Cellular Dynamics international

Application Note

iCell™ Cardiomyocytes:

Assaying Caspase Activity & Apoptosis

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.

Cysteine aspartic acid-specific proteases (caspases) are critical effector molecules of programmed cell death and are one of the most commonly used markers in the field of mammalian apoptosis (6). Both intrinsic and extrinsic pathways of apoptosis can activate the caspase signaling cascade. The intrinsic pathway of apoptosis is initiated through the release of cytochrome c from the mitochondria and its subsequent association with Apaf-1, dATP, and caspase 9 to form the complex known as the apoptosome (7). Formation of the apoptosome initiates caspase 9 to cleave and activate effector caspases 3 and 7. Extrinsic apoptosis signaling, mediated by cell surface receptors, can directly activate caspases 3 and 7 via caspases 8 and 10 without involvement of the mitochondria (8). Caspases 3 and 7 are cellular executioners that cause the destruction of the cell. Cleavage of their substrates lead to the morphological changes associated with apoptosis, including DNA fragmentation, cell shrinkage, chromatin condensation, and membrane blebbing (9).

The Caspase-Glo® 3/7 Assay (Promega) is used to detect effector caspase activity in apoptotic cells (10). Active caspase 3 and 7 cleave a protease-specific DEVD tetrapeptide substrate to release aminoluciferin. Luciferase cleavage of aminoluciferin produces a luminescent signal that is directly proportional to the amount of caspase 3 and 7 activity present. Staurosporine, amoxapine, emetine, and imatinib mesylate (Gleevec) were selected to investigate caspase activation 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:

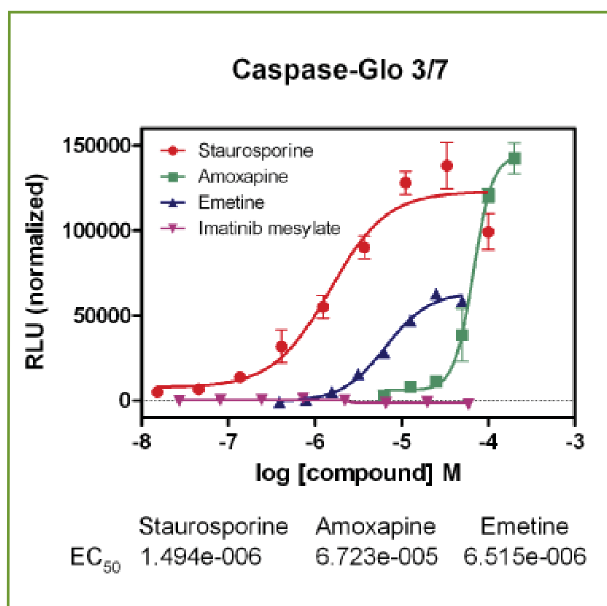
- 10 µL of a three-fold dilution series of staurosporine (AG Scientific #S-1016, final concentration from 15 nM to 100 µM) was added to triplicate wells.
- 10 µL of a two-fold dilution series of amoxapine (Sigma #A129, final concentration from 6.3 µM to 200 µM) was added to triplicate wells.

- 10 µL of a two-fold dilution series of emetine dihydrochloride hydrate (Sigma #E2375, final concentration from 391 nM to 50 µM) was added to triplicate wells.
- 10 µL of a two-fold dilution series of imatinib mesylate (gift from Roche Pharmaceuticals, final concentration from 27 nM to 59 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 treatment. Apoptosis was measured using the Caspase-Glo® 3/7 Assay (Promega #G8091) after 6 hours (staurosporine) or 24 hours (amoxapine, emetine, imatinib mesylate) of compound treatment with a Tecan GENios Pro microplate reader (1 second integration time). Relative luminescence units (RFU) were background-corrected to control wells with cells and vehicle only.

Results & Discussion

Caspase activity can be detected in iCell Cardiomyocytes in response to staurosporine, amoxapine, and emetine using the Caspase-Glo® 3/7 Assay (Figure 1). The EC_{50} value for 6 hour treatment with staurosporine was 1.5 µM. The EC_{50} values for 24 hour treatment with amoxapine and emetine were 67 µM and 6.5 µM, respectively. No caspase 3 or 7 activity was detected at 24 hours in the range of imatinib mesylate tested despite widespread cell death at the highest concentrations.



	Staurosporine	Amoxapine	Emetine
EC_{50}	1.494e-006	6.723e-005	6.515e-006

▲ *Figure 1. Caspase Activity in iCell Cardiomyocytes*

Conclusion

Monitoring caspase activity is critical to detecting and understanding drug toxicity. The use of iCell Cardiomyocytes in preclinical safety testing could accelerate the detection of toxic compounds and provide a mechanism of action for cardiac toxicity. iCell Cardiomyocytes are an amenable cellular tool for in vitro apoptosis assays investigating the activation of caspase pathways.

References

1. Tanaka T, Tohyama S, Murata M, et al. In vitro pharmacologic testing using human induced pluripotent stem cell-derived cardiomyocytes. Biochem Biophys Res Commun 385, 497-502 (2009).
2. Zhang J, Wilson GF, Soerens AG, et al. Functional cardiomyocytes derived from human induced pluripotent stem cells. Circ Res 104, 30-41 (2009).
3. Yokoo N, Baba S, Kaichi S, et al. The effects of cardioactive drugs on cardiomyocytes derived from human induced pluripotent stem cells. Biochem Biophys Res Commun 387, 482-488 (2009).
4. Kettenhofen R and Bohlen H. Preclinical assessment of cardiac toxicity. Drug Discov Today 13, 702-707 (2008).
5. Harding SE, Ali NN, Brito-Martins M, et al. The human embryonic stem cell-derived cardiomyocyte as a pharmacological model. Pharmacol Ther 113, 341-353 (2006).
6. Yuan J and Horvitz HR. A first insight into the molecular mechanisms of apoptosis. Cell S116, S53-S56 (2004).
7. Gustafsson AB and Gottlieb RA. Heart mitochondria: gates of life and death. Cardiovasc Res 77, 334-343 (2008).
8. Wang C and Youle RJ. The role of mitochondria in apoptosis. Annu Rev Genet 43, 95-118 (2009).
9. Danial NN and Korsmeyer SJ. Cell death: critical control points. Cell 116, 205-219 (2004).
10. Caspase-Glo 3/7 Assay Technical Bulletin #TB323. Promega Corporation. Revised June 2009.

For More Information

Cellular Dynamics International, Inc.
525 Science Drive
Madison, WI 53711 USA

T (608) 310 - 5100 | Toll-free US (877) 310-6688
E sales@cellulardynamics.com
W www.cellulardynamics.com

AN-CMCASPASE100305

© Copyright 2010 Cellular Dynamics International, Inc.