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Human stem cell-derived cardiomyocytes detect drug-mediated changes in action potentials and ion currents

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ABSTRACT

Introduction: It has been proposed that proarrhythmia assessment for safety pharmacology testing includes the use of human pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) to detect drug-induced changes in cardiac electrophysiology. This study measured the actions of diverse agents on action potentials (AP) and ion currents recorded from hiPSC-CM.
Methods: During AP experiments, the hiPSC-CM were paced at 1 Hz during a baseline period, and when increasing concentrations of test compound were administered at 4-minute intervals. AP parameters, including duration (APD_{60} and APD_{90}), resting membrane potential, rate of rise, and amplitude, were measured throughout the entire experiment. Voltage clamp experiments with E-4031 and nifedipine were similarly conducted.
Results: E-4031 produced a dose-dependent prolongation of cardiac action potential and blocked the hERG/IKr current with an IC_{50} of 17 nM. At 3 nM, dofetilide significantly increased APD_{90}. Astemizole significantly increased APD_{60} and APD_{90} at 30 nM. Terfenadine significantly increased APD_{90} at concentrations greater than 10 nM. Fexofenadine, a metabolite of terfenadine, did not produce any electrophysiologic changes in cardiac action potentials. Flecainide produced a dose-dependent prolongation of the cardiac action potential at 1 and 3 μM. Acute exposure to nifedipine significantly decreased APD_{60} and APD_{90} and produced a dose-dependent block of calcium current with an IC_{50} of 0.039 μM. Verapamil first shortened APD_{60} and APD_{90} in a dose-dependent manner, until a compensating increase in APD_{90}, presumably via hERG blockade, was observed at 1 and 3 μM. Following a chronic exposure (20–24 h) to clinically relevant levels of pentamidine, a significant increase in action potential duration was accompanied by early afterdepolarizations (EADs).
Discussion: These experiments show the ability of AP measured from hiPSC-CM to record the interactions of various ion channels via AP recording and avoid the limitations of using several single ion channel assays in a noncardiac tissue.

1. Introduction

The generation of pluripotent stem cells by reprogramming mature somatic cells from adult human patients has produced an unlimited supply of stem cells which can be differentiated into a variety of cell types, including induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM). These cells can be used for a variety of applications, such as the production of patient-specific models of arrhythmia; in vitro models to evaluate the effects of drugs for their causative or preventative actions on human disease such as congestive heart failure; and/or as a preclinical safety pharmacology cells to predict the outcome of clinical trials (Davis et al., 2012; Pugsley, Towart, Authier, Gallacher, & Curtis, 2011; Terrenoire et al., 2013; Uesugi, Ojima, Taniguchi, Miyamoto, & Sawada, 2013).

Cardiac safety pharmacology must be determined during nonclinical and clinical drug development to identify changes in cardiovascular function and is mandated by regulatory agencies in the ICH S7B and E14 guidelines (2005). At present, there is no in vitro pre-clinical system using human tissue for cardiac safety testing. The unpredictable availability of human donors, including variations in age, sex, disease and drug treatment, make it impossible to use native human cardiac tissue as a routine tissue source for a testing model. The development of an in vitro system based on hiPSC-CM provides tissue for drug safety assessment using cells from the most relevant species in a timely and reproducible manner (Qu, Gao, Fang, & Vargas, 2013).

In regard to preclinical safety pharmacology, the actions of several reference compounds have been reported on action potentials and ion channels recorded from embryonic H7 stem cells as preclinical

Abbreviations: APD60, action potential duration at 60% repolarization; APD90, action potential duration at 90% repolarization; EADs, early afterdepolarizations; hERG, human ether-a-go-go-related gene; hESC, human embryonic stem cells; hiPSC-CM, human induced pluripotent stem cell-derived cardiomyocytes.

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biomarkers of cardiac safety and efficacy. In general, these experiments accurately predicted the risk of torsades de pointes (TdP) with an overall sensitivity greater than animal tissue assays (Peng, Lacerda, Kirsch, Brown, & Bruening-Wright, 2010). However, a reduced latency for development of channel block and increased potency for IKr block by terfenadine and quinidine was observed in these studies. An additional study using the same embryonic H7 stem cells also reported sensitivity for detecting repolarization delay induced by IKr blockade (Qu et al., 2013). Although, in this study, these cells were less sensitive for detecting Na+ channel blockade. These limitations may result from the embryonic source of these stem cells.

iCell® Cardiomyocytes are generated from an hiPSC line developed from adult volunteers and engineered to permit selection for high cardiomyocyte purity by expressing blasticidin resistance under the control of a cardiac myosin heavy chain promoter (Ma et al., 2011). Study of gene expression demonstrates that these cells express relevant cardiac markers such as ion channels, tissue-specific structural markers, and transcription factors (Puppala et al., 2013). A comprehensive investigation of the electrophysiologic properties of these highly pure hiPSC-CM also demonstrated three separate types of action potentials with atrial-like, nodal-like, and ventricular-like action potentials with gating properties of seven major cardiac ion currents (Ma et al., 2011).

To further validate ventricular-like iCell® Cardiomyocytes as a preclinical model of safety pharmacology, we investigated the actions of diverse reference agents at different concentrations on action potentials and ion currents recorded from ventricular-like cells while paced at a constant pacing rate under physiologic temperatures. We would emphasize that these experiments were designed to measure safety pharmacology in hiPSC-CM, not to investigate specific electrophysiologic mechanisms of drug action. These experiments included agents known to block both individual and multiple cardiac ion channels, inhibit ion channel trafficking and produce EADs. We found that hiPSC-CM can serve as a useful tool in early drug screening or cardiovascular safety frontloading.

2. Methods

2.1. Human heart cell generation and cell culture

Human iPSC-derived ventricular-like cells (iCell® Cardiomyocytes) developed from adult volunteers were obtained from Cellular Dynamics International (CDI, Madison, WI). In brief, as described by Cellular Dynamics International (Madison, WI) this cell line was created by expression of the reprogramming factors *sox7*, *oct4*, *nanog*, and *lin28* using MMLV viral constructs in a human fibroblast cell line. The stem-cell clones were engineered to exhibit blasticidin resistance by inserting the coding region of the *BSD gene* encoding Blasticidin S Deaminase from *Aspergillus terreus*. Upon observation of beating cardiac aggregates, cultures were subjected to 25 ug/ml blasticidin to enrich the cardiomyocyte population. Cardiomyocyte aggregate cultures were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum during cardiomyocyte selection through the duration of the culture prior to cryopreservation. At 30 to 32 days of culture the enriched, stem cell-derived cardiomyocytes were subjected to 0.5% trypsin to obtain single cell suspensions of purified cardiomyocytes, which were >98% cardiac troponin-T (cTNT) positive. These cells (iCell® Cardiomyocytes) were cryopreserved and stored in liquid nitrogen before delivery to Ionic Transport Assays from Cellular Dynamics International, Madison, WI. For additional technical details of stem cell preparation and culture consult www.cellulardynamics.com/products/cardiomyocytes.html.

When delivered to Ionic Transport Assay, Inc., these cells were thawed in CDI plating media and plated onto gelatin coated coverslips for electrophysiology studies. Specifically for this study, single vials containing $\approx 1.5 \times 10^6$ cardiomyocytes were thawed by immersing the frozen cryovial in a 37 °C water bath, transferring thawed cardiomyocytes into a 50 ml tube, and diluting them with 20 ml of room temperature plating medium (iCell® Cardiomyocyte Plating Medium [iCPM]).

When the cells were placed on glass coverslips precoated with 0.1% gelatin, this was defined as culture day 1 for the purpose of this study. Cell culture maintenance media were changed three times each week, being careful to remove most, but not all, of the old media by hand pipetting prior to adding fresh media. After 10 to 30 days in culture, the cardiac cells adhering to the coverslips were placed in a Warner perfusion chamber and maintained at 34.5 °C by the use of a Thermoclamp-1 in-line heater (AutoMate Scientific, CA) with a perfusion rate of 0.8–1.2 ml/min. In general, the perfusate, or external solution, was composed of: 150 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl, 1.0 mM MgCl, 15 mM glucose, 15 mM HEPES, 1 mM Na-pyruvate, with the pH adjusted to 7.2 with 10 N NaOH. The internal, or pipette solution, contained: 150 mM KCl, 5 mM NaCl, 2 mM CaCl, 2 mM MgCl, 5 mM EGTA, 10 mM HEPES, pH adjusted to 7.2 with 10 N KOH. The external perfusate could be collected to measure drug concentrations.

Internal electrode and external perfusion solutions were made from dry powder. Internal pipette solution was made up fresh every Monday; whereas, external perfusion bath solution was made up every morning prior to electrophysiology recording. Drug containing solution was made from the external bath solution by serial dilution of a 100% DMSO stock to the final test concentrations described in Section 3 below.

2.2. Preparation of test compounds

E-4031 and verapamil-HCl were purchased from Enzo Life Sciences (Farmingdale, NY). Astemizole, dofetilide, flecainide and pentamidine were purchased from Tocris-R&D Systems. Fexofenadine and terfenadine were purchased from Sigma-Aldrich (MO, USA). Nifedipine was purchased from Alexis Biochemicals (Farmingdale, NY).

All chemicals were dissolved in DMSO to make stock solutions. The final DMSO concentration ranged from the low dose of E-4031 (i.e., 0.00015% DMSO), to the highest dose of pentamidine (i.e., 0.1% DMSO). Exposure to <0.1% DMSO had no effect on cardiac action potentials (data not shown).

