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Original article

The action potential and comparative pharmacology of stem cell-derived human cardiomyocytes

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ABSTRACT

Introduction: The cardiac action potential (CAP) of stem cell-derived human cardiomyocytes (SC-hCMs) is potentially the most powerful preclinical biomarker for cardiac safety and efficacy in humans. Our experiments tested this hypothesis by examining the CAP and relevant pharmacology of these cells. **Methods:** The electrophysiological and pharmacological profiles of SC-hCMs were compared to rabbit and canine Purkinje fibers (PFs). Ventricular SC-hCMs provided the dominant electrophysiological phenotype (~82%) in a population of ventricular, atrial and nodal cardiomyocytes (CMs). The effects of reference compounds were measured in SC-hCMs using perforated patch, current clamp recording. Selective inhibitors of I_{Kr} , I_{Ks} , $I_{Ca,L}$ and I_{Na} , and norepinephrine (NE), were tested on SC-hCM action potentials (APs). **Results:** AP prolongation was observed upon exposure to hERG channel blockers (terfenadine, quinidine, cisapride, sotalolol, E-4031 and verapamil), with significantly shorter latencies than in PF assays. For the torsadogenic compounds, terfenadine and quinidine, SC-hCM AP prolongation occurred at significantly lower concentrations than in canine or rabbit PF APs. Moreover, the I_{Ks} blocker chromanol 293B prolonged APs from SC-hCMs, whereas both rabbit and canine PF assays are insensitive to I_{Ks} blockers in the absence of adrenergic preconditioning. Early afterdepolarizations (EADs) were induced by 100 nM E-4031 and 100 nM cisapride in the SC-hCM assay, but not in the canine or rabbit PF assay. Selective inhibition of I_{Na} and $I_{Ca,L}$ slowed V_{max} and shortened AP duration, respectively. NE prolonged the AP duration of SC-hCMs. **Discussion:** The CAP of SC-hCMs has been validated as a powerful preclinical biomarker for cardiac safety and efficacy. In addition to its human nature, the SC-hCM AP assay removes diffusion delays, reduces test compound consumption, demonstrates an overall pharmacological sensitivity that is greater than conventional rabbit or canine PF assays, and accurately predicts cardiac risk of known torsadogenic compounds.

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1. Introduction

Torsade de pointes (TdP) is a serious ventricular arrhythmia that can lead to sudden cardiac death. Drug-induced prolongation of the electrocardiographic QT interval can lead to the delays in ventricular repolarization that induce TdP, and is a leading cause of adverse clinical events and withdrawal of drugs (e.g., antipsychotics, anti-histamines, and fluoroquinolone antibiotics) from the market (Brown & Rampe, 2000; Irish Medicines Board, 2002). Therefore, pharmaceutical companies and regulatory agencies view preclinical ventricular repolarization assays as essential for detection of cardiac risk.

The International Conference of Harmonisation S7B guidance outlines a preclinical testing strategy including an *in vitro* I_{Kr} assay and an *in vivo* QT assay in non-rodent animals (typically telemetered canines). However, neither method is free of false positives and negatives (Redfern et al., 2003; Dumotier, Deurinck, Yang, Traebert, & Suter, 2008;

Gintant, Su, Martin & Cox, 2006). Consequently the S7B guidance suggests follow-up studies that may provide greater depth of understanding regarding test substance repolarization risk or QT interval prolongation risk.

One of the most common follow-up assays utilizes an *ex-vivo* Purkinje fiber (PF) preparation. The PF preparation allows detailed electrical measurement of action potential (AP) parameters, with high resolution of all four phases. The PF assay, like preclinical *in vivo* QT assessment, suffers from the limitation of interspecies variability. For example, it is well-established that in the canine PF assay, the AP is insensitive to the specific hERG blocker terfenadine, whereas the rabbit PF AP assay demonstrates terfenadine sensitivity, although only at concentrations ~25-fold higher than the IC_{50} value observed for heterologously expressed hERG channels (Table 1, however see Masumiya et al., 2004). Importantly, the human QT interval is also highly sensitive to terfenadine, and the once widely-prescribed terfenadine brand, Seldane®, was withdrawn from the market due to clinical association with TdP (Brown & Rampe, 2000).

The recent commercial availability of stem cell-derived human cardiomyocytes (SC-hCMs) provides an exciting opportunity to assess

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Table 1
Summary table: comparative pharmacology.

