Cellular Dynamics international

Application Note

iCell™ Cardiomyocytes:

Assaying Cell Viability

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 of iCell Cardiomyocytes after compound exposure can be assessed by using the Promega CellTiter-Glo® Luminescent Cell Viability, a viability assay based on the quantitation of ATP in metabolically active cells (6). Promega's proprietary luciferase enzyme catalyzes the mono-oxogenation of the luciferin substrate in the presence of Mg2+, ATP and molecular oxygen resulting in a light signal that is proportional to the number of viable cells in a cell population.

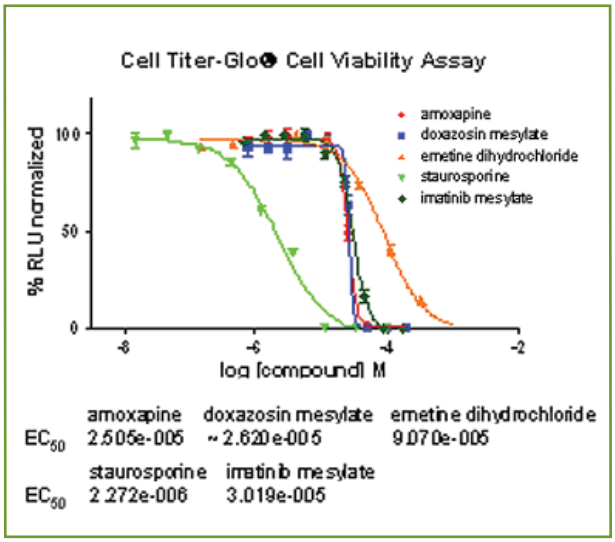
Methods

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, cellular ATP concentrations were assessed using the CellTiter-Glo Luminescent Cell Viability Assay as per the manufacturer's instructions. Luminescence readings were taken on the Tecan GENios Pro microplate reader (1 second integration time/sample).

Results & Discussion

All compounds assayed significantly reduced cell viability with various potencies while the negative controls did not have a significant effect (Figure 1). The EC_{50} values calculated were 2.27 µM for staurosporine, 2.5 µM for amoxapine, 2.62 µM for doxazosin mesylate, 3.02 µM

for imatinib mesylate, and 9.07 µM for emetine dihydrochloride hydrate respectively. The Z' values for all assays were > 0.5 indicating highly robust assays.



▲*Figure 1. Cell Viability Assays with iCell Cardiomyocytes*

Conclusion

iCell Cardiomyocytes are sensitive to known cardiotoxic compounds, as the cell viability assay results measured by the CellTiter-Glo Luminescent Cell Viability Assay demonstrates. Drug compound-induced general cytotoxic effects can be reliably assessed on iCell Cardiomyocytes using the CellTiter-Glo Luminescent Cell Viability Assay.

References

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