2.3. Action potential experimental protocol

Recording electrodes were pulled and filled with internal solution, after which they were measured to have from 2 to 8 MOhms resistance. For action potential recordings, the tip of the recording pipette was filled with normal internal solution. The rest of the electrode was backfilled with internal solution containing 50 μg/ml gramicidin (diluted from a 50 mg/ml gramicidin DMSO stock solution). These electrodes formed gigaohm seals on the cardiac cells with the addition of slight suction. Permeabilization of the patch and access to the internal milieu of the heart cell typically required 20 to 30-minute exposure for good access (≤10 MOhms).

Human iPSC ventricular-like cardiomyocytes first were allowed to spontaneously fire during these experiments; pacing then was started to control the beating rate at 1 Hz. Approximately 20% of these iCell® Cardiomyocytes demonstrate a sinus node-like or atrial-like, electrophysiologic phenotype. These sinus node-like cells were not used in the experiments in this report.

Human iPSC ventricular-like cardiomyocytes were paced at 1 Hz during a 4-minute baseline period, and during increasing concentrations of test compound which were administered at 4-minute intervals. Action potential parameters, including duration (APD_{60} and APD_{90}), resting membrane potential, action potential rate of rise, and action potential amplitude, were routinely measured throughout the entire experiment.

2.4. Voltage clamp experimental protocol

2.4.1. E-4031

Voltage clamp experiments with E-4031 were conducted in a similar manner to the action potential experiments. The gramicidin perforated patch technique provided a more stable and robust voltage clamp recording compared to the standard perforated whole cell patch technique even when 5 mM Mg-ATP was included in the ruptured patch pipette (data not shown). Rundown of the hERG current was not observed in either recording mode, unlike the situation in HEK-293 cells overexpressing the hERG current (Marrannes & De Prins, 2004). The pulse protocol was to hold the cell at −40 mV for 30 s at which time a 4-second depolarizing voltage pulse was delivered to +20 mV. This was followed by a repolarizing voltage pulse to −40 mV that allowed for the assessment of hERG tail current amplitude (deactivation). Once a stable recording was obtained, E-4031 was delivered sequentially at 3, 10, 30, and 100 nM for 4 min at each concentration to obtain a concentration response relationship. Series resistance compensation (85–90%) was used, although the small size and relatively slow kinetics of the hERG current allowed for good voltage clamp control, unlike the large, fast sodium current also seen in these cells (data not shown).

2.4.2. Nifedipine

The perfusate or external solution for nifedipine voltage clamp experiments was composed of: 160 mM Choline Cl (to eliminate contaminating sodium current), 1.8 mM CaCl, 1.0 mM MgCl, 10 mM glucose, 10 mM HEPES, with the pH adjusted to 7.2 with 10 N NaOH. The internal or pipette solution contained: 145 mM CsCl, 5 mM NaCl, 2 mM CaCl, 5 mM MgATP, 5 mM EGTA, 10 mM HEPES, pH adjusted to 7.4 with 10 N CsOH.

The whole cell ruptured patch technique and series resistance compensation were used to ensure good voltage control of the large, fast calcium current seen in these cells. Peak calcium current during a −10 mV pulse was measured and averaged across six cells during a 4-minute baseline control period (no rundown observed). Nifedipine was administered for 4 min for each of the five test concentrations of 0.01, 0.03, 0.1, 0.3, or 1 μM. Calcium current peak amplitude was averaged from five voltage pulses for each nifedipine concentration as well as for the control period.

2.5. Acute pentamidine treatment

Spontaneous activity of all recorded cells was obtained at the start of the experiment. Cells were then paced at 1 Hz during a 5-minute baseline period and increasing concentrations of pentamidine were sequentially applied from 1, 3, 10, 30, and 100 μM for 4 min at each concentration. Action potential parameters including duration (APD_{60} & APD_{90}), resting membrane potential, action potential amplitude, and rate of action potential rate of rise were routinely measured throughout the entire experiment.

2.6. Chronic pentamidine treatment

Cells on cover slips in twenty-four well cell plates were exposed to 1, 3, or 10 μM pentamidine for 20 to 24 h while maintained in low serum media in a CO_2 incubator at 37 °C and 7% CO_2. Individual cover slips were then removed and placed in a Warner perfusion chamber. While in this perfusion chamber, the cells were maintained at 34.5 °C and perfused with drug free, perfusion media for 5 min to enable the recording of spontaneous action potentials. Spontaneous action potential activity was obtained and action potential parameters were measured for all cells treated chronically with pentamidine. For 1 μM pentamidine, additional 1 Hz pacing data was recorded and action potential parameters were likewise measured throughout the experiment.

2.7. Data acquisition and analysis

Action potential recordings were performed using an AM Patch Clamp Amplifier (Model # 2400, AM Instruments, CA, USA) coupled through a 2110a Shielded Connector Block (National Instruments, TX, USA) to a PCI-6014 Basic Multifunction Data Acquisition Board (National Instruments, TX, USA). The average response of at least six recorded action potentials at each concentration was measured. All measured parameters and statistical evaluation of significant test compound actions are given in tabular formats. Data are presented as mean ± standard deviation. Statistical significance was determined with the one way repeated measures (ANOVA), followed by the Dunnett's multiple comparison test when appropriate. Non-linear regression curve fit was used to determine IC_{50} values for E-4031 and nifedipine.

3. Results

3.1. Effects of hERG blockers on action potentials and ion currents recorded from iPSC cardiomyocytes

Standard hERG blockers were selected to investigate the sensitivity of iPSC cardiomyocytes to detect hERG-related repolarization delays. A major advantage of using hiPSC-CM in culture is the ability to routinely record from them using the patch-clamp technique. When stimulating using gramicidin perforation, the action potential duration and shape were essentially unchanged throughout a 40-minute time course (Table 1). In general, these ventricular-like cells were beating at slow rates, i.e. 24–36 beats per min when perforated with gramicidin. Sinus node-like hiPSC-CM were spontaneously beating at faster rates, i.e. 1 Hz, were significantly depolarized, i.e. resting membrane potential of −40 to −60 mv, and demonstrated a smaller action potential amplitude, i.e. 60 to 80 mv. The experiments conducted in this work used ventricular-like hiPSC-CM paced at 1 Hz.

The hERG potassium current is known to rundown over time when recorded from overexpressing cell lines in both manual and automated patch clamp systems and most investigators apply a mathematical correction factor to partially compensate for this effect. If the hERG current were running down in these ventricular-like hiPSC-CMs, the action potential would slowly prolong over time. This was not the case when recording from these cells. If anything was observed, it was a slight shortening of the cardiac action potential duration (Table 1, Time control).

A representative dose response experiment with E-4031 is demonstrated in Fig. 1A. After a 5-minute control recording, increasing concentrations of E-4031 (3, 10, 30, 100 nM) were sequentially applied for 4 min each. This produced a dose-dependent prolongation of the cardiac action potential in this cell. The data in Table 1 demonstrate that acute exposure of hiPSC-CM to E-4031 at concentrations greater than 10 nM significantly increased action potential duration at 90% repolarization; APD_{90} was increased by 72 ± 33% at 30 nM and 106 ± 25% at 100 nM. E-43031 also significantly increased APD_{60} by 42 ± 17% at 30 nM and 60 ± 16% at 100 nM. As seen in the representative action potentials shown in Fig. 1, there was no significant change in resting membrane potential, action potential amplitude, or rate of rise of the action potential after exposure to E-4031. Afterdepolarizations were seen in two of five cells receiving 30 nM E-4031.

Fig. 2A demonstrates block of the hERG/IKr current by 3, 10, 30, and 100 nM E-4031 in hiPSC-CM. The peak of the repolarizing tail current to −40 mV was used to measure the amplitude and block of the hERG/IKr current in these cells. Fig. 2B plots the percent block for hERG/IKr after exposure to 3, 10, 30, and 100 nM E-4031 in four cells. Use of the perforated patch recording technique allowed for precise measurements of the relatively small hERG/IKr current seen in these cells. Significant inhibition of the hERG/IKr current was produced by 10 nM and higher concentrations of E-4031 with an IC_{50} of 17 nM. An excellent correlation was observed between

Table 1
Acute effects of E-4031, dofetilide, terfenadine, fexofenadine, astemizole, nifedipine, flecainide and verapamil on cardiac action potentials recorded from hiPSC-CM.[a, b].

