Cellular Dynamics international

Application Note

iCell™ Cardiomyocytes:

Assaying Cytoxicity

iCell™ Cardiomyocytes, derived from human induced pluripotent stem cells (iPSCs), are suitable for in vitro toxicity screening and drug development. Functionality and relevant responses in pharmacological applications have been recently demonstrated for human iPSC-derived cardiomyocytes (1, 2, 3). Currently used preclinical cardiomyocyte models, such as in vivo animal testing, explanted hearts, cardiac tissue preparations, cardiomyocyte-like cell lines, or primary cardiomyocytes, are plagued by supply limitations, questionable relevance, stability issues, and inconsistency with respect to disease state and genetic background (4, 5).

Cellular Dynamics' iCell Cardiomyocytes overcome the limitations of current models. They are manufactured with high purity in industrial quantities, exhibit properties of native cardiomyocytes, are of human origin, and are amenable to long-term culture. These human iPSC-derived cells are manufactured through reproducible differentiation protocols and have a uniform genetic background to improve consistency across experiments. In addition, iPSC technology holds significant promise for creating cardiomyocyte panels from ethnically diverse populations or simulating cardiac diseases in vitro.

In addition to displaying typical cardiac phenotypes, iCell Cardiomyocytes express cardiac specific transcription factors and structural genes. In addition, functional analysis has shown that iCell Cardiomyocytes have the ionic currents present in adult cardiomyocytes. Together, these findings demonstrate that iCell Cardiomyocytes are more physiologically relevant than in vitro models currently used for non-clinical cardiac safety studies.

Cell viability assays are commonly used in academic, biotech and pharmaceutical research to obtain information on cell health, proliferation and toxicity. The viability and cytotoxicity of iCell Cardiomyocytes after compound exposure can be assessed by using the Promega MultiTox-Fluor Multiplex Cytotoxicity Assay, a ratiometric fluorescent assay that simultaneously measures the relative number of live and dead cells in cell populations by detecting two distinct proteases. A fluorogenic, cell-permeant, peptide substrate glycyl-phenylalanyl-amino-fluorocoumarin (GF-AFC) is used to measure live-cell protease activity which is restricted to viable cells. After entering a viable cell, the GF-AFC substrate is cleaved by the live-cell protease activity resulting in a fluorescent signal proportional to the number of viable cells. Loss of cell membrane integrity renders the live-cell protease inactive. To measure dead cell protease activity, a fluorogenic cell-impermeant peptide substrate called bis-alanyl- alanyl-phenylalanyl-rhodamine 110 (bis-AAF-R110) is used. Upon loss of membrane integrity, protease is released and cleaves the bis-AAF-R110 substrate to generate the fluorescent signal. The impermeable nature of bis-AAF-R110 ensures specificity to dead cells. The products generated by the live and dead-cell protease have different excitation and emission spectra and can therefore be measured simultaneously. The live-cell protease produces the AFC product and the dead-cell protease results in the R110 product which can be measured at 400Ex/505Em and 485Ex/520Em respectively (6).

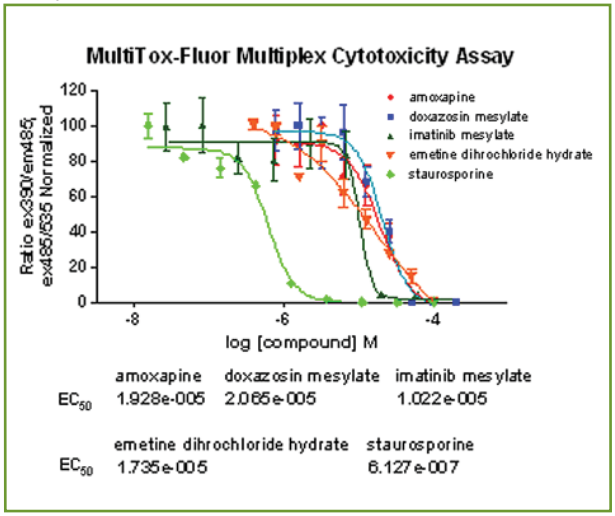
Methods

96-well plates (Corning #3603) were precoated with gelatin (0.1% solution, Sigma #G1890). iCell Cardiomyocytes (99% purity) were seeded in iCell Cardiomyocytes Plating Medium to provide 15,000 plated cells/well in a final volume of 100 µL. 48 hours after plating, wells were washed and cells were fed with 90 µL iCell Cardiomyocytes Maintenance Medium. Nine point drug compound titrations of staurosporine (AG Scientific #S-1016), imatinib Mesylate (gift from Roche Pharmaceuticals), emetine dihydrochloride hydrate (Sigma #E2375), amoxapine (Sigma #A129) and doxazosin mesylate (Sigma #D9815) were prepared at 10X in iCell Cardiomyocytes Maintenance Medium containing 10% of dimethyl sulfoxide (DMSO). 10 µl of each compound dilution were added to triplicate wells bringing the final volume in each well to 100 µl and the final DMSO concentration to 1%. After 24 hours of drug exposure, cell viability and cytotoxicity were assessed using the MultiTox-Fluor Multiplex Assay

per the manufacturer's instructions. Live-cell and dead-cell fluorescence levels were measured at 390Ex/485Em and 485Ex/520Em respectively using a Tecan GENios Pro microplate reader.

Results & Discussion

All compounds assayed (staurosporine, imatinib mesylate, emetine, amoxapine and doxazosin) significantly reduced cell viability while the negative control did not (Figure 1). The EC_{50} values calculated were 613 nM for staurosporine, 10.2 µM for imatinib mesylate, 17.3 µM for emetine dihydrochloride, 19.3 µM for amoxapine and 20.6 µM for doxazosin mesylate, respectively. The Z' values for all assays were > 0.5 indicating highly robust assays.



▲*Figure 1. Cytotoxicity Assays with iCell Cardiomyocytes*

Conclusion

The cell viability and cytotoxicity assay results as measured using the Promega MultiTox-Fluor Multiplex Cytotoxicity Assay demonstrate that iCell Cardiomyocytes are sensitive to known cardiotoxic compounds. Overall, the Promega MultiTox-Fluor Multiplex Cytotoxicity Assay can be used to reliably assess drug compound induced general cytotoxic effects on iCell Cardiomyocytes independent of cell number.

References

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