Compound	Rabbit PF		Canine PF		SC-hCM		hERG IC ₅₀ ^a (μ M)
	Positive ^b	Statistical significance ^c	Positive	Statistical significance	Positive	Statistical significance	
Terfenadine	1 μ M	0.1 μ M	N/A	N/A	0.03 μ M	0.01 μ M	0.004
Quinidine	1 μ M	1 μ M	1 μ M	1 μ M	0.3 μ M	0.1 μ M	0.830
Cisapride	0.1 μ M	0.1 μ M	0.1 μ M	0.1 μ M	0.01 μ M	0.01 μ M	0.026
Sotalol	10 μ M	1 μ M	100 μ M	100 μ M	10 μ M	3 μ M	268
Verapamil	10 μ M	>10 μ M	1 μ M	1 μ M	1 μ M	1 μ M	0.125
Chromanol 293B	N/A ^d	N/A	N/A	N/A	300 μ M	100 μ M	10.7 ^e
E-4031	N/A	N/A	0.01 μ M	0.1 μ M	0.01 μ M	0.003 μ M	N/A
Nifedepine	N/A	N/A	>10 micromolar	>10 micromolar	0.03 μ M	0.03 μ M	N/A

^a Values determined under manual patch clamp using hERG ion channels heterologously expressed in HEK293 cells.

^b Positive effects were defined as >10% change in APD₉₀ at the indicated concentration.

^c Significant difference ($p < 0.05$) at the indicated concentration when compared to vehicle control.

^d Data is not available.

^e KvLQT/mink IC₅₀, all other values are hERG IC₅₀.

AP parameters in human heart cells. Quoting the S7B guidance: “Novel ion channel activity assays can be useful in preliminary screening of test substances to identify lead candidates. It is important to demonstrate concordance between conventional and new technologies before adopting new technologies for regulatory purposes.” In light of this guidance, there were two goals in this study: first, to develop an SC-hCM-based AP assay and assess the utility of the assay as a screening tool; second, to compare the pharmacological response of the SC-hCM AP assay to the conventional and industry-standard canine and rabbit PF AP assays.

2. Methods

2.1. Preparation and cell culture, SC-hCM

SC-hCMs were generated at Geron Corporation (Menlo Park, CA, USA) from the human embryonic stem cell line H7 by sequential treatment with activin A and BMP 4 essentially as described in Laflamme et al. (2007). ChanTest obtained SC-hCMs from Geron Corporation and maintained them in culture (37 °C, 5% CO₂) for up to 6 weeks before dispersal. Culture media consisted of GIBCO™ RPMI Medium 1640 supplemented with GIBCO™ B-27 Serum-Free Supplement (Invitrogen Corporation, Carlsbad, CA, USA), and was exchanged every other day. Cells for electrophysiological recording were plated onto Matrigel™-coated 35-mm plastic dishes (Matrigel™ preparation per Manufacturer instructions, BD Biosciences, San Jose, CA, USA) at a density of approximately 1000 cells/cm². Cells in stock plates were dispersed by sequential 20-minute incubations first with Versene (Gibco 15040, Invitrogen Corporation, Carlsbad, CA, USA), followed by Accutase (Innovative Cell Technologies, Inc, San Diego, CA), and then multiple 20-minute incubations with Worthington collagenase type-2 (Worthington Biochemical Corporation, Lakewood, NJ, USA) (2 mg/mL prepared in GIBCO™ RPMI Medium 1640) with mild trituration. Cells were placed in a 37 °C incubator between media exchanges. After each 20-minute exposure to collagenase, suspended cells were collected into a 15 mL polystyrene conical tube, fresh collagenase was added to the stock plate, and the process repeated until ~80–90% of cells were collected. Cells were then centrifuged at 300 rpm for 15 min (Thermo Scientific CL10 Centrifuge, OG26/1 rotor), resuspended in GIBCO™ RPMI Medium 1640 supplemented with GIBCO™ B-27 Serum-Free Supplement and plated onto 35 mm cell culture dishes until use for electrophysiological recordings.