	n	RMP (mV)[c, f]	APD_{60} (ms)[d, h]	APD_{90} (ms)[d, g]	APA (mV)[c, i]	Rate of rise (V/S)[e, j]
Time control						
Control	8	−68 ± 9	314 ± 67	427 ± 65	103 ± 9	42 ± 10
4 min	8	−68 ± 9	305 ± 64	414 ± 64	103 ± 8	43 ± 13
8 min	8	−69 ± 9	303 ± 68	415 ± 69	102 ± 11	43 ± 13
12 min	8	−67 ± 10	303 ± 72	423 ± 74	101 ± 12	43 ± 13
16 min	8	−67 ± 10	303 ± 75	424 ± 75	99 ± 12	43 ± 14
E-4031						
Control	5	−77 ± 5	290 ± 30	431 ± 54	101 ± 6	33 ± 8
3 nM	5	−74 ± 5	299 ± 43	456 ± 77	104 ± 7	39 ± 4
10 nM	5	−73 ± 6	332 ± 62	512 ± 96	105 ± 5	41 ± 8
30 nM	5	−70 ± 8	412 ± 77*	736 ± 141***	108 ± 4	44 ± 4
100 nM	5	−67 ± 8	464 ± 70**	879 ± 27***	106 ± 7	40 ± 4
Dofetilide						
Control	9	−78 ± 6	389 ± 81	541 ± 50	117 ± 11	58 ± 15
1 nM	9	−77 ± 5	388 ± 82	554 ± 36	111 ± 7	58 ± 16
3 nM	9	−76 ± 9	439 ± 85	633 ± 89*	110 ± 11	58 ± 17
10 nM	9	−64 ± 9*	498 ± 105	803 ± 118***	100 ± 15*	52 ± 18
30 nM	9	−44 ± 17***	770 ± 172***	935 ± 38***	85 ± 21***	47 ± 17
Terfenadine						
Control	6	−72 ± 7	259 ± 77	459 ± 19	96 ± 11	66 ± 14
3 nM	6	−72 ± 8	258 ± 74	473 ± 35	96 ± 12	65 ± 14
10 nM	6	−71 ± 9	259 ± 76	491 ± 30	96 ± 12	65 ± 15
30 nM	6	−69 ± 8	265 ± 77	560 ± 39***	95 ± 14	67 ± 15
100 nM	6	−65 ± 8	280 ± 75	652 ± 48***	95 ± 14	64 ± 11
Fexofenadine						
Control	5	−68 ± 3	322 ± 39	454 ± 47	100 ± 10	36 ± 8
0.1 μM	5	−69 ± 2	314 ± 40	439 ± 51	101 ± 10	41 ± 6
0.3 μM	5	−68 ± 2	317 ± 44	440 ± 56	101 ± 11	43 ± 15
1.0 μM	5	−68 ± 3	323 ± 50	447 ± 63	102 ± 9	49 ± 16
3.0 μM	5	−68 ± 3	320 ± 41	446 ± 56	103 ± 9	42 ± 10
Astemizole						
Control	5	−81 ± 6	295 ± 35	438 ± 45	99 ± 5	49 ± 5
3 nM	5	−81 ± 5	337 ± 32	448 ± 42	104 ± 8	41 ± 13
10 nM	5	−81 ± 5	335 ± 29	508 ± 40	108 ± 5	41 ± 15
30 nM	5	−75 ± 4	409 ± 39***	702 ± 47***	108 ± 2	44 ± 19
Nifedipine						
Control	6	−78 ± 4	410 ± 42	529 ± 45	112 ± 7	104 ± 22
0.01 μM	6	−76 ± 4	358 ± 35*	474 ± 49*	110 ± 8	102 ± 21
0.03 μM	6	−72 ± 5	321 ± 52*	406 ± 56*	106 ± 7	97 ± 21
0.1 μM	6	−67 ± 5**	252 ± 52**	347 ± 73**	104 ± 11	93 ± 19
0.3 μM	5	−56 ± 7***	172 ± 46***	257 ± 65***	93 ± 13	88 ± 21
Flecainide						
Control	5	−68 ± 7	327 ± 27	463 ± 38	100 ± 13	56 ± 21
0.1 μM	5	−70 ± 7	318 ± 17	476 ± 30	95 ± 13	52 ± 25
0.3 μM	5	−65 ± 11	330 ± 30	493 ± 56	94 ± 15	45 ± 11
1.0 μM	5	−63 ± 12	352 ± 28	533 ± 51**	88 ± 22	39 ± 4
3.0 μM	5	−59 ± 18	369 ± 45**	592 ± 30***	84 ± 24	36 ± 6
Flecainide						
Control	4	−77 ± 13	250 ± 39	386 ± 57	105 ± 12	41 ± 9
3.0 μM	4	−74 ± 11	335 ± 64	546 ± 76*	108 ± 13	45 ± 6
10.0 μM	4	−68 ± 13	391 ± 66	700 ± 98***	105 ± 19	27 ± 6*
Verapamil						
Control	7	−73 ± 6	461 ± 54	583 ± 45	106 ± 12	62 ± 40
0.03 μM	7	−72 ± 5	442 ± 63	553 ± 54*	104 ± 11	66 ± 42
0.1 μM	7	−71 ± 6	423 ± 47*	549 ± 33*	105 ± 10	71 ± 49
0.3 μM	7	−68 ± 8*	364 ± 70*	517 ± 59*	103 ± 12	45 ± 34
1.0 μM	7	−66 ± 7*	315 ± 88*	541 ± 72	97 ± 17	50 ± 38
3.0 μM	7	−64 ± 2*	338 ± 74*	576 ± 73	91 ± 24	47 ± 31

[a] 4-minute exposure at each concentration.
[b] Data are expressed as mean ± standard deviation (X ± SD).
[c] RMP and APA values are in mV.
[d] APD_{60} and APD_{90} are in ms.
[e] Rate of rise is in V/S.
[f] RMP: resting membrane potential.
[g] APD_{90}: action potential duration at 90% repolarization.
[h] APD_{60}: action potential duration at 60% repolarization.
[i] APA: maximum amplitude of action potential from resting membrane potential (action potential amplitude).
[j] Rate of rise: rate of rise of the action potential.
* Indicates change value is statistically significant (*$p \leq 0.05$, ** $p \leq 0.01$, ***$p \leq 0.001$).

the blocking effects of the hERG/IKr current in the voltage clamp experiments with the prolongation in action potential duration observed using the same concentrations of E-4031.

The data in Table 1 and Fig. 1B demonstrate that acute exposure of hiPSC-CMs to dofetilide significantly increased APD_{90} at concentrations greater than 1 nM. APD_{90} was increased by 10 ± 8% at 3 nM,

43% ± 25 at 10 nM, and 70 ± 16% at 30 nM. Dofetilide also significantly increased APD_{60} by 99 ± 77% at 30 nM. The resting membrane potential depolarized at 10 and 30 nM dofetilide and reduced the peak amplitude of the action potential at 10 and 30 nM dofetilide. These latter changes were accompanied by a significant prolongation in the action potential duration and a decreased resting membrane potential at the time of the ensuing 1 Hz stimulation.

The data in Table 1 and Fig. 1C demonstrate that acute exposure of hiPSC-CM to terfenadine at concentrations greater than 10 nM significantly increased action potential duration at 90% repolarization. APD_{90} was increased 22 ± 7% at 30 nM and 42 ± 5% at 100 nM (Fig. 1C). Terfenadine did not change APD_{60}; nor did it significantly change the resting membrane potential, the peak amplitude of the action potential, or the rate of rise of the action potential.

After control recording, hiPSC cardiomyocytes were exposed to increasing concentrations of fexofenadine, (0.1 μM, 0.3 μM, 1.0 μM and 3.0 μM) that were sequentially applied for 4 min each. No significant changes were observed in resting membrane potential, APD_{90}, APD_{60}, action potential amplitude, or rate of rise of the action potential after exposure to these concentrations of fexofenadine (Table 1). These concentrations of fexofenadine were substantially higher than the concentrations of terfenadine, i.e. 30 nM, which prolonged the action potential duration of hiPSC cardiomyocytes in this study.

A representative dose response experiment with astemizole is demonstrated in Fig. 1D. After a control recording, increasing concentrations of astemizole (3, 10, and 30 nM) were sequentially applied for 4 min each. This produced a dose-dependent prolongation of the cardiac action potential in this hiPSC-CM. The data in Table 1 demonstrate that acute exposure of hiPSC-CM to astemizole at a concentration greater than 10 nM significantly increased action potential duration at 90% repolarization. APD_{60} increased by 39 ± 13% and APD_{90} was increased by 61 ± 12% at 30 nM. As seen in the representative action potentials shown in Fig. 1D, there were no significant changes in resting membrane potential, action potential amplitude, or rate of rise of the action potential after exposure to astemizole.

3.2. Effects of a calcium channel blocker on action potentials and ion currents recorded from hiPSC cardiomyocytes

Nifedipine, a Cav1.2 calcium channel blocker, was used to measure the sensitivity of hiPSC-CM to detect calcium channel mediated blockade (Sheng et al., 2012). The data in Table 1 and Fig. 3 demonstrate that acute exposure of hiPSC-CM to nifedipine significantly decreased APD_{90} by 10 ± 5% at 0.01 μM, 23 ± 11% at 0.03 μM, 34 ± 14% at 0.1 μM, and 50 ± 16% at 0.30 μM nifedipine. Nifedipine also significantly decreased APD_{60} by 13 ± 5% at 0.01 μM, 21 ± 12% at 0.03 μM, 38 ± 14% at 0.1 μM, and 56 ± 15% at 0.30 μM nifedipine. Significant decreases in resting membrane potential also were observed after exposure to 0.1 μM or higher concentrations of nifedipine. No significant decreases in the action potential amplitude or the rate of rise of the action potential were observed after exposure to nifedipine.

