Screening for EADS and pro-arrhythmic potential with human iPSC derived cardiomyocytes using a multiwell Microelectrode Array (MEA)

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Abstract
Cardiotoxicity is the most common reason for attrition due to toxicity. Current in vitro cytotoxicity assays identify only the most overt toxic compounds, while assays to measure specific liabilities such as hERG and other ion channels may fail to detect a response which relies on a combination of endpoints or a delayed response due to expression. Toxicity determination relies heavily on the later preclinical phase animal studies which have much higher costs associated and can have species specific results which may not model human responses. Therefore, an ideal assay for predicting cardiotoxicity would involve screening earlier on a platform measuring the field potential in human cardiomyocytes and which can identify the pro-arrhythmic potential of compounds.
eCiphrCardio is a multiwell MEA assay which measures the field potential of PSC derived human cardiomyocytes. This assay provides a summation of the effects of the compounds on all ion channels, receptors, and pathways which affect cardiac function. This data reported is field potential duration (FPD) (QT), beat rate, Fast Na+ amplitude and slope. The measurement of these effects can be used to predict whether a compound driving a T-wave is consistent with known responses from various reference compounds that have been evaluated in the system previously. A major liability in cardiotoxicity is associated with hERG inhibition which is linked to QT prolongation. As this MEA assay is capable of identifying T-wave, prediction of a hERG liability can be demonstrated by a reduction in the amplitude of the T-wave as well as increase in the FPD. It is also capable of identifying Early After Depolarizations (EADs) which are common for some hERG inhibitors and can stimulate Torsades de Pointes like responses. The assay is also label free so it can also be performed over multiple time points and days to measure effects which are delayed and which patch clamp cannot detect.
eCiphrCardio is a powerful, predictive assay for cardiac liabilities.

Materials and Methods

Preparation of iCell® Cardiomyocytes:
Six-well tissue culture plates are pre-coated with 0.1% Matrigel™. Twelve hours prior to plating, the plates are rinsed and coated with iCell plating medium. Later, iCell® Cardiomyocytes are plated at 20,000 cells per well in iCell Maintenance Medium. These are incubated for a further 5-8 days until a stable beating phenotype is achieved and cells are ready for drug treatment. The medium is decanted and replaced with vehicle, positive control, or test compound at five concentrations in duplicate.

Electrophysiological recordings and data analysis:
The 48 well multi-electrode arrays are recorded prior to dosing (baseline) and at 45 minutes using the Axion Biosystems Maestro MEA system and AxiS software. The average number of beats, Na+ spike amplitude (mV) and FPD per well will be calculated along with an IC50 for each endpoint.

Compound Treatment:
Cells were treated with multiple concentrations of compounds. The electrical activity was recorded prior to dosing (baseline) and at 45 min using the Axion Biosystems Maestro MEA system. An IC50 value from each compound was determined.

Calcium Channel Effectors in eCiphrCardio

Figure 2. MEA traces of 48 well derived cardiomyocytes treated with Calcium Channel compounds.

Figure 3. Fast Na+ Channel Blockers

Conclusions

• Cardiac toxicity is a key reason for drug attrition at all stages of the drug discovery process. Identifying potential cardiac toxicity earlier in the drug discovery process can save both time and development costs.

• Axion Biosystem’s Maestro MEA system is an innovative platform for screening compounds for effects on cardiac toxicity

• Cyprotex’s eCiphrCardio uses microelectrode array (MEA) recording to monitor electrophysiological activity by measuring beat rate, action potential duration, amplitude and conduction velocity.

• eCiphrCardio assesses phenotypic changes in field potential of cardiomyocytes.

• eCiphrCardio can identify EAD formation in response to some hERG inhibitors which are often linked to Torsades de Pointes risk.

• This assay is a measurement of the contribution of all ion channels involved in the field potential and not just from a single channel (ie. hERG).

• This cardiac assay provides a unique in vitro system for preclinical drug discovery, cardiotoxicity assessment, disease modeling and high throughput phenotypic screening of drug candidates.

• Highly reproducible with a strong concordance with the in vivo cardiac effects of multiple classes of compounds.

• eCiphrCardio is a powerful in vitro assay for identifying cardiac liabilities earlier in the drug discovery pathway

References

Peaker J. (2002). Toxicol Lett., 127(1-3); 279-294