2.2. PF isolation

Canine PFs were excised from adult ventricles by standard methods (Gintant, Limberis, McDermott, Wegner, & Cox, 2001). Purpose-bred beagle dogs (8 to 20 kg, Marshal Farms USA Inc. NY) were housed in AAALAC accredited facilities at Northeastern Ohio Universities College

of Medicine (NEOUCOM), Rootstown, OH. The weight, date of birth, and sex of each animal were recorded and documented in the study file. On the day of testing, a dog was anesthetized with sodium pentothal and heparinized. The heart was rapidly removed and placed in chilled, oxygenated, storage solution (standard PFT supplemented with 8 mM KCl) and transported to ChanTest on wet ice. Free-running Purkinje fibers from both ventricles were removed along with their muscle attachments by the following method: The heart was submerged in a pre-chilled and pre-gassed (95% O₂ and 5% CO₂) Tyrode's solution which was placed on ice and continuously oxygenated throughout the dissection. The atria were removed by cutting around the atrio-ventricular groove. The left ventricular free-wall was cut near the center in the base to apex direction, and the ventricle carefully spread open with minimal stretching. The papillary muscles were located and their trabecular connections severed. The largest, minimally branched Purkinje fibers were removed by cutting segments of muscle ends using fine dissection scissors, followed by careful transfer to gassed (95% O₂, 5% CO₂) storage Tyrode's solution (4 mM KCl, room temperature). Two or more fibers were obtained from the left ventricle. The right ventricular free-wall was cut in a similar manner to the left ventricular free-wall, except that the cut was offset toward the septum to avoid fiber damage. One or two fibers from the right ventricle were removed and placed in storage Tyrode's at room temperature until use. Under these conditions, the Purkinje fibers generally remained healthy for at least 8 h.

Rabbit PFs were isolated and prepared by the following procedures. Atria were separated from the ventricles by cutting around the atrio-ventricular groove. The right ventricle was isolated by cutting the right ventricle free-wall along the inter-ventricular groove from the base to apex. With minimal stretching, the right ventricle free-wall was released by cutting the tricuspid valve, all trabecular connections, and free-running Purkinje fibers at their attachment to the septum. The freed right ventricle was transferred with a spatula to gassed (95% O₂, 5% CO₂) storage Tyrode's solution (4 mM KCl, room temperature). To expose Purkinje fibers from the left ventricle, the septum was cut along the ventral edge from the base to apex or the left ventricle was cut in half by cutting the left ventricular free-wall and the septum along the ventral edge in the base to apex direction. The open left ventricle was carefully transferred with a spatula to the storage Tyrode's solution. Under these conditions, the Purkinje fibers generally remained healthy for 6–8 h. Purkinje fibers from the right ventricle were not regularly used for experiments.

2.3. Formulations

All chemicals used in solution preparation were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise noted and were of ACS reagent grade purity or higher. Stock solutions of the test articles were

prepared either in dimethyl sulfoxide (DMSO) or in water and stored frozen, unless otherwise specified. Test article concentrations were prepared fresh daily by diluting stock solutions into either PF Tyrodes (PFT) solution (for canine PF assays) and rabbit PF Tyrodes (rPFT) solution (for rabbit PF assays), or HEPES-buffered physiological saline (HB-PS) solution (for the SC-hCM assay). HB-PS was composed of (in mM): NaCl, 137; KCl, 4.0; CaCl₂, 1.8; MgCl₂, 1; HEPES, 10; Glucose, 10; pH adjusted to 7.4 with NaOH (prepared weekly and refrigerated until use). PFT was composed of (in mM): NaCl, 131; KCl, 4.0; CaCl₂, 2.0; MgCl₂, 0.5; NaHCO₃, 18.0; NaH₂PO₄, 1.8; Glucose, 5.5. rPFT was composed of (in mM): NaCl, 131; KCl, 5.4; CaCl₂, 2.0; MgCl₂, 0.5; NaHCO₃, 18.0; NaH₂PO₄, 1.8; Glucose, 5.5. The PFT and rPFT solutions were aerated with a mixture of 95% O₂ and 5% CO₂ (pH 7.2 at room temperature). Since previous results have shown that 0.3% DMSO does not affect the AP, all test and control solutions contained no more than 0.3% DMSO.