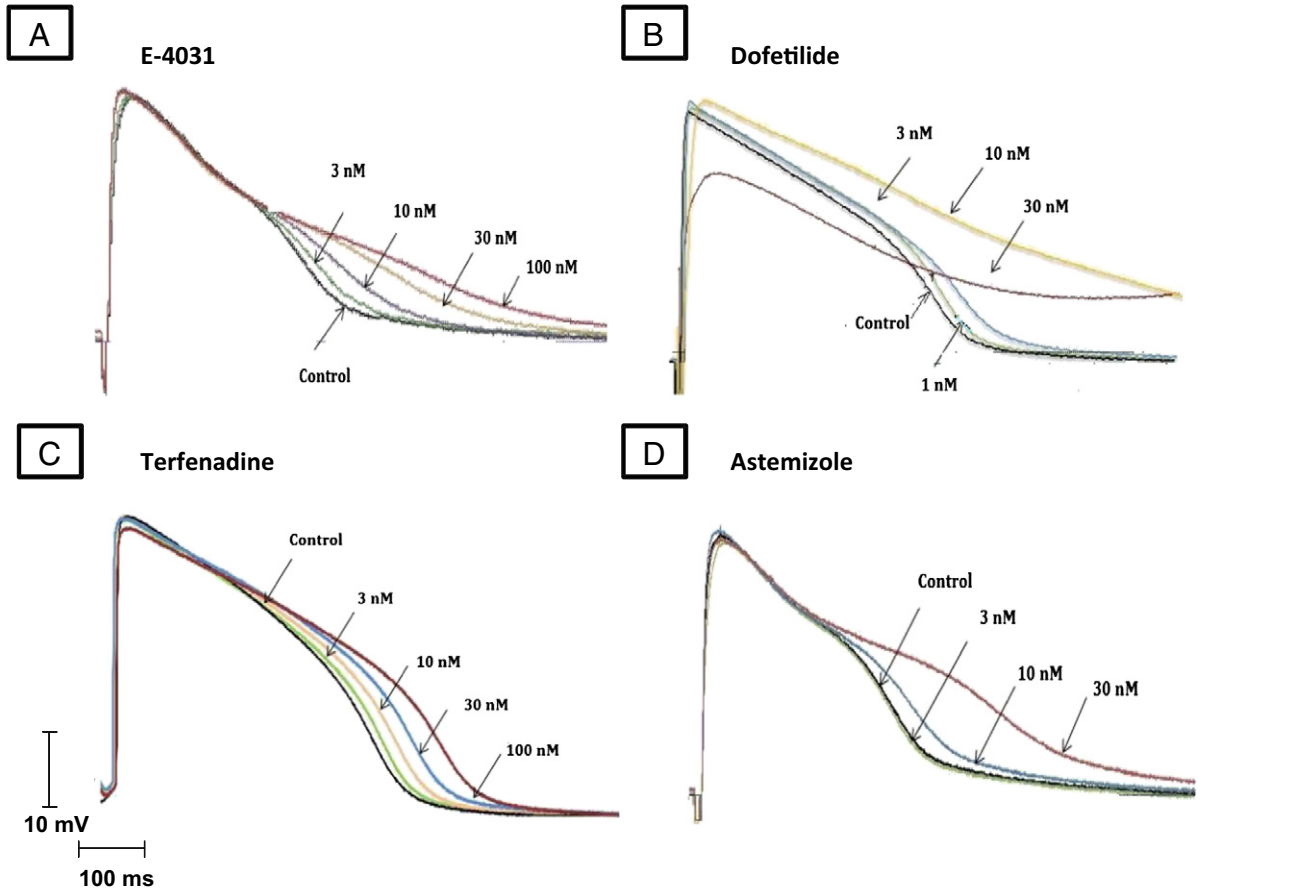

Fig. 1. E-4031, dofetilide, terfenadine and astemizole increased ventricular action potential duration. A. Increasing concentrations of E-4031 were applied to a hiPSC-CM. Records are superimposed between Control (black) and 3 (green), 10 (gray), 30 (yellow), and 100 nM E-4031 (red). B. Increasing concentrations of dofetilide were applied to a hiPSC-CM. Records are superimposed between Control (black) and 1 (green), 3 (blue), 10 (yellow), and 30 nM dofetilide (red). C. Increasing concentrations of terfenadine were applied to a hiPSC-CM. Records are superimposed between Control (black) and 3 (green), 10 (yellow), 30 (blue), and 100 nM terfenadine (dark red). D. Increasing concentrations of astemizole were applied to a hiPSC-CM. Records are superimposed between Control (black) and 3 (green), 10 (blue), and 30 (red) nM astemizole.

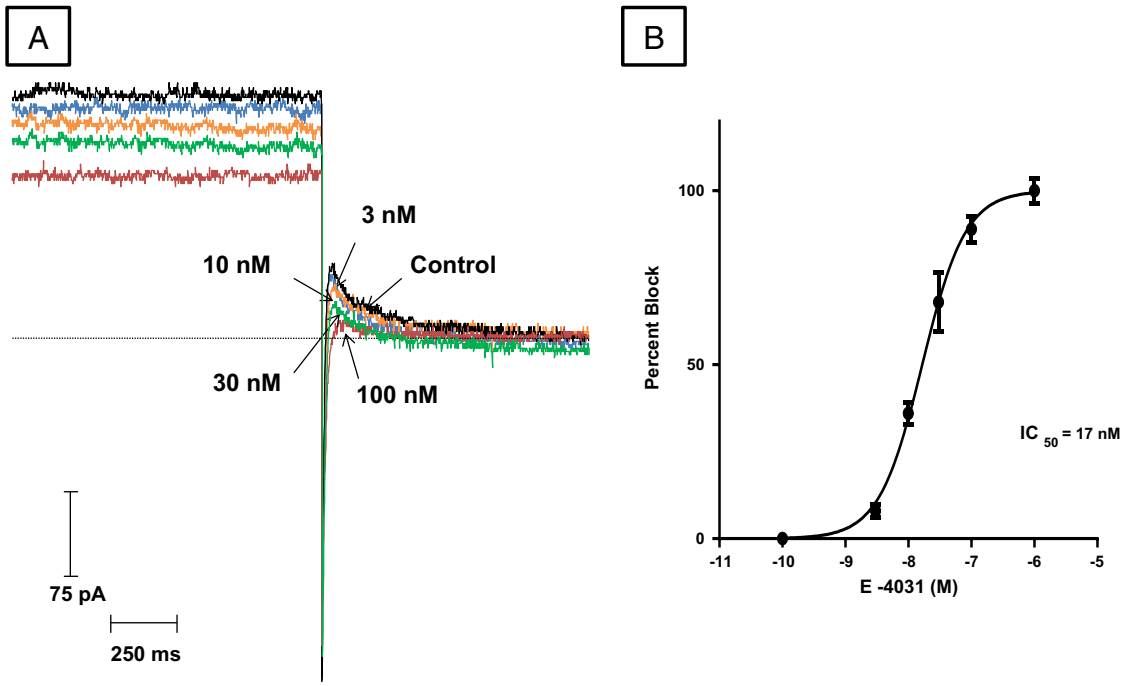

Fig. 2. E-4031 inhibits hERG currents in a dose-dependent manner in four hiPSC-CM. A. Cells were maintained at a holding potential of –80 mV and a 150 ms depolarizing pulse to +20 mV was applied at 20 s intervals. The membrane potential was then returned to −40 mv to generate outward tail currents. B. The X axis of this plot displays % hERG block during 3, 10, 30, and 100 nM E-4031. The first and last data points are a theoretical minimum and maximum inhibition (i.e., 0% and 100%, respectively). The 95% confidence intervals for the IC_{50} are 15 nM to 19 nM, and an R^2 value of 0.99.

Fig. 4A shows the dose-dependent block of calcium current from a hiPSC-CM cell following perfusion of 0.01, 0.03, 0.1, 0.3, and 1.0 μM nifedipine. Fig. 4B shows the dose response fit for the average percentage block of nifedipine in the six cells tested. The nifedipine IC_{50} was calculated to be 0.039 μM (39 nM) for block of the calcium current in these human cardiac cells. The data in Table 2 demonstrate that acute exposure of hiPSC-CM to nifedipine dose-dependently decreased the calcium current in six cells. Table 2 shows peak calcium current data normalized with respect to total cell capacitance (pA/pF) for control and nifedipine treated cells. This compensates for the difference in cell size as larger cells will have large whole cell capacitance (pF) and larger calcium currents relative to smaller cells. Dividing the peak calcium current amplitude (pA) by the whole cell capacitance (pF) compensates for the difference in cell size. Table 2 shows the channel average percent block of the calcium current for the six cells tested.

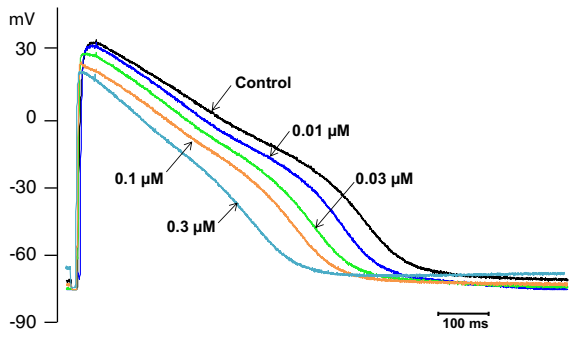

Fig. 3. Nifedipine decreases ventricular action potential duration. Increasing concentrations of nifedipine were applied to a hiPSC-CM to produce significant reductions in APD_{60} and APD_{90}. Records are superimposed between Control (black) and 0.01 (dark blue), 0.03 (green), 0.1 (orange), and 0.3 μM nifedipine (light blue).

3.3. Effects of a potassium blocker and sodium blocker on action potentials recorded from iPSC cardiomyocytes

Flecainide was selected to measure the sensitivity of hiPSC-CMs to detect simultaneous potassium channel mediated repolarization blockade and sodium channel depolarization blockade respectively. Fig. 5A shows the effect of acute exposure to increasing concentrations of flecainide (0.1, 0.3, 1.0 and 3.0 μM). A dose-dependent prolongation and subsequent triangulation of the cardiac action potential was reproducibly observed. The data in Table 1 demonstrate that acute exposure to flecainide significantly increased APD_{60} by 12 ± 11% at 3.0 μM and APD_{90} by 17 ± 6% at 1.0 μM and 30 ± 6% at 3.0 μM. Table 1 also demonstrates that these doses of flecainide reduced the rate of rise of the AP upstroke, but these decreases were statistically insignificant. Since flecainide at these doses did not have significant effects on the rate of rise of the AP, additional experiments were conducted to test the actions of 3.0 and 10 μM flecainide on the rate of rise of the action potential. As observed in the previous experiments, significant prolongation, i.e. 42 ± 12%, of the cardiac action potential was observed at 3.0 μM flecainide (Fig. 5B). The data in Table 1 also demonstrate that acute exposure to 10.0 μM flecainide significantly increased APD_{90} by 82 ± 15% and depressed the rate of rise of the AP upstroke. Flecainide at these doses did not have significant effects on resting membrane potential, or action potential amplitude.

