

iCell™ Cardiomyocytes:

Assaying Mitochondrial Membrane Potential

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.

Mitochondrial function is critical for cardiomyocyte viability through ATP synthesis, ion homeostasis, and the regulation of apoptosis and necrosis (6). The maintenance of the mitochondrial inner membrane potential ($\Delta\Psi_m$) is a critical component of cell health, and its integrity is often used as a measure of cellular viability (7). Loss of $\Delta\Psi_m$ results in decreased ATP production and can promote a cascade of pro-apoptotic factors. Mitochondrial toxicity

is linked to many of the drugs receiving Black Box Warnings from the FDA, and at least three drugs have been pulled from the market because of organ toxicity directly related to the collapse of $\Delta\Psi_m$ (8).

The Cell Meter™ JC-10 Mitochondrial Membrane Potential Assay Kit is used to detect the loss of $\Delta\Psi_m$ (9). In healthy cells, JC-10 selectively accumulates in mitochondria as orange "J-aggregates." As the inner membrane potential is lost in apoptotic or necrotic cells, the monomeric form of JC-10 is released into the cytoplasm and the cells fluoresce green. The shift of fluorescence in cells from orange to green indicates apoptosis or necrosis. Valinomycin and staurosporine, two compounds known to disrupt $\Delta\Psi_m$ in other cell types (10, 11), were selected to investigate their response in iCell Cardiomyocytes.

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. The following compounds were administered:

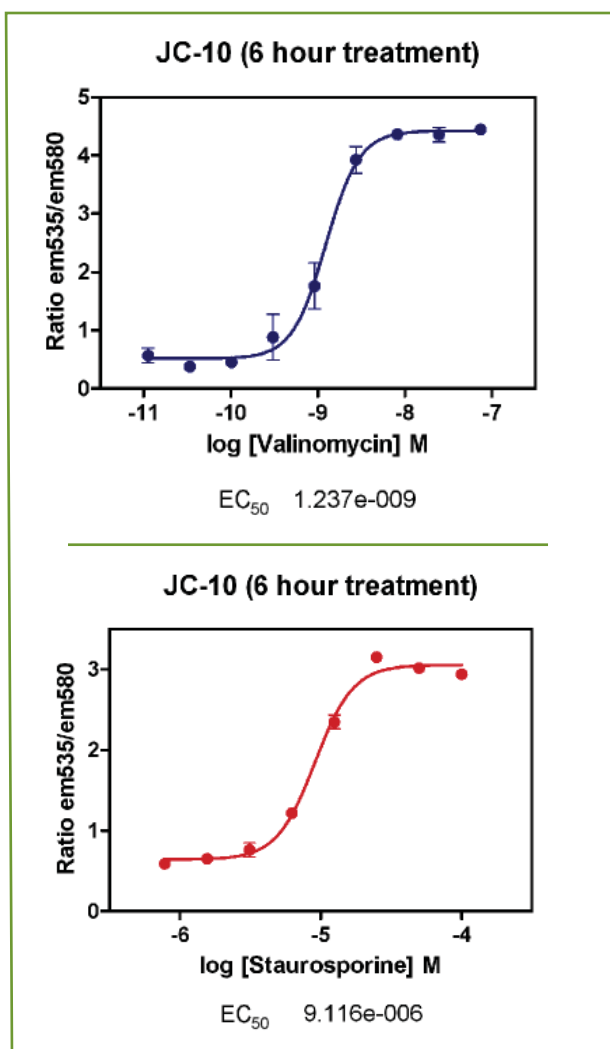
- 10 μL of a three-fold dilution series of valinomycin (Fluka #94675, final concentration of 11 pM to 74 nM) was added to triplicate wells.
- 10 μL of a two-fold dilution series of staurosporine (AG Scientific #S-1016, final concentration of 781 nM to 100 μM) was added to triplicate wells.

Compound dilutions were performed in iCell Cardiomyocytes Maintenance Medium with 10% DMSO for a final concentration of 1% DMSO during the treatment. Mitochondrial membrane potential was measured using the Cell Meter™ JC-10 Mitochondrial Membrane Potential Assay Kit (ABD Bioquest #22800) following six hours of

compound treatment using a Tecan GENios Pro microplate reader (ex490em535 and ex490em580). Relative fluorescence units (RFU) were background-corrected to the levels in control wells containing media only. Data was reported as a ratio of em535 to em580 after background correction.

Results & Discussion

The loss of mitochondrial membrane potential can be detected in iCell Cardiomyocytes in response to valinomycin and staurosporine using the Cell Meter™ JC-10 Mitochondrial Membrane Potential Assay Kit. The EC₅₀ values for a six hour treatment with valinomycin and staurosporine were 1.2 nM and 9.1 μM, respectively (Figure 1).



▲ Figure 1. $\Delta\Psi_m$ Activity in iCell Cardiomyocytes

Conclusion

The compromise of mitochondrial function and ATP production can have significant impact on tissues with

high-energy demands like the heart. Because cardiotoxicity is a major concern in drug development, it is critical to screen candidate drugs for their effects on $\Delta\Psi_m$. iCell Cardiomyocytes are an amenable cellular platform for in vitro toxicity assays investigating the integrity of the $\Delta\Psi_m$.

References

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