Live Cell Beating Assay Using Human iPSC-derived Cardiomyocytes for Evaluation of Drug Efficacy and Toxicity

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Abstract

A large percentage of new drugs fail in clinical studies due to cardiac toxicity. Therefore, development of a more predictive in vitro assay is needed. Human cardiomyocytes derived from stem cell sources can greatly accelerate the discovery of drugs and improve drug safety by offering more clinically relevant cell-based models than those presently available. Derived from human induced pluripotent cells and highly purified, CelliC® Cardiomyocytes are especially attractive because they express ion channels and demonstrate beating and action potentials similar to primary cardiac cells. Here we describe cell-based assays for measuring the impact of pharmacological compounds on the rate and magnitude of beating cardiomyocytes with different instrument platforms.

Introduction

Preclinical safety is an important part of drug discovery and development. Early assessment of cardiac toxicity would allow the industry to reduce the number of drugs failing in clinical trials because of unacceptable toxicity. An emerging application for iPSC-derived cardiomyocytes is for use as a model cell-based system for testing functional effects of ion channel blockers, GPCR antagonists, or other prospective drugs on cardiac contractility and toxicity. Cellular Dynamics International’s (CDI) CelliC® Cardiomyocytes are highly purified human cardiomyocytes derived from induced pluripotent cells. Using these cells in conjunction with automated imaging platforms we have shown dose-dependent atypical patterns and changes in cell beating caused by number of cardioactive and cardiotoxic compounds.

Methods

Cell Preparation and Imaging

Cell cardiomyocytes were received frozen from CDI. Cells were thawed and plated according to recommended protocol.

Flux Assay

1. Calcium solution was added to the plates and incubated 1 hour at 37°C in 5% CO2.
2. Compound plates were preincubated at 37°C inside the FLIPR Tetra instrument and compound addition was done simultaneously to all wells.
3. Fluo4 solution plates were loaded and a pre-drug read was acquired in absence of compounds.
4. Data was acquired at 8 fps
5. Calcium index was measured during compound addition and at prescribed time frames after the compound addition (read time – 2 min).

High Throughput FLIPR Tetra System Cardiac Beating Assay

A complementary method uses the FLIPR Tetra system to monitor changes in intracellular Ca2+ fluxes associated with cardiomyocyte contractions using the FLIPR® Calcium 5 Xl Assay. The FLIPR system allows automatic addition of reagents and compounds, simultaneous with reading from 96 or 384 well formats. This has been found to be very advantageous for the cardiac beating assays because it reduces well-to-well variability caused by reading at different time points. The absolute beat rates were found to be very similar to that measured by imaging methods. Temporal response curves for analysis and visualization of beating can be acquired in ~2 min per plate making this assay suitable for high throughput screening of compound libraries.

Abstract

Cardiac toxicity is a serious drug safety concern because it can cause arrhythmias or heart failure. We have developed automated methods for the ImageXpress Micro and FLIPR Tetra systems that enable image acquisition and automatic determination of beating rate of live cardiomyocytes from a series of time-lapse images. One protocol captures mechanical movement of cells; a second monitors changes in intensity of Ca2+ fluxes synchronous with beating. Both methods allow visual monitoring of drug impact on the beat rate, rhythm and amplitude in 96 or 384 well formats. The system allows saving data as a video, presenting intensity curves, and automatic analysis of beat rates.

Visualizing beating cardiomyocytes by Ca2+ fluxes

Cell-based model for testing cardiac drugs

Dose with epinephrine followed by cardiotoxic drugs such as DMSO or doxorubicin

Epinephrine increased by beat rate.

Epinephrine (didoxine: Concentration vs MeanValue)

Epinephrine showed dose dependency in 96 well plates.

Cell-based model for testing cardiac drugs

Evidence of a surrogate marker for cardiac toxicity assay

The assay system allows detection of atypical patterns caused by compounds known to be associated with long QT syndrome (e.g., cipride and doxazosin) and Na+ channel blockers (e.g., lidocaine). Measuring peak width, peak spacing and other parameter would allow prediction of drugs inducing long QT syndrome, arrhythmia and other potentially dangerous features.

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Summary

We demonstrate live-cell assays for measuring the impact of pharmacological compounds on the rate and magnitude of beating cardiomyocytes using FLIPR Tetra and ImageXpress Micro Systems

Development of Predictive Cell-Based Assays

Development of new, more potent and safer drugs requires an in vitro system where efficacy and safety can be tested. Positive and negative ionotropes are used in clinics to treat heart failure (tachycardia, arrhythmia or other cardiac diseases. We have demonstrated effects of several positive (isoproterenol, dopamine, etc.) and negative (–) (i.e., blockers) chronotropes on cardiac rates and determined EC50 at the expected ranges. Image based assays using calcium flux and iPSC derived cardiomyocytes are suitable for that task and could be used to estimate efficacy and approximate dosing prior to clinical studies. Results from assays run on the FLIPR Tetra system are shown below.

Positive and negative chronotropic effects

FAIL EARLY: Identification of compounds that affect beat rate and rhythm of iPSC cardiomyocytes

We have shown dose-dependent atypical patterns and changes in cell beating caused by several known cardiotoxic compounds including tetrodotoxin, Ca2+ and Na+ channel blockers. Potentially toxic compounds can be easily detected in the assay by their effect on the beat rate pattern.

Surrogate markers for cardiac toxicity assay

The assay system allows detection of atypical patterns caused by compounds known to be associated with long QT syndrome (e.g., cipride and doxazosin) and Na+ channel blockers (e.g., lidocaine). Measuring peak width, peak spacing and other parameter would allow prediction of drugs inducing long QT syndrome, arrhythmia and other potentially dangerous features.