Assessment of in vitro compound-induced pro-arrhythmia in human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes using multiple assay platforms

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Abstract

Introduction
NIH-sponsored cardiomyocytes hold great potential for safety pharmacology testing. Therefore, they are currently evaluated within the CiPA (Comprehensive in vitro Preclinical Assessment) consortium as models for prediction of drug-induced arrhythmias.

Objective
Here, we assessed the effects of a set of low (verapamil, mexiletine, nifedipine, and diltiazem), intermediate (amiloride), and high (Mexiletine, dofetilide) risk CIPA compounds on the electrophysiology of hiPSC-derived ventricular cardiomyocytes (Pluricyte® Cardiomyocytes). Cardiomyocytes were cultured in well-defined Pluricyte® CardioECR Medium, and compound-effects were assessed using MEA analysis and Ca²⁺-flux assays, to evaluate them as a relevant in vitro model for preclinical cardiac safety assessment.

Results
Both MEA analysis and Ca²⁺-flux assays are suitable to detect pharmacological responses in Pluricyte® Cardiomyocytes. As expected, high and intermediate risk compounds appeared to cause more dramatic effects (prolonged field potential durations and even Torsade-de-Pointes-like arrhythmias) than the low risk compounds at concentrations close to clinical plasma concentrations.

Conclusion
Our data support the use of Pluricyte® Cardiomyocytes to predict cardiac safety of pharmaceuticals in humans, thereby improving preclinical testing strategies for the assessment of cardiac safety, which is in line with the new regulatory approach embodied by the CiPA initiative.

MEA analysis and Ca²⁺-flux assays for the assessment of electrophysiology and Ca²⁺-transients of Pluricyte® Cardiomyocytes

Screening of compound-induced alterations of calcium transients in Pluricyte® Cardiomyocytes measured using the FDSS®/µCell system

Figure 1. A typical waveform of a Pluricyte® Cardiomyocyte monolayer acquired through MEA analysis. Cell index (i.e. impedance) signal (top) and ECR (i.e. field potential) signal (bottom) obtained using the xCellence® RTCA CardioECR instrument (ACEA Biosciences). The field potential shows a robust inward spike amplitude and a well-pronounced repolarization peak.

Figure 2. A typical baseline measurement of the fluorescent signal resulting from calcium transients in spontaneously contracting Pluricyte® Cardiomyocytes obtained with the FluoMmaster® instrument. Parameters that can be analysed are indicated. For this assay, the FDSS®/µCell system was used to detect calcium-transients (Molecular Devices).

In vitro effects of CIPA compounds on Pluricyte® Cardiomyocyte electrophysiology – with respect to clinically relevant concentrations

Figure 5. Pharmacological responses of Pluricyte® Cardiomyocytes to low, intermediate, and high risk CIPA compounds determined with xCellence® RTCA CardioECR Field Potential and Impedance analysis. Diltiazem (in blue) determined as a low risk CIPA compound, induced a concentration-dependent increase in the field potential duration, a decreased ECR signal, and a decreased ECR signal amplitude, which was in line with decreased calcium transient amplitudes (Figure 2). A one-way ANOVA test with post hoc Dunnett’s multiple comparisons test was performed to determine significance at increasing compound concentrations.

Figure 4. Calcium transient analysis of Pluricyte® Cardiomyocytes upon incubation with cardiac toxic compounds. Overview of different cardiovascular compounds and their effects on beat rate (peak frequency) and peak amplitude, expressed as percentage change when compared to the baseline (DMSO control). In addition, the incidence of beating irregularities, arrhythmias/depolarization events, and stopping of beating (within 5 minutes after compound application) was determined. N=4 wells for each condition.

Conclusion

• MEA analysis of Pluricyte® Cardiomyocytes shows monolayer field potentials with well-pronounced de- and repolarization peaks for easy detection and analysis of the field potential duration.
• Calcium-transient analysis in Pluricyte® Cardiomyocytes using the FDSS®/µCell system provides a robust, medium-to-high-throughput screening method for cardiac safety, which can be used already at an early stage of drug development.
• Pluricyte® Cardiomyocyte-based MEA assays show predictive and reproducible responses to low, intermediate, and high-risk level CIPA compounds.
• As expected, high and intermediate risk CIPA compounds appeared to cause more dramatic effects (prolonged field potential durations and even Torsade-de-Pointes-like arrhythmias) than the low risk compounds at concentrations close to clinical plasma concentrations.
• Our data support the use of hiPSC-derived cardiomyocytes to predict cardiac safety of pharmaceuticals in humans at an early stage of development, in line with the new regulatory approach embodied by the CiPA initiative.

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Assessment of the effects of low, intermediate, and high risk CIPA compounds on Pluricyte® Cardiomyocyte Electrophysiology using MEA analysis

A. Example of low-risk CIPA compound: L-type Ca²⁺-blocker diltiazem

B. Example of intermediate-risk CIPA compound: hERG K⁺ blocker astemizole

C. Example of high-risk CIPA compound: hERG K⁺ blocker dofetilide

Figure 6. The overview of the effects of a set of CIPA compounds on Pluricyte® Cardiomyocytes measured using xCellence® RTCA CardioECR and shown with respect to their clinically relevant concentrations.

Verapamil, a dual inhibitor of the outward hERG potassium current and the L-type calcium channel, had minimal effects on FFDC and spike amplitude at clinically relevant concentrations. Mexiletine, a dual inhibitor of the inward sodium current and the outward hERG potassium current also had minimal effects on FFDC and spike amplitude at clinically relevant concentrations. Both compounds showed no TdP-like arrhythmia behavior at any of the concentrations tested.

Next, an intermediate (Astemizole) and a high-risk (Dofetilide) compound were tested. In contrast to the low-risk compounds, and especially for Astemizole, dose-dependent prolongations of the FFDC and arrhythmias close to clinically relevant plasma levels were observed. For the high-risk compound Dofetilide, all tested concentrations induced arrhythmias at a concentration of 1 µM. In case of arrhythmias or stop of beating, FFDCs were quantified prior to the onset these phenomena.

Conclusive Remarks