics International CDI, Madison, WI, USA) were seeded as described previously (Rast et al., 2015). Cor.4U and CorV.4U (Axiogenesis, Cologne, Germany) were seeded according to the manufacturer's protocol. In brief, frozen cells were thawed and cultivated in 6-well plates at a density of $1.5 * 10^6$ cells/well for 2 days, after which they were enzymatically detached and plated on Geltrex (Life Technologies)-coated 96-well plates equipped with planar recording electrodes (Multi Channel Systems MCS, Reutlingen, Germany) at 10^4 cells/5 μL. The cells were allowed to recover and to form a functional syncytium for four days before usage.

Pluricytes (Pluriomics, Leiden, The Netherlands) were seeded according to the manufacturer's protocol. In brief, cells were thawed and seeded directly into fibronectin-coated 9-well MEA chips (MCS) at a density of $30 * 10^3$ cells/electrode array (a spot of 2 mm diameter within each well). Cells were used for experiments on days 5 to 8 after plating.

Primary ventricular chick embryonic cardiomyocytes were generated as described in Connolly, Clark, Curtis, Dow, and Wilkinson (1990); in brief, ventricular cardiomyocytes were isolated from 13 day-old chicken embryos using enzymatic digestion. Cells were plated at a density of 10^5 cells/5 μL on polyethylenimide-coated 96-well plates equipped with planar recording electrodes (MCS) and recorded after five days in culture.

Measurements with iCells and Pluricytes were performed as described previously (Rast et al., 2015). Measurements with Cor.4U, CorV.4U, iCells^2, and primary chick embryonic cardiomyocytes were performed as described in Meyer, Boven, Guenther, and Fejtl (2004). Briefly, cardiomyocytes cultivated on a 96-well plate were inserted into the respective recording system (MCS). Recordings were obtained at 37 °C in a humidified carbogen atmosphere (95% O_2 and 5% CO_2), using a bandpass filter of 1 Hz–3 kHz and a sampling rate of 10 kHz. The cells were allowed to rest for 15 min before the experiments, followed by a control recording of 2 min. Compound application was followed by a 5 min incubation period and additional 2 min of recording. For experiments with increasing concentrations compounds were applied cumulatively.