2.4. Data acquisition and analysis, SC-hCM

All SC-hCM experiments were performed at near-physiological temperature (35 ± 2 °C). Internal solution for perforated patch whole cell recording (SC-hCMs) was (composition in mM): 130 KCl, 5 MgCl₂, 5 EGTA, 10 HEPES, pH adjusted to 7.2 with KOH. The internal solution was supplemented with 240 µg/mL amphotericin B to permeabilize the cell membrane at the recording site (Rae, Cooper, Gates, & Watsky, 1991). The internal solution was prepared in batches, stored frozen, and freshly thawed on each day of use. After obtaining a gigaseal with a glass patch pipette, capacity current transients in voltage clamp mode were monitored for change in access resistance. An access resistance of less than 10 MΩ indicated achievement of a perforated patch recording. The patch clamp amplifier was switched to current clamp mode. For all experiments except for recordings of spontaneous APs (i.e., the representative E-4031 recording shown in Fig. 1B, and the representative norepinephrine recording shown in Fig. 5E), a brief current pulse (up to 3 ms duration) at approximately twice the voltage threshold for excitation was used to evoke APs at a cycle length of 1 s (1 Hz). The majority of recorded SC-hCMs showed spontaneous activity (beating cells were identified under the microscope and selected for analysis), but could be paced at 1 Hz; SC-hCMs that could not be paced at 1 Hz were not included in the analysis.

Patch clamp recording was performed using an Axopatch 200B amplifier and pCLAMP 9.2 software (MDS Inc., Union City, CA). Analog signals were low-pass filtered at 10 kHz before digitization at 50 kHz (DT3010 Data translation, Marlboro, MA), and stored on hard disk using a PC-compatible computer controlled by Notocord-Hem 3.5 software (Notocord Systems SA, Croissy, France). Data were stored on the ChanTest computer network (and backed-up nightly) for off-line analysis. Data analysis was performed using Notocord-Hem 3.5 software and Microsoft Excel 2003.

One or more test concentrations were sequentially applied (from lowest concentration to highest concentration) to SC-hCMs. Steady state was obtained for at least 30 s before application of higher concentrations. The average responses from five recorded APs were analyzed for each concentration. The following parameters were quantitated: APD₆₀ and APD₉₀ (AP duration at 60% and 90% repolarization, respectively, ms), RMP (resting membrane potential, mV), APA (AP amplitude, mV), and upstroke velocity (V_{max} , mV/ms). APD₆₀, APD₉₀ and V_{max} are presented as percent change ($\Delta\%$) from baseline at each concentration. RMP and APA data are presented as change in membrane potential (Δ mV). All data are presented as mean ± standard error of the mean (SEM), except for the vehicle control data (Table 4), which is presented as mean ± standard deviation (SD). Pharmacological effects were compared to baseline using a paired two-tailed *t*-test, with statistically significant differences defined by $p < 0.05$. AP parameter changes >10% were considered physiologically significant.