3.4. Effects of a hERG blocker and calcium channel blocker on action potentials recorded from iPSC cardiomyocytes

Verapamil, a blocker of L-type Ca channels and hERG, was selected to measure the sensitivity of hiPSC-CMs to detect the interactions of this concomitant blockade on ventricular repolarization. Table 1 contains the average action potential parameters for seven dose response experiments following exposure to 0.03, 0.1, 0.3, 1.0, or 3.0 μM verapamil. As seen in the representative action potentials shown in Fig. 6A, there was no significant change in AP amplitude, or rate of rise of the action

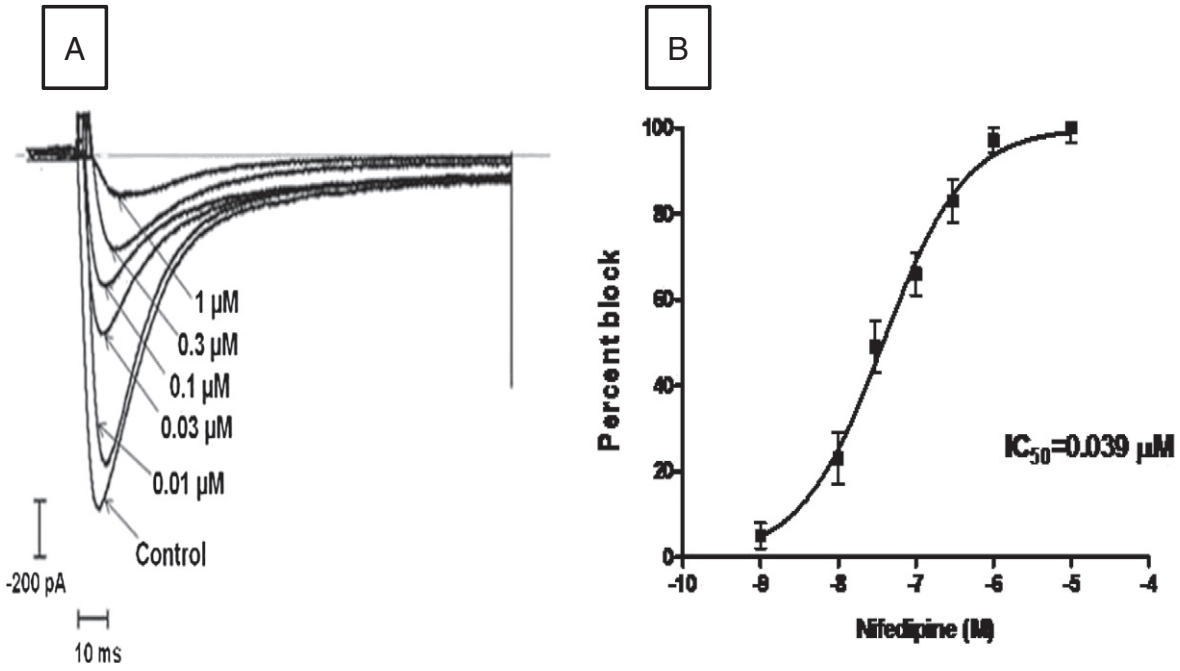

Fig. 4. Nifedipine blocks calcium current in a hiPSC-CM. A. Increasing concentrations are superimposed for control, 0.01, 0.03, 0.1, 0.3 and 1.0 μM nifedipine. B. Nifedipine dose response fit of peak calcium current block. The 95% confidence intervals for the IC_{50} are 0.030 to 0.059 μM, and 0.7 to 1.0 for the Hill slope.

potential after exposure to 0.03 or 0.1 μM verapamil. However, during exposure to 0.3, 1.0, and 3.0 μM verapamil, the resting membrane potential of the cell significantly depolarized. Figs. 6A and 7 demonstrate that administration of 0.1, 0.3, 1.0, and 3.0 μM verapamil (4-minute exposure at each concentration) also produced a marked shortening of APD_{60} with a maximum of 31% at 1 μM. A smaller, but statistically relevant, decrease in APD_{90} was observed at the lower verapamil concentrations (0.03, 0.1, and 0.3 μM) with a maximum shortening of 11% at 0.3 μM. However, at 1 μM and 3 μM verapamil, APD_{90} returned to baseline values with a prolonged decaying tail of AP repolarization. As verapamil was washed out of the tissue bath, APD_{60} and APD_{90} returned toward control, baseline levels (Fig. 6A [blue trace], Fig. 7). Following this wash step, this cell was exposed to 30 nM E-4031, producing a prolongation of APD_{90} (Fig. 6B). Another cell responded to 0.03 μM, 0.1 μM, 0.3 μM, 1 μM, and 3 μM verapamil with the characteristic triangulation and decaying tail of repolarization (Fig. 6C and 7). After verapamil washout and recovery of APD_{60} and APD_{90}, this cell was exposed to 0.3 μM nifedipine resulting in APD_{60} and APD_{90} shortening. Removal of nifedipine and subsequent application of 30 nM E-4031 prolonged both APD_{60} and APD_{90} in this cell (Fig. 6D, Fig. 7). These experiments with nifedipine and E-4031 demonstrate that the electrophysiologic changes observed with verapamil were not a consequence of current "rundown." The L-type calcium and hERG currents remained viable at the end of these experiments after previous exposure to verapamil.

3.5. Effects of a hERG channel trafficking inhibitor on action potentials recorded from iPSC cardiomyocytes

Pentamidine was selected to measure the sensitivity of hiPSC-CM to detect the inhibition of hERG ion channel trafficking.

3.6. Acute treatment

Fig. 8 shows hiPSC-CM progressively perfused with increasing concentrations of 1 μM, 3 μM, 10 μM, and 30 μM pentamidine every 4 min when stimulated at 1 Hz. The acute application of these doses of pentamidine had no effect on the action potential, although application of hERG blocker E-4031 at the end of the experiment, greatly prolonged the AP duration, demonstrating the presence of the hERG current (Fig. 8). Experiments were conducted in three additional hiPSC-CM and significant changes in action potentials were not recorded from these cells stimulated at 1 Hz (data not shown).

3.7. Chronic treatment

Unlike the hiPSC-CM receiving acute treatment with pentamidine, which did not demonstrate changes in action potential duration, marked increases in APD_{60} and APD_{90} were observed with chronic (20 h, overnight) treatment of pentamidine (i.e., >50% increase at

Table 2
Peak calcium current normalized to cell capacitance (pA/pF) in control and nifedipine containing solutions as well as percentage block of cardiac cell calcium current[a].

	1	2	3	4	5	6	Mean ± SD (pA/pF)	Mean ± SD (%)
Nifedipine								
Control	−9.20	−9.03	−9.26	−9.11	−8.77	−9.14	−9.09 ± 0.2	0
0.01 μM	−6.83	−6.58	−6.52	−6.82	−7.50	−7.54	−7.01 ± 0.4***	23 ± 6
0.03 μM	−4.31	−4.22	−4.19	−5.36	−4.84	−5.14	−4.68 ± 0.5***	49 ± 6
0.1 μM	−2.37	−3.20	−3.10	−3.36	−2.84	−3.53	−3.07 ± 0.4***	66 ± 5
0.3 μM	−0.90	−1.25	−1.48	−1.54	−2.06	−1.89	−1.52 ± 0.4***	83 ± 5
1 μM	−0.14	−0.15	−0.45	−0.21	−0.29	−0.34	−0.26 ± 0.1***	97 ± 1

[a] Data are expressed as mean ± standard deviation (X ± SD).
* Indicates change value is statistically significant (***$p \leq 0.001$).

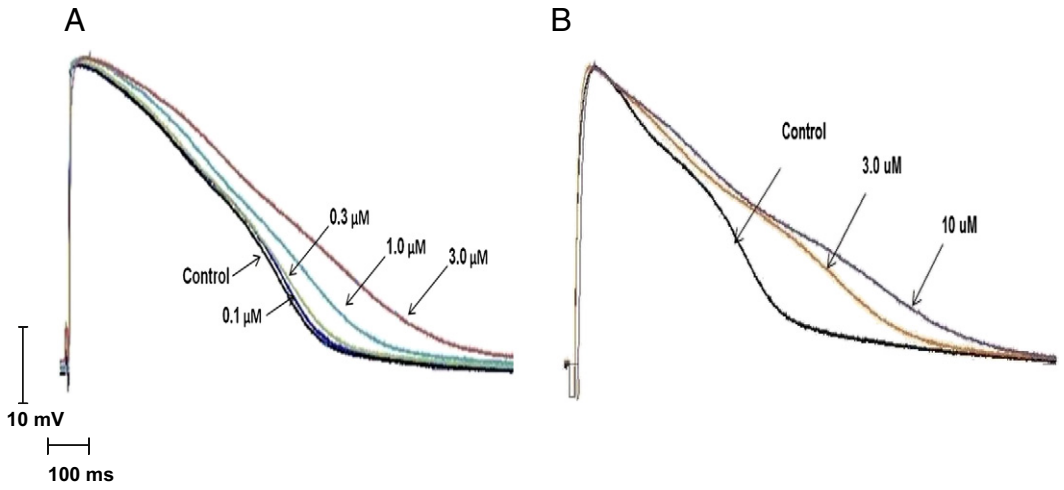

Fig. 5. Flecainide increased action potential duration and reduced the rate of rise of the action potential. Increasing concentrations of flecainide were applied to a hiPSC-CM. A. Records are superimposed between Control (black) and 0.1 μM (green), 0.3 μM (gray), 1.0 μM (blue) and 3.0 nM (red) flecainide. B. Higher concentrations of flecainide were tested to determine concentrations that caused significant inhibition of action potential rate of rise. Records are superimposed between Control (black), 3 μM (red) and 10 μM (purple) flecainide.

