Introduction

The human heart is an electrically driven pump with high fidelity autonomic rhythm, which beats about 3 billion times in a normal life span. If normal pumping rhythm and function is severely interrupted for more than a few minutes (arhythmia) irreversible multi-organ damage and death occurs. Consequently, cardiotoxicity is one of the major causes of failed drug development and withdrawal (Stevens and Baker, 2009). Hence, consistent preclinical in vitro cardiotoxicity tests are required to improve drug safety and reduce the attrition and/or the cost of drug development.

Methods

16 and/or 48-well multielectrode array (MEA) plates (Axion Biotechnologies) were prepared by pipetting 4µl droplets of fetal bovine serum (FBS) onto each electrode and incubating for 5 min at room temperature. Following adsorption of FBS to the surface the droplet was replaced with 4µl sterile saline solution (12.5ug/ml, BD Biosciences) and plates incubated at 37°C for 2h with 95% humidity to prevent the drying of sterile saline droplet. CytoLinx Plus CMs or CDI (Ca²⁺)-cardiomyocytes were seeded directly from thawed cryopreservation plates onto MEA plates using 4µl of cell suspension at 1.5 x 10⁶ viable cells/ml. 60,000 cells per well in RPM1 1640/B27 medium for CytoLinx cells and plating medium for CDI (Ca²⁺) with 95% humidity to prevent the drying of cardiomyocytes droplet. Once cell attachment was complete (2–3h) wells with CytoLinx cells were filled with RPM1 1640/B27 medium and wells with CDI were filled with CDI maintenance medium taking care not to disturb the plated cells. The plates were then incubated at 37°C/5% CO₂. ICP measurements were recorded from spontaneously beating hESC-hPSC-CM monolayers using a Maestro MEA system (Axion Biotechnologies) at 37°C. Data was sampled at 12,5 Hz and filtered with a Butterworth 1 Hz to 2 Hz band-pass filter.

Results

hESC-CM Cardiac Field Potential Waveform Data

Figure 2: Graphical representation of the effect of time-matched vehicle control, astemizole, bepridil, cisapride, dofetilide, E-4031, mexiletine, nitrendipine, quinidine, and sotalol additions on interspike interval, spike amplitude and cFPD.

hPSC-CM Cardiac Field Potential Waveform Data

Figure 3: Graphical representation of the effect of time-matched vehicle control, astemizole, bepridil, cisapride, dofetilide, E-4031, mexiletine, nitrendipine, quinidine, sotalol, and verapamil additions on interspike interval, spike amplitude and cFPD.

Table 1: Effect of time-matched vehicle control, astemizole, bepridil, cisapride, dofetilide, E-4031, mexiletine, nitrendipine, quinidine, and sotalol additions on interspike interval, spike amplitude and cFPD.

Table 2: Effect of time-matched vehicle control, astemizole, bepridil, cisapride, dofetilide, E-4031, mexiletine, nitrendipine, quinidine, sotalol, and verapamil additions on interspike interval, spike amplitude and cFPD.

Summary

A prospective comparison of hESC-CM and hiPSC-CM cells was made using a multielectrode array system (Axion Biotechnology) cardiac field potential waveforms to measure the intra-spike interval, spike amplitude and field potential duration and thus verify the utility and reliability of these cardiomyocytes with known hERG, Nav1.5 and Cav1.2 inhibitors.

Astemizole, bepridil, cisapride, dofetilide, E-4031, Quinidine and dl-Sotalol prolonged cFPD and at 1000nM reduced spontaneous activity in both hESC-CM and hiPSC-CM cells.

Mexiteline, a sodium channel inhibitor did not induce cFPD prolongation in hESC-CM and hiPSC-CM; but at high concentrations (3.3 to 30µM) suppressed spontaneous activity in hiPSC-CM cells.

Calcium channel inhibitors Nifedipine, Nitrendipine and Verapamil induced shortening of cFPD and at higher concentration (>1000nM) suppressed spontaneous activity in both hESC-CM and hiPSC-CM cells.

The hESC-CM and hiPSC-CM cells were qualitatively and quantitatively consistent in the electrophysiological responses to hERG, Nav1.5 and Cav1.2 inhibitors.

Literature


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