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

L. Guo, M. Fournis, J. Hamre, L. Batista*, T. Bastogne*, Y. Zhuge*, J.C. Wu*, S. Eldridge†, M. Davis‡

Laboratory of Investigative Toxicology, Frederick National Laboratory for Cancer Research/Leidos Biomedical Research, Inc., Frederick, MD 21702; *University of Lorraine, INRIA, Vandœuvre-lès-Nancy, France
‡Stanford Cardiovascular Institute, Stanford University, Stanford, CA 94305

1DCTD, National Cancer Institute, Bethesda, MD 20892

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 (hiPSC-CMs) cultured in a single 384-well plate. We measured intracellular Ca²⁺ transients, caspase 3/7 activity, and plasma membrane permeabilization sequentially in the same plate via a series of assay readouts. A set of cardiac ion channel modulators (doxilide, stolace, nilotinib, mexiletine, and mexiletine) and chemotherapeutics (tamoxifen, doxorubicin, and vincristine) was tested at clinically relevant concentrations for effects on intracellular Ca²⁺ transients after a short-term (30 min) exposure, and plasma membrane permeabilization and caspase 3/7 activity after a long-term (72 h) exposure. Intracellular Ca²⁺ transients were monitored by fluorescent images taken with a high-speed camera in beating cardiomyocytes loaded with Cal590-CA²⁺ dye, permeabilized plasma membrane (for dead-cell detection) was identified with live-stain DRAQ7™ nuclear dye and activation of caspase 3/7 was determined biochemically with the Caspase-3/7™ Assay kit. Multiple endpoints derived from Ca²⁺ transients, including beat rate, calcium transit duration (CTD) measured at 30% or 90% from peak and corrected by inter-peak interval (IP9), along with CTD triangulation, beat rhythm, short- or long-term variability of CTD06 and IP9 Poincaré plots, were used to assess drug effects on intracellular Ca²⁺ cycling and arrhythmogenicity. Increases in positive slope starting for DRAQT™ and caspase 3/7 activity represented structural cardiotoxicity. We found that increased CTD triangulation, development of arrhythmic events and both the short- and long-term variability of CTD9 or IP9 were robust indicators of functional effects. Positive nuclear staining for DRAQT™ was a robust indicator of structural effects. Accordingly, doxilide and stolace 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 frequently a 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 (e.g., sunitinib, dasatinib and nilotinib). As this adverse effect can be manifested by either structural damage (i.e., cardiomyopathy and heart failure) or functional alteration (e.g., arrhythmia and sudden cardiac death), evaluation on risks to induce both structural and functional cardiotoxicity should be included in preclinical safety profiling of each new anti-cancer drug prior to the first dose in human.

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-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, 2D Matrigel (Fisher/Corning), B27-insulin, DMEM/F12 (Gibco/Life Sciences), Accutase (Sigma), Cal-590-CA²⁺ dye (AAT Bioquest), DRAQ7™ DNA dye (abcam), Caspase-Glo 3/7 Assay kit (Promega), Doxilide, stolace, nilotinib, mexiletine, tamoxifen, vincristine, and vincristine soybean (NCI Compound Repository)

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

Parameters:
Cell plated Replated Assays for Ca²⁺ transients DNA stain + Caspase 3/7

Data analysis:
Measurement of beat-to-beat Ca²⁺ transients was performed by CYBERnano i-Cardo 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

Results

Ca²⁺ transients of beating cardiomyocytes:
Figure 1. Ca²⁺ cycling imaged at 31 fps from a single view-field

Analysis:
Figure 2. Ca²⁺ transients analyzed by CYBERnano i-Cardo

Figure 3. Representative traces of typical effects on Ca²⁺ transients

Figure 4. Beat variability analysis on Poincaré plots of CTD09

Figure 5. Representative images of nuclear stains

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 were more specific than CTD lengthening to predict arrhythmogenicity

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

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