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Jr. Investigator Travel Award Recipient

2015 JR. INVESTIGATOR POSTER CONTEST

2ND PLACE

Cardiotoxicity t… n human iPSC-derived cardiomyocytes for the CiPA pi… using all-optical electrophysiology

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CiPA HESI Q - STATE BIOSCIENCES

Introduction

The goal of the Comprehensive *in vitro* Proarrhythmia Assay (CiPA) initiative is to create a new model for evaluating potential cardiotoxic effects of compounds through improved cellular assays coupled with *in silico* modeling. A primary endpoint of this effort is to evaluate human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes (CMs) as a cellular substrate for cardiotoxicity testing. Studies have shown that hiPSC-CMs recapitulate a number of phenotypic properties of native cardiac tissue and are sensitive to different classes of ion channel modulators.

At Q-State Biosciences, we have developed an all-optogenetic platform called Cardiac Optopatch that enables simultaneous stimulation and detection of electrical activity in hiPSC-derived CMs. Stimulation is achieved with a channelrhodopsin variant, CheRiff, which provides a stable electrophysiological background. Simultaneous fluorescent detection of action potential (AP) waveforms and calcium transients (CTs) is achieved using a fast, sensitive dual-reporter fusion protein called CaVlar (calcium and voltage indicator).

As part of the CiPA pilot study, we used this platform to test the effects of eight ion channel modulatory compounds (blinded) on cell lines from two providers, CDI and Axiogenesis. Measurement of electrophysiological parameters included changes in AP waveforms (APD30, APD60, APD90, triangulation, AP rise time, spontaneous beat rate) and CTs (amplitude) at the spontaneous beat rate and at two paced frequencies (1Hz, 2Hz). These results demonstrate the utility of Cardiac Optopatch to be used for assessment of drug responses in hiPSC-derived cardiomyocytes.

Day 6 Day 14 Day 3 Day 7 Optopatch Imaging

Q-State Optogenetic Constructs

CaVlar CheRiff QuasAr2 eGFP GCaMP6f

Cardiac Optopatch: CheRiff stimulates action potentials, CaVlar (Ca^{2+} and voltage indicator) enables fluorescent readout of electrophysiology.

Raw Fluorescence Traces

QuasAr2 GCaMP6f

Ca²⁺ Voltage Pace

1 Hz Pacing 2 Hz Pacing 2 sec

Comparison: Voltage Sensitive Dyes

QuasAr2 FluoVolt

FluoVolt + QuasAr2 FluoVolt - QuasAr2

Time (s)

Top panel: Recorded fluorescence of a cell both expressing QuasAr2 and labeled with FluoVolt shows **negligible difference in the AP waveform**. Bottom panel: The FluoVolt fluorescence signal is unperturbed in the presence (green) and absence (cyan) of QuasAr2 expression.

Drug ID	Target channel	Vial code	Conc. 1 (uM)	Conc. 2 (uM)	Conc. 3 (uM)	Conc. 4 (uM)
Ranolazine dihydrochloride	Late Nav1.5	351	1	3	10	30
Quinidine	Na^+	213	0.3	1	3	10
Nifedipine	Ca^{2+}	213	0.01	0.03	0.1	0.3
Moxifloxacin hydrochloride	hERG	354	3	10	30	100
Mexiletine hydrochloride	Na^+	210	1	3	10	30
JNJ 303	Iks	216	0.01	0.03	0.1	0.3
Flecainide Acetate	Late Nav1.5	217	0.1	0.3	1	3
E-4031 dihydrochloride	hERG	218	0.003	0.01	0.03	0.1

…were performed for CDI and …cytes at the spontaneous beat …rates …APD30, APD50, APD90, rise time, …amplitude, triangulation index …ADs for all three beat rates

No significant changes in AP parameters were detected when tested with a DMSO control. CDI CMs showed a **faster upstroke** speed, whereas **Axiogenesis** CMs showed a **faster spontaneous beat rate** on average.

2. Extraction of Average Waveform

3. Statistical Analysis of Waveforms

K⁺ channel modulators

Drug 254: Moxifloxacin – hERG channel block

CDI Axiogenesis

hERG channel block with **moxifloxacin** leads to **prolonged APs**.

Drug 256: JNJ 303 – IKs block

CDI Axiogenesis

IKs channel block with **JNJ 303** shows **modest effects** on AP parameters.

Drug 258: E-4031 dihydrochloride – hERG channel block

CDI

E-4031 increases APD90, rise time and decreases Ca^{2+} amplitude. EADs are observed at 30 nM where cells no longer pace at 1 Hz.

Axiogenesis

E-4031 increases APD90, rise time and decreases Ca^{2+} amplitude. At 10 nM cells no longer pace at 2 Hz and beating stops at 100 nM.

Na^+ channel modulators

Drug 251: Ranolazine dihydrochloride – late NaV1.5 block

CDI Axiogenesis

Ranolazine increases rise time, consistent with late channel block. In both cases, **APD90 broadens** with increasing concentration of drug.

Drug 252: Quinidine – Na^+ channel block

CDI

Quinidine increases APD90, rise time and decreases Ca^{2+} amplitude. EADs are observed at 3 μM.

Axiogenesis

Quinidine increases APD90, rise time and decreases Ca^{2+} amplitude. At 10 μM beating stops.

Drug 255: Mexiletine hydrochloride – Na^+ channel block

CDI Axiogenesis

Mexiletine increases AP width and rise time.

Drug 257: Flecainide acetate – Late NaV1.5 block

CDI Axiogenesis

Flecainide increases AP width and rise time. CDI cells showed EADs at 3 μM.

Ca^{2+} channel modulators

Drug 253: Nifedipine – Ca^{2+} channel block

CDI Axiogenesis

Nifedipine decreases AP width and Ca^{2+} amplitude, consistent with Ca^{2+} channel block. Axiogenesis cells ceased to beat at 30 nM.

Conclusions

- Both CDI and Axiogenesis CMs expressed Cardiac Optopatch vectors efficiently, enabling high SNR, simultaneous recordings of AP waveforms and calcium transients. Both cell types could be paced at different stimulus frequencies using this platform.
- Axiogenesis CMs showed a faster spontaneous beat rate compared to CDI CMs. CDI CMs showed a faster upstroke velocity.
- Both cells lines showed AP prolongation upon addition of hERG blockers (moxifloxacin, E-4031). Neither cell line showed significant changes upon addition of JNJ 303, an IKs blocker.
- Na^+ channel blockers (ranolazine, flecainide, mexiletine, quinidine) increased rise time and AP prolongation, larger in effect for CDI cells.
- The calcium channel blocker nifedipine reduced CT amplitude and AP width.
- EADs were detected in CDI CMs for quinidine, flecainide and E-4031.
- These results demonstrate the utility of Cardiac Optopatch to be used for assessment of drug responses in hiPSC-derived cardiomyocytes.

How much of a cardiomyocyte can we …
Setting up metrics for cardiac cell mo…

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Introduction

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Safety Pharmacology Society

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