Cardiotoxicity Assessment of HESI Reference Compounds Using HiPSC-CMs

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Introduction

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) are increasingly being used for preclinical in vitro cardiac toxicity testing. Combined with various high-throughput assay platforms, hiPSC-CMs have the potential to enhance drug-induced cardiotoxicity detection and prediction, particularly for drugs with cardiotoxic liability that cannot be easily identified with the current preclinical safety evaluation in animals due to species differences. Moreover, patients with prolonged exposure to numerous drugs, such as anthracyclines, tyrosine kinase inhibitors (TKIs), and drugs that disrupt ion channel trafficking, often exhibit some late onset cardiotoxic effects. Therefore, new preclinical assays are required to assess effects of chronic drug exposure on structural and functional changes of the cardiovascular system.

The Comprehensive in Vitro Proarrhythmia Assay (CiPA) myocyte study organized by the Health and Environmental Sciences Institute (HESI) demonstrated the value of hiPSC-CMs and various high-throughput assay platforms for drug-induced QT prolongation and Torsades de Point (TdP) prediction. The results of the CiPA initiative have been used to provide guidance for the use of hiPSC-C.Ms in the ICH S7B Q & A guideline for acute Proarrhythmia evaluation. In 2022, HESI organized another multi-site blinded study to evaluate the ability of current hiPSC-CM in vitro assays to detect chronic cardiac toxicity. Twelve well-characterized cardiotoxins with different mechanisms were selected, coded, and tested using different platforms following the same drug preparation protocol at multiple sites. Herein, we report the responses of hiPSC-CMs to the 12 HESI compounds tested at NCTR as part of the multi-site study using an impedance-based technology.

Materials and Methods

- **Cell Culture** - hiPSC-CMs ($iCell_2$) were purchased from FujiFilm Cellular Dynamics international (FCDI, Madison, WI, USA). Thawed cells were plated on a fibronectin-coated Cardio E-plate (96-well) at approximately 2 x 10^4 plateable cells per well using iCell-plating medium. The culture was maintained with iCell maintenance medium (iCMM) for 7-8 days and 100% media exchanges were performed every other day before drug treatment.
- **Treatment with Blinded Compounds and Positive Control** - Compounds were serially diluted with DMSO, followed by a 1000-fold dilution into iCMM, as per HESI's instructions. The hiPSC-CMs were exposed to four concentrations of blinded compounds (Table 1), the positive control (1µM doxorubicin), as well as vehicle control (0.1 DMSO%), for a total of 120 hours, with drugs refreshed every 48 hours. The blinded compound details were revealed only after uploading the raw data to HESI from all test sites.
- **Agilent xCELLigence RTCA Cardio System** - Impedance signals were recorded at 0, 1, 24, 48, 72, 96, and 120 hours post drug exposure and analyzed using the RTCA Cardio software version 1.2. All data are presented as mean ± S.D, and the data is normalized to the values at baseline (pre-drug treatment).

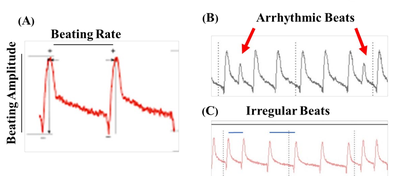

Figure 1- Examples of impedance parameters analyzed: (A) Beat amplitude, beat rate, and (B & C) Arrhythmias that increase beating rhythm irregularity (BRI).

Experimental Protocol for Impedance Assay

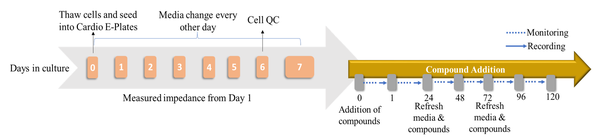

Figure 2 Schematic view of the drug application and measurement schedule used for real-time monitoring and data recoding of hiPSC-CMs impedance signals with the RTCA Cardio System.

