High throughput cardiac safety and toxicity evaluation of human iPSC-derived cardiomyocytes in a pro-maturation environment

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Abstract

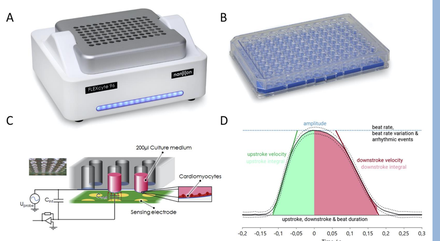
In pre-clinical drug development, cardiac contraction analysis of potential drug candidates is one of the crucial steps to ensure a successful and reliable transition to clinical stages. The use of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) continues to increase in the assessment of safety and toxicological side effects of newly developed compounds, due to their reproducibility and low ethical concern. However, their premature phenotype causes issues concerning non-physiological responses for preclinical drug development. Additionally, acute testing within limited timescales (min to h) after compound application remains the primary application so far, partly due to the inability of common cell-based assays to analyze cellular behavior reliably over prolonged periods of time.

The aim of this study was to evaluate the applicability of hiPSC-CM contractility measurements for acute safety and chronic toxicological assessment using the high-throughput FLEXcyte 96 system. Treatment of hiPSC-CM with positive inotropic compounds exhibited physiological responses when plated on FLEXcyte plates confirming the pro-maturation effect of the native-like environment given by the membranes. Additionally, 15 kinase inhibitors and 3 anthracyclines with well-known cardiotoxic profiles were selected to evaluate the reproducibility of clinical data.

Technology

The FLEXcyte technology is based on a special 96 well plate that contains high-precision, ultra-thin and hyperelastic silicone membranes instead of stiff plastic surfaces as basis for human iPSC-CMs. This FLEXcyte 96 plate is analysed in the FLEXcyte 96 device (Fig.1A), an add-on system for the CardioExcyte 96 (Nanion Technologies).

In the FLEXcyte 96-well plate (Fig.1B), the cells adhere as monolayers on flexible substrates. While being deflected by the weight of the culture medium, rhythmic contraction of the cardiomyocytes lifts the membranes in the 96-well upwards. These changes in deflection are quantified by means of capacitive distance sensing (Fig.1C). The unique Mean Beat Function of the software automatically visualizes the average beat of traces from one well per sweep, enveloped by the standard deviation. Additional parameters like amplitude, rising and falling times as well as beat duration are analysed via the obtained mean beat while the beat rate is examined separately (D) (Gossmann et al., 2016, Gossmann et al., 2020).



Method

Human iPSC-CMs (iCell® CM², Fujifilm Cellular Dynamics) were cultured on FLEXcyte 96 well plates at 100k per well according to manufacturers' guidelines in 200 µL maintenance medium. Cells were seeded 6 days before compound treatment to allow proper monolayer and network formation. A final media change was conducted 4-6 hours before drug application. For the experiments, 50 µL of the cell culture medium was removed and replaced with 50 µL medium containing 4x concentrated compound, resulting in the desired final compound concentration. Measurements were performed over a period of 5 days. (Fig.2)

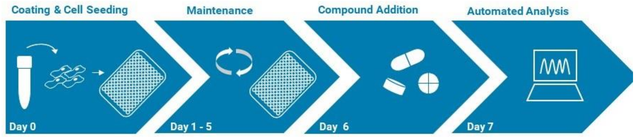


Figure 2. FLEXcyte 96 Workflow

Summary of the Result

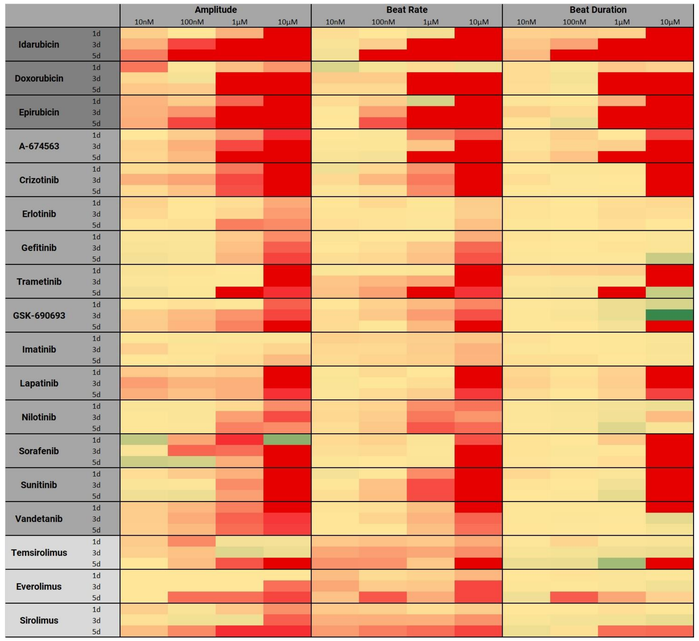


Figure 3. Heatmap of chronic cardiotoxic effects of kinase inhibitors and anthracyclines. hiPSC-CMs analysed after 1 d, 3 d and 5 d of compound treatment on the FLEXcyte 96. Shown parameters are amplitude, beat rate and beat duration. The heat map colours indicate increasing effects (green) of hiPSC-CMs, stable conditions (yellow) as well as decreasing reactions (red) up to ceasing effects (deep red). Erlotinib, imatinib, everolimus, sirolimus and temsirolimus are known compounds with low cardiotoxic potential and served as negative control. Anthracyclines are highlighted in dark grey, TKIs in grey and mTOR inhibitors in light grey.