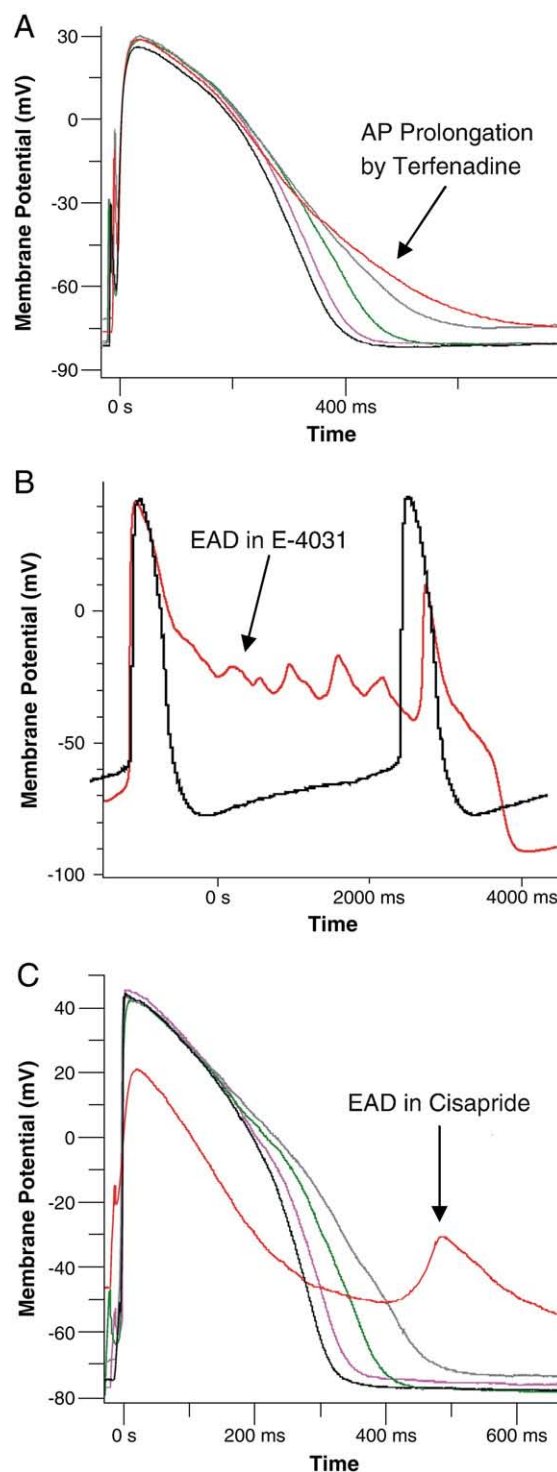


Fig. 1. AP Prolongation and torsadogenicity induced by HERG channel blockers terfenadine, E-4031, and cisapride. The selective HERG channel blocker terfenadine, which does not induce AP prolongation in canine PFs, was applied to ventricular cardiomyocytes at concentrations of 3, 10, 30 and 100 nM (purple, green, gray and red traces, respectively). Concentration-dependent AP prolongation was observed (panel A). The selective HERG channel blockers E-4031 and cisapride were applied to SC-hCMs. 100 nM E-4031 induced early afterdepolarizations (EADs), a trigger for TdP (panel B). In the example shown, the SC-hCM was spontaneously beating. Cisapride at 3, 10 and 30 nM (purple, green and gray traces, respectively) prolonged the APD, while 100 nM cisapride induced EADs (panel C).

2.5. Data acquisition and analysis, PF

All PF assays were performed at physiological temperature (37 ± 1 °C). PFs were placed in a recording chamber (approximate volume, 5 mL) mounted on a heated platform, and superfused at approximately 5 mL/min with vehicle control solution. The bath temperature was controlled using a combination of in-line solution pre-heater, chamber platform heater, and feedback temperature controller. Bath temperature was measured using a thermistor probe. Intracellular membrane potentials were recorded using conventional intracellular microelectrodes pulled from borosilicate glass capillary tubing on a micropipette puller (Sutter Instruments P-97, Novato, CA), filled with 3 M KCl solution and connected via Ag–AgCl wire to an intracellular electrometer amplifier (Warner Instruments IE 210, Hamden, CT). Membrane potential was referenced to an Ag–AgCl electrode. APs were evoked by repetitive electrical stimuli (1–3 ms duration, approximately 1.5 times threshold amplitude). A bipolar, insulated (except at the tip) platinum wire electrode was used to deliver pulses generated by a photo-isolated, electronic stimulator (Dagan Corp. S-900, Minneapolis, MN). Analog signals were low-pass filtered at 10 kHz before digitization at 50 kHz (DT3010 Data translation, Marlboro, MA), and stored on hard disk using a PC-compatible computer controlled by Notocord-HEM 3.5 software (Notocord Systems SA, Croissy, France).

Concentration-response and rate-dependence were determined by the following test procedure. PFs were paced continuously at a basic cycle length (BCL) of 2 s for at least 30 min (recovery and stabilization) before obtaining baseline AP responses. Only fibers with resting potentials more negative than -80 mV and normal AP morphology ($APD_{90} = 250$ – 450 ms for canine PF or $APD_{90} \geq 150$ ms for rabbit PF) were used (Gintant et al., 2001). Acceptable fibers were then stimulated continuously at BCL of 2 s for at least 20 min. Baseline APD rate-dependence under control conditions were measured using stimulus pulse trains consisting of approximately 50 pulses at BCL of 2, 1 and 0.5 s. After returning to BCL of 2 s, test articles were applied at the lowest concentration for at least 20 min to allow equilibration, and the stimulus trains repeated. The entire sequence (~ 20 min of equilibration followed by three cycles of stimulus trains at decreasing BCL) was repeated at cumulatively increased test article concentration. The average responses from the last five recorded APs from each stimulus train were analyzed for each test condition. Values collected at 1 s BCL in PF assays were used to compare with SC-hCM assay values. Data were analyzed as described for the SC-hCM assay.

