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Assessing functional and structural cardiotoxicity in cultured human iPSC-cardiomyocytes in a single plate format

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Abstract

A comprehensive profiling of cardiotoxicity early in drug discovery and development can aid in reducing late-stage attrition and establishing risk mitigation strategies during clinical development. In most cases, multiple assay platforms and instrument-specific plate formats are required for this type of approach. In this study, we evaluated both functional and structural endpoints associated with cardiotoxicity in human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) cultured in a single 384-well plate. We measured intracellular Ca2+ transients, caspase 3/7 activity and permeabilization sequentially in the same plate via a series of assay readouts. A set of cardiac ion channel modulators ( dofetilide, sotalol, nilotinib, and mexiletine) and chemotherapeutics (tacrolimus, doxorubicin, and vincristine) was tested at clinically relevant concentrations for effects on intracellular Ca2+ transients after a short-term (30 minutes) exposure, and plasma membrane permeabilization and caspase 3/7 activation after a long-term (72 hours) exposure. Intracellular Ca2+ transients were monitored by fluorescent images taken with a high-speed camera in beating cardiomyocytes loaded with Ca2+ dye, permeabilized plasma membrane (for dead-cell detection) was identified with live-stain DRAG2™ nuclear dye and activation of caspase 3/7 was determined biochemically with the Caspase-Glo® 3/7 Assay kit. Multiple endpoints derived from Ca2+ transients, including beat rate, calcium transient duration (CTD) measured at 30% or 90% from peak and corrected by inter-peak interval (IP), and CTD transilation, heart rhythm, short- or long-term variability of CTD06 and IP, Ficollate plots, were used to assess drug effects on intracellular Ca2+ cycling and arrhythmogenicity. Increases in positive nuclear staining for DRAG2™ and caspase 3/7 activity represented structural cardiotoxicity. We found that increased CTD transilation, development of arrhythmic events and both the short- and long-term variability of CTD06 or IP were robust indicators of functional effects. Positive nuclear staining for DRAG2™ was a robust indicator of structural effects. Accordingly, dofetilide and sotalol were identified as primarily arrhythmogenic; doxorubicin was primarily structurally toxic, while nilotinib and vincristine were both arrhythmogenic and structurally toxic. The use of these endpoints in a single plate format simplifies the cardiotoxicity assessment and does not require multiple cell plates for measurements.

Introduction

Cardiotoxicity is a frequently dose-limiting toxicity associated with many highly efficacious chemotherapeutics that include both classic cytotoxic or cytostatic agents, such as doxorubicin or other anthracycline analogs, and newly developed targeted anti-cancer molecules such as protein kinase inhibitors (i.e. sunitinib, dasatinib and nilotinib). As this adverse effect can be manifested by either structural damage (i.e. cardiomyopathy and heart failure) or functional alteration (i.e. arrhythmia and sudden cardiac death), evaluation on risk to induce both structural and functional cardiotoxicity should be included in preclinical safety profiling of new anti-cancer drug prior to the first dose in human.

Human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) represent a novel cellular model system to test for cardiotoxicity and are being used increasingly with a wide variety of analytic platforms in study of cardiac biology and drug safety testing. In this study, we developed an image-based, multiplex assay that enables interrogation of both functional and structural toxicity endpoints in a single plate format.

Methods & Materials

Cells:

Cryopreserved iPSC-cardiomyocytes were provided by Dr. Joseph C. Wu and Stanford Cardiovascular Institute (SCVI) Biobank.

Reagents:

RPMI 1640, BD Matrigel (Fisher/Corning), B27-insulin, DMEM/F12 (Gibco/Life Technologies), Accutase (Sigma), Cal-520™ Ca2+ dye (AAT Bioquest), DRAQ7™ DNA dye (abcam), Caspase-Glo® 3/7 assay kit (Promega), Doxilide, sotalol, nilotinib, mexiletine, tacrolimus, doxorubicin, nilotinib, sunitinib and vincristine (NCI Compound Repository)

Biomarkers:

Ca2+ transients: contractile function, relaxation-delay, arrhythmia
DNA stain: permeabilization of plasma membrane (cell death)
Caspase 3/7 activity: apoptosis activation

Workflow:

Cells plated                                      Replated               Assays for Ca2+ transients

6-well plate                                      1 week                                  1 week
384-well optical plate                            10 min and 72 hrs

DNA stain + Caspase 3/7

IN-Cell Analyzer (HCA)

Tecan plate reader

Data analysis:

Measurement of beat-to-beat Ca2+ transients was performed by CYBERnano (iCardio platform); treatment-related changes in each endpoint were shown as % of the baseline value. Cells stained positive with DRAQ7 were shown as % of total nuclear (DAPI) counts and Caspase 3/7 activity was quantified as the luminescence intensity in each well. Statistical analysis was conducted with Student’s t-test.

Table 1. Summary of effects on Ca2+ transients parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Condition</th>
<th>Cell density</th>
<th>IC50 (μM)</th>
<th>Emax (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dofetilide</td>
<td>Control</td>
<td>1x</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>1000 RFU</td>
<td>10x</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Nilotinib</td>
<td>Control</td>
<td>1x</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>1000 RFU</td>
<td>10x</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Discussion & Conclusion

Ca2+ transients were sensitive to ion channel modulators, with changes in beat rate and Ca2+ transients amplitude; EADs, beat-to-beat variability and triangulation of CTD effects were more specific than CTD lengthening to predict arrhythmogenicity.

Caspase 3/7 activity was a sensitive indicator of insults to iPSC-CMs but increased nuclear stains of impermeable DNA dye was more robust to label structural cardiotoxicity.

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