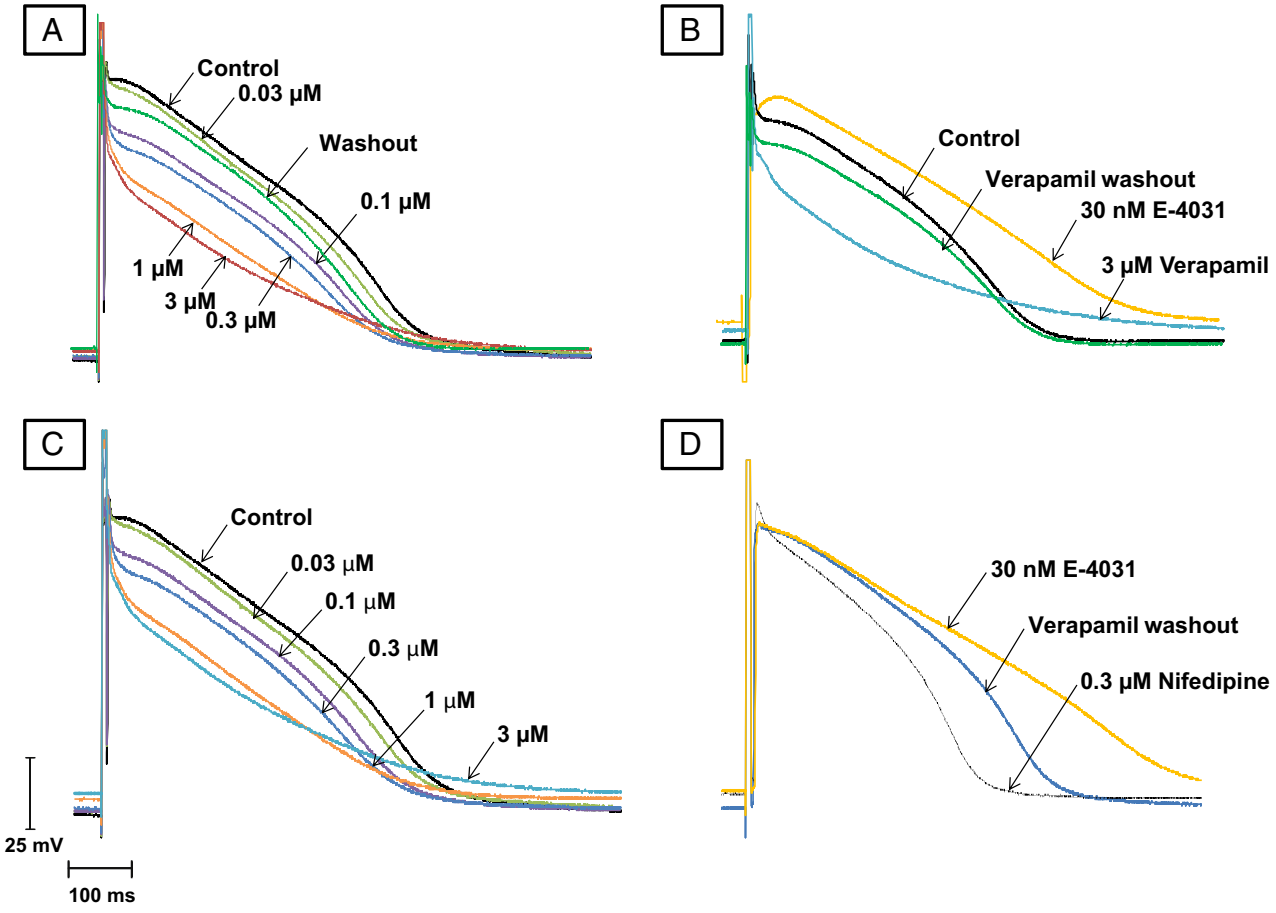

Fig. 6. A. Verapamil first shortens the APD_{60} which returns to normal duration with washout (blue trace). At 1 μM (orange trace) and 3 μM (red trace) verapamil, the APD_{90} lengthens and then returns to normal duration with washout (blue trace). Control trace (black trace) is baseline, color lines show shortening of action potential duration after exposure to 0.03, 0.1, 0.3, 1, and 3 μM verapamil. B. (Same cell as Fig. 6A) Severe shortening of action potential duration after exposure to 3 μM verapamil (blue trace), followed by verapamil washout (green trace). Final exposure of the cell to 30 nM E-4031 (yellow trace) produced lengthening of the action potential duration. C. (Another cell) Verapamil first shortens the $APD_{60,}$ after exposure to 0.03 (green trace), 0.1 (purple trace), and 0.3 μM verapamil (dark blue trace). At 1 μM (orange trace) and 3 μM (light blue trace) verapamil APD_{90} lengthens. Control trace (black trace) is baseline, color lines show shortening of action potential duration after exposure to 0.03, 0.1, 0.3, 1, and 3 μM verapamil. D. (Same cell as Fig. 6C). After washout of verapamil (blue trace), exposure to 0.3 μM nifedipine (gray trace) shortened action potential duration; subsequent washout of nifedipine restored action potential duration, and additional exposure to 30 nM E-4031 (yellow trace) significantly increased action potential duration.

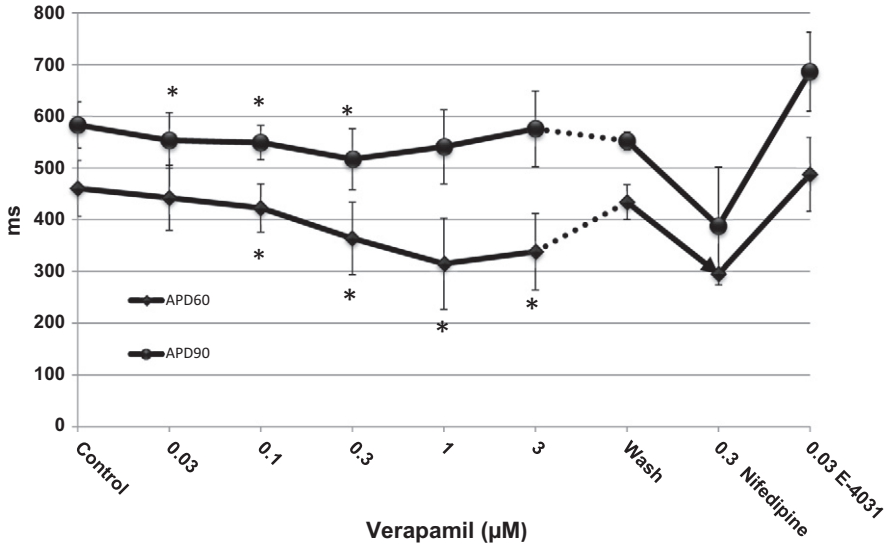

Fig. 7. Verapamil shortens the APD_{60} after exposure to 0.03, 0.1, 0.3, and 1.0 μM verapamil. Although APD_{90} also shortens at 0.03, 0.1, 0.3 μM verapamil, it returns to normal duration at 1.0 μM and 3.0 μM verapamil. Washout of verapamil returns both APD_{60} and APD_{90} to control levels. Subsequent exposure to 0.3 μM nifedipine shortens action potential duration and final exposure of the cell to 0.030 μM (30 nM) E-4031 lengthens action potential duration.

1 μM pentamidine; >400% increase at 3 μM pentamidine [Table 3]). Fig. 9B shows representative spontaneous action potentials from hiPSC-CM that had been exposed to 3 μM pentamidine for 20 h while maintained in a cell incubator at 7% CO_2. This recording was performed in the absence of pentamidine with control drug-free external solution continually perfusing the hIPSC CM. After this chronic, overnight treatment, the action potential duration was greatly prolonged when compared to control untreated hiPSC-CM (Fig. 9A), and the waveform clearly shows EADs producing arrhythmic cardiac activity. With the exception of 1 μM pentamidine, it was not possible to pace these chronically treated hiPSC-CM due to their significant AP duration prolongation. Even when paced at 1 Hz, hiPSC-CM chronically treated with 1 μM pentamidine had significantly prolonged APD_{60} and APD_{90} (Fig. 10).