Table 1- HESI Reference Compounds Chart

Compound Name	Tested Dose range (µM)	Clinical C"max"-total (µM)	Mechanism of Cardiotoxicity	Black Box Warning
Doxorubicin	0.1, 0.3, 1, 3	1.6	Energetics/Mitochondrial toxicant	Left ventricular failure, arrythmias, Cardiomyopathy
Erlotinib	0.23, 0.77, 2.3, 7.7	3.15-4.8	Mitochondrial toxicant	Acute myocardial infarction
Sunitinib	0.01, 0.1, 0.3, 1	0.18-0.25	Mitochondrial toxicant	Left ventricular ejection fraction (LVEF), Torsade de Pointes, and hypertension
Pentamidine	0.1, 0.3, 1, 3	1.8	Ion channel trafficking	Hypertension and arrhythmia
Arsenic Trioxide	0.016, 0.048, 0.16, 0.48	0.91-12.1	Ion channel trafficking	QT prolongation and congestive heart failure
BMS-986094	0.1, 0.3, 1, 3	0.3	Contractility	Left ventricular ejection fraction (LVEF)
Milrinone	0.1, 0.3, 3, 10	1.18/0.62	Contractility	Ventricular arrythmias
Nilotinib	0.01, 0.1, 0.3, 1	0.84-4.27	Contractility	QT prolongation
Endothelin-1	300 pM, 1nM, 30nM, 100nM	2.2pM (5.4pg/mL)	A tool drug to induce hypertrophy	Cardiac hypertrophy
Vinblastine	0.0003, 0.003, 0.03, 0.3	0.035	myofilament	Hypertension
Vincristine	0.0003, 0.003, 0.03, 0.3	0.007	myofilament	Hypertension
Vinorelbine	0.1, 0.3, 1, 3	0.811	myofilament	Tachycardia

Table 1- HESI reference compound used in the in vitro chronic cardiotoxicity study. The doses highlighted in yellow color is the concentration close to Cmax. **Dose range above Clinical Cmax; dose range below Clinical Cmax**.

Drugs that Interfere with Ion Channel Trafficking

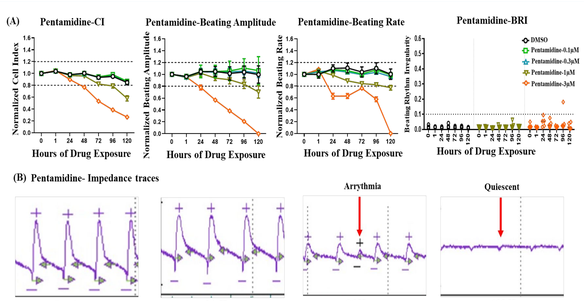

Figure 3 (A) Pentamidine induced irregular beats and reduced cell viability, amplitude and beating rate at the highest concentration (3µM) tested in the study. (B) Examples of impedance traces of hiPSC-CMs before and after pentamidine (3 µM) treatment.

Drugs that Affect Mitochondrial Activity

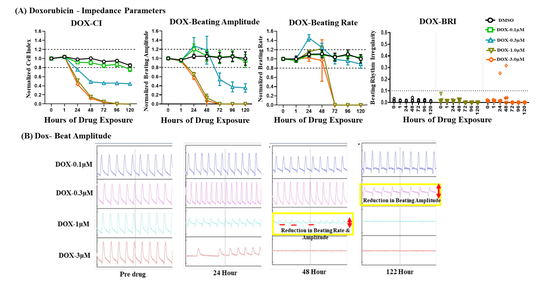

Figure 4 (A) Summary of the concentration- and time-related changes of the cell index (CI, an indicator of cytotoxicity), beating rate, beating amplitude, and beating rhythm irregularity (BRI) post-doxorubicin (DOX) exposure. (B) Examples of impedance traces of hiPSC-CMs before and after DOX treatment.