3. Results

3.1. SC-hCMs are differentiated into ventricular, atrial, and nodal cell types

APs were recorded from a total of 125 hESCs. Spontaneously beating cells were selected for electrophysiological evaluation. An APD_{90} value of 150 ms was used to classify atrial and nodal versus ventricular type cardiomyocytes (representative recordings are shown in Fig. 2) based on hierarchical cluster analysis of an initial population (40 cells) of SC-hCMs (data not shown), and our observation that a noticeable AP plateau (Phase 2) could generally be observed in cells with APD_{90} values greater than approximately 150 ms. The presence of prominent pacemaker morphology, slow upstroke velocity and regular, rapid continuous AP activity was used to distinguish nodal from atrial type cardiomyocytes. The majority (82%) of cells were ventricular type (Table 2). Of the 23 cells with APD_{90} values < 150 ms, one cell showed clear pacemaker activity and was designated nodal type, whereas 22 cells were designated atrial type. To further characterize the cells, in addition to APD_{90} , the following parameters were quantified: RMP, APA and V_{max} (Table 2).

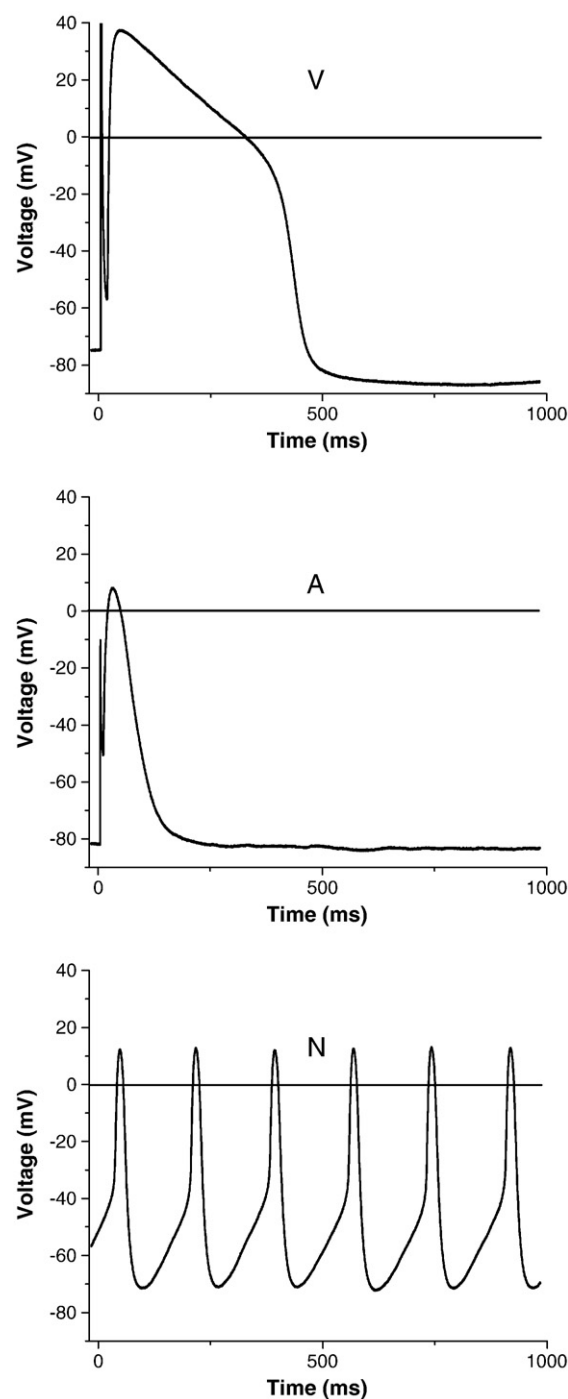


Fig. 2. Sample ventricular (V), atrial (A) and nodal (N) APs. APs were recorded from a total of 125 human stem cell-derived cardiomyocytes using perforated patch current clamp technology. An APD_{90} value of 150 ms was used to discriminate atrial and nodal from ventricular type cardiomyocytes. The majority (82%) of cells were ventricular type. Of the 23 cells with APD_{90} values < 150 ms, one cell showed clear pacemaker currents and was designated nodal type, whereas 22 cells were designated atrial type.