4. Discussion

Ventricular repolarization time is determined by the duration of the transmembrane action potential (APD) of the ventricular myocardium and is reflected as the QT interval on the body surface electrocardiogram (Sanguinetti, Jiang, Curran, & Keating, 1995). Delayed ventricular repolarization that manifests as QT interval prolongation on the electrocardiogram is associated with the development of TdP that can result in recurrent fainting and sudden death in humans. A number of medications have been found to prolong ventricular repolarization, leading to QT prolongation (Champeroux et al., 2005; Gintant, 2011). Indeed, many non-cardiac agents, such as cisapride, terfenadine, erythromycin, and sparfloxacin, have been removed from the market or relabeled for restricted use because of their proarrhythmic potential due to prolongation of the QT interval. In addition, drug induced shortening of the QT interval also has become a concern of safety pharmacology (Lu et al., 2008; Schimpf et al., 2012; Shah, 2010). Before first testing in human subjects, regulatory guidelines currently recommend preclinical assessment of potential new drugs for QT prolongation with the resultant risk of TdP in humans (ICH S7B Oct, 2005; ICH S7B Nov, 2005).

The experiments in this study used the in vitro perforated patch-clamp preparation to study the effects of various compounds on ventricular-like action potentials recorded from hiPSC-CM, paced at 1 Hz and maintained at physiologic temperature. Additional experiments were performed to measure the effects of E-4031 and nifedipine on potassium and calcium ion currents, respectively, recorded from the hiPSC-CM.

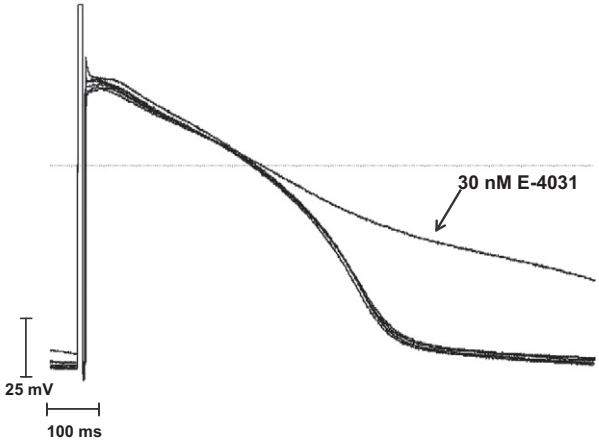

Fig. 8. Acute application of 1, 3, 10, and 30 μM pentamidine had no effect on the action potential duration recorded from a hiPSC-CM when stimulated at 1 Hz. Action potentials at each pentamidine concentration are superimposed. Application of the hERG blocker E-4031 (30 nM) at the end of the experiment in the presence of 30 μM pentamidine greatly prolonged the duration of the action potential duration, demonstrating the presence of the hERG current.

4.1. Action potential prolongation related to hERG inhibition

In clinical studies, both dofetilide and terfenadine have been shown to produce significant QT prolongation (Monahan et al., 1996; Naccarelli et al., 2003). Although E-4031 has not been tested extensively in patients, it is a well-known inhibitor of hERG producing action potential prolongation and QT prolongation (Okada, Ogawa, Sadanaga, & Mitamura, 1996; Spector, Curran, Keating, & Sanguinetti, 1996).

In the present study, ventricular-like action potentials responded to hERG inhibition by 30 nM E-4031, 30 nM astemizole, 3 nM dofetilide, or 30 nM terfenadine with significant increases in APD_{90}. These potencies are similar to previous studies with human embryonic stem cells (hESC), in which 2 nM dofetilide and 30 nM terfenadine produced significant increases in AP duration and inhibited the hERG ion channel (Peng et al., 2010; Qu et al., 2013). Likewise, in another study using hiPSC-CM, 30 nM E-4031 produced significant increases in action potential duration at 90% repolarization (Ma et al., 2011).

Table 3
Chronic effects of pentamidine on cardiac action potentials recorded from spontaneously beating hiPSC-CM[a].

	n	RMP (mV)[b, d]	APD_{60} (ms)[c, f]	APD_{90} (ms)[c, e]	APA (mV)[b, g]	Spontaneous beating frequency (Hz)
Pentamidine						
Time control	4	−57 ± 13.6	560 ± 153.4	696 ± 129.3	85 ± 13.9	0.3 ± 0.1
1 μM	3	−65 ± 19.7	832 ± 95.2*	1019 ± 106.4*	92 ± 10.7	0.4 ± 0.1
3 μM	4	−53 ± 22.4	3366 ± 586.8*	3748 ± 465.5*	81 ± 4	0.2 ± 0
10 μM	1	−52	3040	3550	41	0.22

[a] Data are expressed as mean ± standard deviation (X ± SD).
[b] RMP and APA values are in mV.
[c] APD_{60} and APD_{90} are in ms.
[d] RMP: resting membrane potential.
[e] APD_{90}: action potential duration at 90% repolarization.
[f] APD_{60}: action potential duration at 60% repolarization.
[g] APA: maximum amplitude of action potential from resting membrane potential (action potential amplitude).
* Indicates change value is statistically significant (*$p \leq 0.05$ versus time control).

The electrophysiologic actions of astemizole have not been reported with stem cell cardiomyocyte preparations. However, significant hERG blockade and action potential prolongation, i.e. 10 nM in guinea pig cells and rabbit Purkinje fibers has been reported for astemizole (Salata et al., 1995, Cavero, Mestre, Heuillet, & Roach, 1999). Similar action potential prolongation was seen in these experiments with astemizole and hiPSC-CM.

To summarize, in the present study, all hERG blockers prolonged action potential duration in hiPSC–CM at a pharmacologic dose range consistent with inhibition of the hERG ion channel in other preparations. Voltage clamp experiments with hiPSC-CM demonstrated that E-4031 prolonged action potential duration at concentrations which inhibited the hERG ion channel.

Although additional changes in cardiac electrophysiology were not observed with E-4031 or terfenadine, in the case of dofetilide, excessive prolongation of the action potential at 90% repolarization to more than 900 msec produced a significant depolarization of resting membrane potential. This was accompanied by a decrease in the amplitude of the action potential when the cells were stimulated at 1 Hz.

Despite previous reports that terfenadine does not prolong action potential in canine or porcine Purkinje fibers and rabbit ventricle, action potential prolongation with 30 nM terfenadine was observed in these experiments with hiPSC-CM (Gintant, Limberis, McDermott, Wegner, & Cox, 2001; Hondeghem, Dujardin, Hoffmann, Dumotier, & De Clerck, 2011). This dose of 30 nM terfenadine is similar to the IC_{50} of 38 nM for hERG reported by Qu et al. (2013) using HEK cells in the PatchXpress. This makes hiPSC-CM a sensitive model to assess repolarization delay with test compounds such as terfenadine.

Fexofenadine is a metabolite of terfenadine that does not prolong the QT interval in patients (Markham & Wagstaff, 1998; Pratt et al., 1999). After exposure to fexofenadine, no significant changes were observed in resting membrane potential, APD_{90}, APD_{60}, action potential amplitude, or rate of rise of the action potential (Table 1). These concentrations of fexofenadine, 0.1, 0.3, 1.0 and 3 μM, were substantially higher than the concentrations of terfenadine, i.e. 30 nM, which prolonged the action potential duration of hiPSC cardiomyocytes in this study.

In summary, selective hERG blockers such as E-4031, astemizole, dofetilide, or terfenadine tested on hiPSC-CM demonstrated a significant, specific prolongation of APD_{90} at concentrations that directly block the hERG current. As shown in Table 4, the concentrations of these drugs which significantly prolonged action potential duration in hiPSC-CM are similar to the plasma concentrations which produced QT prolongation in clinical trials. This demonstrates the ability of hiPSC-CM as an assay predictive of QT prolongation.

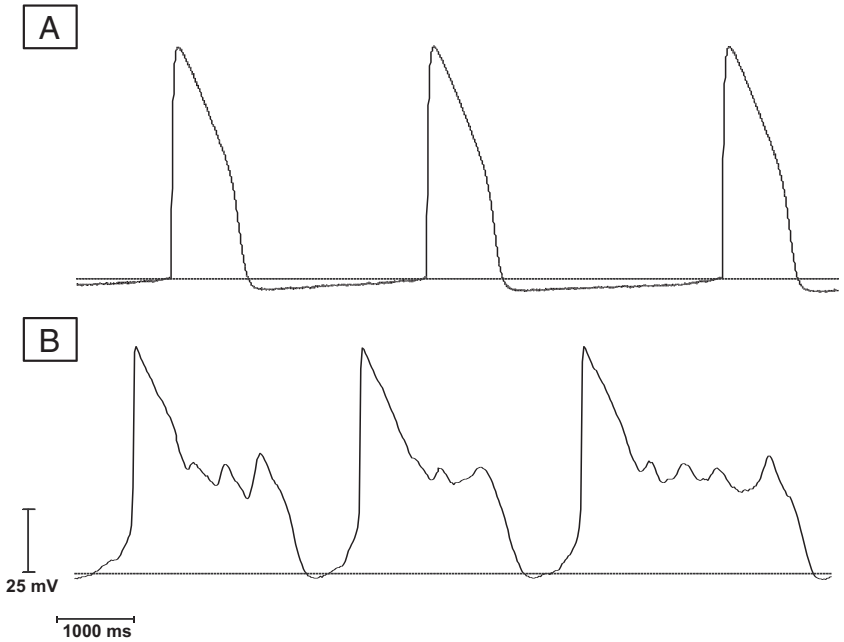

Fig. 9. Chronic application of pentamidine greatly prolonged the duration of the hiPSC-CM. A. Upper trace shows recording from time control hiPSC-CM spontaneously firing. The hiPSC-CM is depolarized and paced from a distant spontaneously beating heart cell. B. Lower trace shows recording from hiPSC-CM treated with 3 μM pentamidine for 20 h. Although the pentamidine has been removed from the perfusion solution, these hiPSC-CM are depolarized and spontaneously beating. Severe action potential prolongation and EADs are evident with each beat.