Drugs that Cause Structural-Related Changes

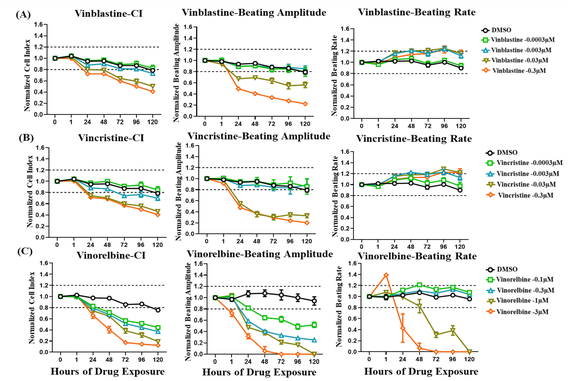

Figure 5. Concentration-dependent cardiotoxic effects of drugs that cause myofilament changes. (A & B) Vinblastine and vincristine reduced cell viability, beating amplitude and increased beating rate at higher test concentrations. (C) Vinorelbine induced irregular beats (data not shown) and dose-dependent cytotoxicity, reduced beating rate and beating amplitude at exposures at ~1.2x Cmax exposure.

Endothelin-1 –A Tool Drug to Induce Hypertrophy

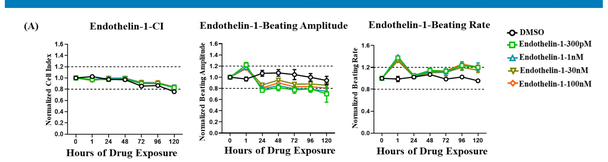

Figure 6. Endothelin-1 (ET-1) is a potent endogenous vasoconstrictor, and it can induce cardiac hypertrophy in vitro. ET-1 slightly increased beating rate and induced irregular beats at the lowest concentration tested in this study.

Drugs that Alter Contractility

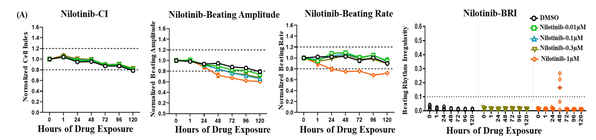

Figure 7. (A) Nilotinib reduced beating rate, beating amplitude, and induced arrhythmia at the test concentrations approximating clinical Cmax (<1x).

Summary

Mechanism of Toxicity	Compound	Cell Index	Beating Amplitude	Beating Rate	Irregular Beating activity /Arrhythmia	Conc. (Fold to Cmax)
Mitochondrial toxicity	Doxorubicin	Decrease	Decrease	Decrease	Yes	0.3µM (0.2x)
	Erlotinib	No change	Decrease	No change	Not observed	7.7µM (1.6x)
	Sunitinib	No change	Increase	Decrease	Yes	1µM (4x)
Ion channel	Pentamidine	Decrease	Decrease	Decrease	Yes	3µM (1.6x)
	Arsenic Trioxide	No change	No change	No change	Not observed	0.48µM (< 1x Cmax)
Contractility	Nilotinib	No change	Decrease(1µM)	Decrease	Yes	1µM (0.2x)
	Milrinone	No change	No change	No change	Not observed	10µM (8.5x)
	BMS-986094	Decrease	Decrease	Increase	Not Observed	0.3µM (1x)
Structural/myofilaments	Endothelin-1	No change	Increase (1hr)	Increase (1hr)	Yes	300pM (136x)
	Vincristine	Decrease	Decrease	Increase	Not observed	0.03µM (4.2x)
	Vinblastine	Decrease	Decrease	Increase	Not observed	0.03µM (0.9x)
	Vinorelbine	Decrease	Decrease	Decrease	Yes	1µM (1.2x)

Conclusion

- The inter-plate variability of hiPSC-CMs treated with 1mM of doxorubicin (the positive control) exhibited a small inter-plate variability in the impedance assay.
- Overall, most of the HESI reference compounds showed some changes in impedances signals.
  - Arsenic trioxide and milrinone showed little effects, as arsenic trioxide was tested at relatively low concentrations (<1x Cmax) due to a solubility issue and the positive inotropic effect of milrinone cannot be detected with less mature iPSC-CMs in conventional 2D culture.
- Drug testing with the hiPSC-CM impedance assay may provide some useful information for chronic cardiotoxicity prediction; however, further characterizations are needed to identify the related electrophysiological, structural, energetic, and contractility changes of cardiomyocytes upon chronic drug exposure.
- The data will be compared with the results from the other study sites to build the confidence in applying hiPSC-CMs for chronic cardiotoxicity detection and prediction.

Disclaimer

The information in this poster is not a formal dissemination of information by the FDA and does not represent agency position or policy.

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