References

Gossmann et al., 2016 Mechano-Pharmacological Characterization of Cardiomyocytes Derived from Human Induced Pluripotent Stem Cells

Gossmann et al., 2020 Integration of mechanical conditioning into a high throughput contractility assay for cardiac safety assessment

Sharma et al., 2017 High-throughput screening of tyrosine kinase inhibitor cardiotoxicity with human induced pluripotent stem cells

Acknowledgments

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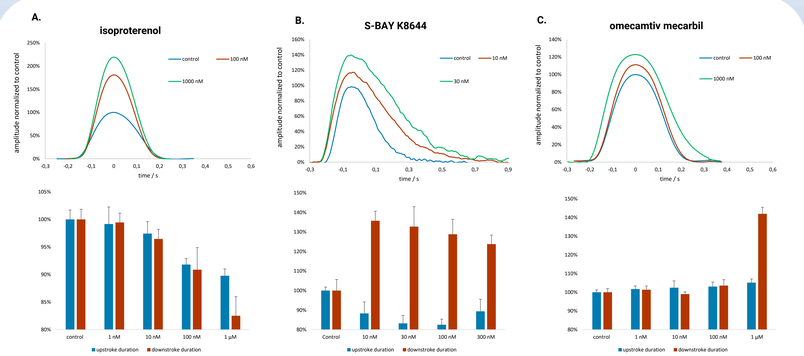

Figure 4. Adult-like responses of hiPSC-CMs upon acute positive inotropic compound application. Recorded beat shape and corresponding beat duration / beat amplitude analysis of hiPSC-CM cultured on FLEXcyte 96 well plates after treatment with isoproterenol (A), S-Bay K8644 (B) and omecamtiv mecarbil (C). **A**. hiPSC-CM (Cardiosight®-S, Nexel) beat shape after treatment with 100 nM and 1000 nM isoproterenol in comparison to control. Beat amplitude and beat duration analysis of hiPSC-CM after treatment with increasing concentrations of isoproterenol (1 nM – 1000 nM) in comparison to control. **B**. hiPSC-CM (iCell® CM2, FCDI) beat shape after treatment with 10 nM and 30 nM S-Bay K8644 in comparison to control. Beat amplitude and beat duration analysis of hiPSC-CM after treatment with increasing concentrations of S-Bay K8644 (10 nM – 300 nM) in comparison to control. **C**. hiPSC-CM (Cardiosight®-S, Nexel) beat shape after treatment with 100 nM and 1000 nM omecamtiv mecarbil. in comparison to control. HiPSC-CM beat amplitude and beat duration analysis of omecamtiv mecarbil treatment (1 nM – 1000 nM) in comparison to control.

Results

In total, 15 kinase inhibitors and 3 anthracyclines were analysed upon cardiotoxic side effects using human iPSC-CMs on the FLEXcyte 96. Adult-like positive inotropic compound reactions of hiPSC-CMs cultured on physiological FLExcyte plates were shown with isoproterenol, S-Bay k8644 and omecamtiv mecarbil.

Known cardiotoxic anthracyclines such as doxorubicin and epirubicin show expected toxic effects, ranging from the reduction in contractility at nanomolar concentrations to ceased beating at micromolar concentrations (deep red). Negative controls with known low cardiotoxic risk such as erlotinib, imatinib, everolimus, sirolimus and temsirolimus only showed toxic side effects at super-therapeutic concentrations in a time-dependent manner (Fig.3).

Isoproterenol increased the contraction amplitude in a dose-dependent manner to approx. 210% of control at 1 µM. At the same time, the durations of contraction and relaxation phase were shortened, indicating an increase in acceleration and deceleration of the contraction. In contrast, S-Bay K8644 elicited a symmetry change in the beat shape. While the contraction phase was unaffected, the increase in intracellular calcium concentration resulted in a prolongation of the relaxation phase. As a result, the total duration of the contraction-relaxation-cycle was increased to 120% of control. The amplitude reached 150% of control at 30 nM. Omecamtiv mecarbil increased both the contraction amplitude as well as the duration of the contraction-relaxation-cycle, indicating the positive inotropic effect based on activation of the myosin complex. The symmetry of the contraction-relaxation-cycle was not changed. The mature physiological responses of hiPSC-CM shown here are triggered by compounds acting at different target levels, underlining the pro-maturation effects of the FLEXcyte 96 which cannot be elicited with other assays commonly used for drug development purposes (Fig.4).

Conclusion

The displayed adult-like hiPSC-CM responses upon positive inotropic compound treatment underline the pro-maturation effect of the physiological environment created by the flexible membranes. The time and dose-dependent cardiotoxic progression profiles of anthracyclines and TKIs assessed, indicate the suitability of the FLEXcyte technology for (sub)chronic safety and toxicity evaluation of new drug candidates.

The combination of human iPSC-CMs and the FLEXcyte 96 technology allows for cardiac risk assessment using a predictive human cell model on a high-throughput format.

The FLEXcyte technologies' comprehensive goal on a larger scale is to advance translational studies for contractile cardiotoxicity, replace/minimize animal use in drug development, and reduced risk of adverse cardiac side effects in clinical trials.