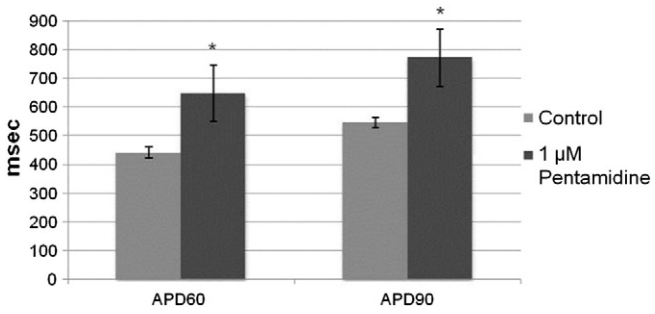

Fig. 10. Chronic application of pentamidine greatly prolonged the APD_{60} and APD_{90} duration of the hiPSC-CM. Control is from time matched hiPSC-CM paced at 1 Hz not exposed to pentamidine. Chronic (20 h, overnight) pentamidine treatment, under these 1 Hz pacing conditions, significantly increased APD_{60} and APD_{90}.

4.2. Action potential shortening related to calcium channel inhibition

Nifedipine is a calcium channel inhibitor which disrupts the movement of calcium through the calcium channel ICa-L (Cav1.2). This is the voltage gated calcium channel which maintains the plateau of the cardiac action potential at approximately 60% repolarization of action potential duration (Zhang, Zhou, Gong, Makielski, & January, 1999). The experiments in this study demonstrate the ability of 0.01 μM (10 nM) nifedipine to significantly shorten the duration of the action potential duration at 60% and 90% repolarization. This potency is similar to the previous study with hESC myocytes in which 0.03 μM (30 nM) nifedipine produced significant decreases in action potential duration (Peng et al., 2010). In comparison, canine Purkinje fibers only show significant APD_{60} and APD_{90} shortening at high concentrations of nifedipine, i.e., 100 nM.

In summary, nifedipine, a selective calcium channel blocker, demonstrated a significant shortening of APD_{60} and APD_{90} at therapeutic concentrations. Voltage clamp experiments demonstrated that nifedipine shortened action potential duration at concentrations which inhibited the calcium (Cav1.2) ion channel.

4.3. Action potential changes related to sodium and hERG inhibition

Measurement of action potential parameters identifies the risk for test compound-induced inhibition of ventricular depolarization and repolarization. As a physiologic parameter, action potential duration is the net result of many interdependent membrane ion channels. One concern behind the concomitant measurement of these parameters is that despite a block of multiple ion channels, action potential duration may not change significantly. This would be predictive of no prolongation of QT interval or no cardiac arrhythmias (Redfern et al., 2003).

Unlike the compounds discussed above, flecainide is a "mixed" ion channel blocker, with significant block of hERG potassium and sodium channels. Flecainide, i.e., 4–10 μM blocks hERG currents in HEK293 cells, feline and guinea pig ventricular myocytes (Paul, Witchel, & Hancox, 2002; Follmer & Colatsky, 1990). Similar actions resulting in an action potential prolongation at a heart rate of 1 Hz were seen in the present study with at 1 μM flecainide.

Flecainide, 10 μM, also blocks the fast inward sodium current (I_{Na}), causing the phase 0 depolarization of the cardiac action potential to decrease resulting in a decreased rate of rise of the action potential (Ducroq, Printemps, Guilbot, Salvetat, & Le Grand, 2007). Although resting membrane potential, action potential amplitude, and action potential upstroke velocity were not reported for hESM by Qu, Y. et al., they concluded that hESM are less sensitive for detecting sodium channel blockers (2013). This is consistent with the depolarized resting membrane potential and diminished sodium current reported in hESM (Peng et al., 2010). However, in the present study, 10 μM flecainide produced a significant block of the action potential rate of rise while simultaneously prolonging the duration of the action potential.

In summary, flecainide, a compound with sodium channel and hERG channel blocking actions lengthened the APD_{90} and depressed the upstroke of the action potential in hiPSC-CM. These actions are consistent with the electrophysiologic actions of flecainide seen in the clinic and they demonstrate the ability of hiPSC-CM action potentials to detect drug-induced actions on different ion channels (Salerno et al, 1986).

Table 4
Comparison of drug concentrations that induced action potential prolongation on hi-IPSC-CM and in vivo QT intervals.

Compound	hiPSC CM action potential actions	In vivo QT actions
E-4031	30 nM, APD +	12–27 nM, QT + Nakamura et al. (2014)
Dofetilide	3 nM, APD +	4–9 nM, QT + De molis et al. (1996)
Terfenadine	30 nM, APD +	90 nm, QT + Monahan et al. (1996)
Astemizole	30 nM, APD +	16 nM, QT + Tabo et al. (2008)

APD + — prolongation of action potential duration.
QT + — prolongation of QT interval.

4.4. Action potential changes related to calcium and hERG channel inhibition

Verapamil, a blocker of L-type Ca channels (Cav1.2), the voltage gated calcium channel which maintains the plateau of the cardiac action potential prior to recovery of the cardiac action potential, and hERG, the potassium channel responsible for the recovery of the cardiac action potential, was selected to measure the sensitivity of hiPSC-CMs to detect the interactions of this dual blockade on ventricular repolarization (Zhang et al., 1999). The expectation behind this measurement is that with a simultaneous block of these ion channels, action potential duration will not change significantly with no potential prolongation of QT interval or clinical cardiac arrhythmias (Redfern et al., 2003).

In the present study, ventricular-like action potentials responded to calcium channel ICa-L (Cav1.2) inhibition by verapamil first with a significant shortening of APD_{60} and APD_{90}, despite the presence of hERG blockade. APD_{90} shortened in a dose-dependent manner until a compensating increase in APD_{90}, presumably via hERG blockade, was observed at higher doses of 1 μM verapamil.

In summary, verapamil, a compound with calcium channel and hERG channel blocking actions, initially shortened the APD_{60} and APD_{90}. At higher concentrations, APD_{90} lengthened to control levels. This is consistent with the clinical data showing no significant QT prolongation or TdP with clinical application of verapamil (Aiba et al., 2005; Kannankeril, Roden, & Darbar, 2010).

4.5. Pentamidine

Although direct blockade of the hERG channel can lead to QT prolongation and TdP, disruption of hERG protein trafficking to the cell surface with pentamidine producing reduced expression of functional ion channels also can lead to QT prolongation (Kuryshev et al., 2005). As seen in the representative action potential shown in Fig. 8, there was no change in the ventricular-like action potentials after all acute doses of pentamidine. However, following chronic exposure to clinically relevant peak serum levels of pentamidine (i.e, 1 to 5 μM), a significant increase in AP duration was observed in hiPSC-CM (Lidman, Bronner, Gustafsson, & Rombo, 1994). This substantial increase in action potential duration was accompanied by EADs. These signs of proarrhythmia have not previously been reported for hiPSC-CM with pentamidine. These actions are consistent with the clinical actions of pentamidine to produce QT prolongation and arrhythmias after chronic pentamidine administration to patients (Lidman et al., 1994).

4.6. Limitations of our study

This initial analysis of hiPSC-CM as a tissue for potential safety pharmacology assay of drug effects on cardiac action potential electrophysiology is limited by the number of compounds tested in this study. A larger set of positive, false positive and false negative compounds is necessary to establish hiPSC-CM as a validated model of safety pharmacology.

A variable which must be controlled in the use of these hiPSC-CM is the electrophysiologic variability seen with these cells. We selected a phenotypic subset of ventricular-like hiPSC-CM, but future work with these tissues, will include a development of a more "consistent" phenotype.

It must be noted that the experiments in this report used relatively large ½ log incremental concentrations of test agent. This range of doses provides a safety pharmacology assessment of the ion channel targets of a test compound. This does not provide a careful mechanistic evaluation of the electrophysiologic mechanism(s) of drug actions. Additional experiments at therapeutic concentrations are necessary to determine the exact electrophysiologic mechanisms of therapy; such concentrations possibly would possibly be within a log range of test doses.

Larger sets of compounds have been evaluated with hiPSC-CM in high-throughput systems with less electrophysiologic detail (Harris et al., 2013; Navarrete et al., 2013). While solely measuring field potential duration, external electrical signals of action potential duration, these studies also have demonstrated the ability of hiPSC-CM to evaluate safety pharmacology of reference compounds in regard to QT prolongation. In order to validate such high-throughput assays, it will be necessary to insure the use of ventricular-like hiPSC-CM.

4.7. Conclusions

This study demonstrates the interaction of various ion channels on cardiac repolarization in hiPSC-CM. In addition, it provides physiologically relevant pharmacologic testing of hiPSC-CM and minimizes the in vivo diffusion barriers, or pharmacokinetic/metabolism factors, that may prevent a test compound from reaching its site(s) of action. The action potentials recorded in this study showed sensitivity of hiPSC-CM to multiple drugs, including inhibitors of cardiac potassium, sodium and/or calcium ion currents. In addition, chronic treatment with pentamidine, a known inhibitor of protein trafficking, caused significant prolongation of action potential duration with resultant EADs at a concentration similar to that seen in patients where pentamidine induced arrhythmia has been observed. These experiments show the value of hiPSC-CM action potentials to measure the interactions of the various ion channels and avoid the limitations of using several single ion channel assays in a noncardiac tissue.

Conflict of interest statement

All of the authors state that there are no conflicts of interest.

Sources of funding

At the time that these experiments were conducted, all the authors were employees of Ionic Transport Assays. All cells and materials used in the experiments in this report were purchased by Ionic TransportAssays from commercial vendors.

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