Introduction

Drug-induced delayed cardiac repolarization, a recognized risk factor for pro-arrhythmia, has become the single most common cause for the withdrawal of prescription drugs. The ability to identify detrimental off-target effects earlier has the potential to improve drug safety and reduce the cost of drug development. The vast majority of drugs known to prolong the repolarization of the cardiac membrane preferentially inhibit the delayed rectifier current (I_Kr) by binding to the HERG K+ channel. Consequently, functional in vitro assays for predicting a drug’s potential to delay cardiac repolarization typically include evaluating HERG K+ channel block in transgenic cell lines, or action potential duration assays with primary canine or rabbit Purkinje fibres. The predictive value of these existing assays is limited, however, due to species differences and the lack of complex on channel interactions in cell lines over-expressing HERG K+ channel. The introduction of assays utilizing human embryonic stem cell-derived (hESC) cardiomyocytes could potentially address the shortcomings of these existing models and form the basis of more predictive assays. Here, we describe the use of hESC-derived cardiomyocytes on the multielectrode array platform (MEA) to assess the prospect of using the measured extracellular field potential as a pre-clinical cardio-toxicity screen.

Methods

Preparation of Cyttiva™ Plus cardiomyocytes: To induce CM phenotype, hESCs (H7 cell line) were subjected to a controlled differentiation process. Briefly, the hESCs were adapted to alternative growth conditions, subjected to growth factor induction, followed by a period of cardiomyocyte maturation. At the end point of differentiation, cardiomyocytes were harvested and cryo-preserved at 106 cardiomyocytes per vial.

Seeding MEA plates: Cyttiva™ Plus cardiomyocytes were seeded direct from thaw onto 12-well MEA plates (Avon Biosystems) at a density of 60,000 cells per well. On day 4 post-thaw half the seeding medium was replaced with fresh medium.

Figure 1. Time-line depicting cell seeding and maintenance for MEA experiments

MEA recordings – MEA recordings were made on day 5 post-thaw at 37°C following the compound-addition protocol outlined below.

Figure 2. Time-line depicting MEA experimental protocol

Cytiva™ Plus cardiomyocyte characterization

Figure 3. Cyttiva™ Plus cardiomyocytes on day 5 post-thaw on a 12-well MEA plate (Avon Biosystems)

Figure 4. Cyttiva™ Plus cardiomyocytes on day 5 post-thaw stained for troponin I (peroxidase and DAB immunostain; blue)

Figure 5. Spontaneous Ca2+ transients in Cyttiva™ Plus cardiomyocytes on day 5 post-thaw imaged using an IN Cell 2200 Analyzer with Fluor4-AFC. Coloured curves indicate images from respective points in the Ca2+ transient.

Effect of drugs known to prolong QT interval on Cyttiva™ Plus cardiomyocyte FPD

To validate Cyttiva™ Plus cardiomyocytes as a potentially useful new in vitro test system for drug-induced delayed cardiac repolarization, dose escalation studies were performed for a number of drugs known to prolong the QT interval in therapeutic or side effect. For selective HERG K+ channel blockers, a similar rank-ordering of compounds was found for both the propensity of the drug to induce FPD prolongation and the drug’s HERG IC50 value.

Multi-parameter analysis of MEA drug data

Correlation plots of FPD against spike amplitude for range of drug concentrations tested. Drugs with similar mechanisms of action show similar correlation graphs. Also drugs with similar Redfern 1999 classification scores (1) have similar profiles.

Figure 6. Cyttiva™ Plus cardiomyocytes form spontaneous beating monolayers by day 5 post-thaw with the expected morphology

Figure 7. Drug potency to modulate FPD from embryo-derived cardiomyocytes

Pharmacological modulation of the Cyttiva™ Plus cardiomyocyte waveform

Many different ion currents contribute to the measured extracellular field potential. Blockade of a specific ion channel results in the characteristic modulation of the Cyttiva™ Plus cardiomyocyte waveform as outlined below.

Figure 8. Drug potency to modulate FPD from embryo-derived cardiomyocytes

Summary

Cyttiva™ Plus cardiomyocytes are hESC-derived cardiomyocytes that exhibit the appropriate morphology and electrophysiological responses. Cyttiva™ Plus cardiomyocytes:
- Form spontaneously beating monolayers with the following extracellular field potential characteristics:
  - Spike amplitude = 2.3 mV
  - Beat period = 1.3 s
  - CV of best period = 0.006
- Can distinguish between specific ion channel blockers. Blockade of I_Na, I_Ca, I_Kr, or I_Ks results in a characteristic modulation of the Cyttiva™ Plus waveform as illustrated.
- Can predict the potency of drugs that prolong the QT interval.

We propose that an MEA assay based on hESC-derived cardiomyocytes could complement or potentially replace some of the pre-clinical cardiac toxicity screening tests currently used for lead optimization and further development of new drugs.

References


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Jan Turner, Hayley Tinkler, Liz Roquemore, Nick Thomas, Mike Clements* GE Healthcare, Amersham Place, Little Chalfont, Buckinghamshire, England, UK HP7 9NA. Tel: +44 1494 2052 6169; Fax: +44 1494 2052 6203. * e-mail: michael.clements@ge.com