Multiparametric assessment of cardiotoxicity of chemotherapeutic drugs in human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes

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Abstract

Drug-induced cardiotoxicity, particularly following anti-cancer therapy, is a major concern in drug discovery and development. Cardiotoxicity can be functional (affecting the electrophysiology or contractility of the myocardium) or structural (morphological damage to cardiomyocytes) by nature. To prevent drug-induced cardiotoxicity in the clinic, there is an urgent need for physiologically relevant in vitro models that can predict both functional and structural drug-induced cardiotoxic effects.

We aimed to develop a multiparametric approach to assess drug-induced cardiotoxicity in hiPSC-derived cardiomyocytes (Pluricyte® Cardiomyocytes) on a functional as well as structural level. For this, drug-induced effects of chemotherapeutic drugs (i.e., staurosporine, sunitinib and lapatinib) were assessed at different exposure times using various assays, such as multielectrode array (MEA) and impedance technology, cardiac troponin I (cTnI) release and ATP assays.

Multiparametric analyses revealed various pharmacological responses of Pluricyte® Cardiomyocytes to the different test compounds. Treatment with staurosporine and sunitinib resulted in acute functional cardiotoxic effects, while lapatinib only showed effects on functionality after longer incubation times. We also observed different responses in structural toxicity: Lapatinib had no effect on cTnI release or cell viability after 24 hr of incubation, while sunitinib and staurosporine induced a dose-dependent increase in cTnI release. The dose-dependent increases in cTnI release were in line with reduced ATP levels induced by both compounds.

Taken together, Pluricyte® Cardiomyocytes can capture clinically relevant cardiotoxic effects of chemotherapeutics. Importantly, our multiparametric approach to examine cardiotoxicity in hiPSC-derived cardiomyocytes allows a comprehensive assessment for predicting cardiotoxic risks of compounds.

Human Pluricyte® Cardiomyocytes are fully functional and show a relatively high level of maturity

Figure 1. Characteristics of Pluricyte® Cardiomyocytes cultured in Pluricyte® Cardiomyocyte Medium.

A: A typical action potential of Pluricyte® Cardiomyocytes (measured by PhysioStim, Lautrec, France), demonstrating a low resting membrane potential (~70mV) and fast upstroke velocity. As expected, L-type calcium channel blocker nifedipine decreased the APD20 (65%), APD90 (46%) and APA (6.2%).

B: Pluricyte® Cardiomyocytes exhibit a high degree of ultra-structural sarcomere organization as determined by immunofluorescence (Green: alpha actinin; Red: myosin heavy chain).

Sunitinib and staurosporine induce acute functional toxicity in Pluricyte® Cardiomyocytes

Figure 2. Pluricyte® Cardiomyocyte monolayer show clear impedance-field potential signals obtained by MEA/impedance technology.

Cell Index (i.e. impedance, a surrogate marker for contractility) signal (top) and ECF (i.e. field potential) signal (bottom) enable simultaneous assessment of the excitation-contraction coupling and electrophysiology. The field potential traces show robust and well-pronounced depolarization (D) and repolarization (R) peak amplitudes. Data was obtained using the xCelligence® RTCA Cardio/Electro instrument (ACEA Biosciences).

Figure 3. Acute effects of sunitinib and staurosporine on electrophysiology and impedance in Pluricyte® Cardiomyocytes.

3 µM sunitinib did not affect impedance or field potential signals of Pluricyte® Cardiomyocyte monolayers after 20 min. of incubation. By contrast, treatment with 5 µM sunitinib and 3 µM staurosporine induced acute cardiotoxic effects. More specifically, sunitinib caused proarrhythmic events, while staurosporine resulted in an immediate drop in impedance and disturbed field potential signals. Data was obtained using the xCelligence® RTCA Cardio/Electro instrument (ACEA Biosciences).

The tyrosine kinase inhibitor, lapatinib, alters the excitation-contraction coupling in Pluricyte® Cardiomyocytes after long-term incubation

Figure 4. Long-term effect of lapatinib on the impedance peak width of Pluricyte® Cardiomyocytes.

A: Lapatinib induced an increase in impedance peak width after 16 hr (54%, P<0.01) to 24 hr (114%, P<0.0001) post-addition, suggesting a long-term effect on the relaxation time of Pluricyte® Cardiomyocytes.

B: Overlay of impedance waveforms showing an increase in peak width in Pluricyte® Cardiomyocytes after long-term incubation with 3 µM lapatinib. One waveform represents the average of all waveforms recorded during one measurement of 60 seconds.

Data was obtained using the xCelligence® RTCA Cardio/Electro instrument (ACEA Biosciences). All error bars represent SDs. Significant values indicated are the result from Dunnett’s multiple comparisons test, after performing two-way ANOVA. ** P<0.01, **** P<0.0001.

Sunitinib and staurosporine induce structural toxicity in Pluricyte® Cardiomyocytes after long-term incubation

Figure 5. Cardiac Troponin I (cTnI) release (A) and ATP levels (B) in Pluricyte® Cardiomyocytes after long-term incubation with 3 µM staurosporine and 5 µM sunitinib.

No clear effects of lapatinib were observed in both cTnI release and ATP assays up to 24 hr treatment of Pluricyte® Cardiomyocytes. Sunitinib showed significant decrease in cell viability and increase in cTnI release only at high concentrations (~5 µM). Staurosporine showed the highest level of toxicity, which is in line with the observed acute effects on electrophysiology and impedance as depicted in figure 3. Moreover, staurosporine showed a clear dose response effect in cTnI release and ATP levels after 16 hr and 24 hr of incubation.

Dashed line indicates baseline cTnI levels of untreated Pluricyte® Cardiomyocytes. Correlation analysis to cell loss is crucial for accurate determination of the IC50 value of the compounds.

Concluding Remarks

• Pluricyte® Cardiomyocytes cultured in Pluricyte® Cardiomyocyte Medium show a ventricular, relatively mature phenotype.

• Effects of lapatinib could only be detected after long-term measurements, indicating that potential cardiotoxic effects of compounds can be missed when performing solely acute measurements.

• Troponin I release assay can capture potential structural toxic effects of compounds, which cannot be detected with only MEA technology.

• Studying acute and long-term cardiotoxic effects on a functional as well as a structural level allows a better understanding of the wide range of drug-induced cardiotoxicities that can occur. Hence, our multiparametric approach can provide a more detailed cardiotoxicity profile of novel compounds, which can further improve the safety pharmacology decision-making at an early stage of drug development.

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Slimpickings

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