3.2. Comparative pharmacology, I_{Kr} (hERG) blockers

The hERG cardiac potassium channel underlies I_{Kr} and is inhibited by a wide variety of compounds. Therefore, the hERG channel blockers terfenadine, quinidine, cisapride, sotalol and E-4031 were applied to SC-hCMs and AP parameters were measured. AP durations at 60% repolarization (APD_{60}) and 90% repolarization (APD_{90}) for each test compound are presented in Table 3. For comparison, the APD_{60} and APD_{90} response to the same set of test articles was measured in rabbit

Table 2
Characterization of SC-hCMs.

Cell type	Number of cells	% Total	APD ₉₀ (ms)	V _{max} (V/s)	RMP (mV)	APA (mV)
			(Mean ± SEM)	(Mean ± SEM)	(Mean ± SEM)	(Mean ± SEM)
Ventricular	102	82	355.9 ± 12.5	12.1 ± 0.9	−73.1 ± 1.2	102.3 ± 1.6
Atrial	22	18	95.7 ± 6.1	14.5 ± 5.3	−62.7 ± 2.5	86.3 ± 3.0
Nodal	1	<1	N/A	N/A	N/A	N/A
Total number of cells (n)	125	100	125	125	125	125

or canine PFs (Table 3). APD prolongation in SC-hCMs was observed upon exposure to each of the five hERG channel blockers. Sensitivity (threshold 10% APD prolongation) of SC-hCMs was higher than observed in either the canine or rabbit PF assay for torsadogenic compounds terfenadine, quinidine and cisapride. SC-hCM sotalol sensitivity was higher than observed in canine PF, and equivalent to rabbit PF. E-4031 sensitivity in SC-hCMs and canine PFs was observed at 10 nM, though APD prolongation was markedly greater (~2-fold) for SC-hCMs compared to canine PFs. In addition, early afterdepolarizations (EADs) were induced by 100 nM E-4031 and 100 nM cisapride in the SC-hCM assay, but not in the rabbit and/or canine PF assay. The comparative pharmacology data is summarized in Table 1.

3.3. Blocking rate is faster in the SC-hCM than in the PF AP assay, and the SC-hCM assay is stable

One limitation of PF AP assays is that the PF is encased in a collagenous sheath that may present a barrier to diffusion to the majority of Purkinje cells (Sommer & Johnson, 1968). Test article access to the PF may therefore be prevented, or, at a minimum, exposure durations are necessarily long to ensure test article equilibration (≥20 min). On the other hand, SC-hCMs are isolated

single cells that can be directly exposed to test compound, and therefore are expected to show more rapid equilibration with test compounds. The onset of AP prolongation was therefore measured in the rabbit PF and SC-hCM AP assays by applying sotalol, a hERG blocker commonly used as a positive control. As shown by the representative traces in Fig. 3, the onset of APD₉₀ prolongation in response to 50 μM sotalol was significantly shorter in SC-hCMs (steady state achieved in approximately 5 min) compared to rabbit PF (steady state achieved in approximately 15 min). This same trend was observed for other AP modifiers (data not shown), despite the fact that the perfusion system flow rate was 5-fold faster in the PF assays (~5 mL/min) compared to the SC-hCM assay (~1 mL/min).

Another limitation of PF AP assays is that the AP parameters can change over the relatively long time course of pharmacological experiments. To compare AP parameter stability between PF and SC-hCM assays, APD₆₀, APD₉₀, RMP, APA, and V_{max} were measured in SC-hCMs and rabbit PFs in the absence of test substance (Table 4). Changes in the AP parameters varied relatively widely during rabbit PF vehicle control experiments over the course of a typical 60 minute PF experiment (standard deviation values ranged from 7.2 to 37.4, n = 43). In contrast, changes in AP parameters showed relatively small variance in the SC-hCM vehicle control experiments for a typical 20 minute experiment (standard deviation values ranged from 1.0 to 9.8, n = 4). For direct comparison to the SC-hCM data, the 20 minute

Table 3
AP prolongation by hERG inhibitors.

	SC-hCM				Rabbit PF				Canine PF			
	μM	N	APD ₆₀ (Δ%)	APD ₉₀ (Δ%)	μM	N	APD ₆₀ (Δ%)	APD ₉₀ (Δ%)	μM	N	APD ₆₀ (Δ%)	APD ₉₀ (Δ%)
Terfenadine	Control ^a	4	219.6 ± 46.6	327.2 ± 50.8	Control	4	251.6 ± 43.6	296.6 ± 49.8	Control	2	233.7 ± 18.6 ^b	290.4 ± 21.4 ^b
	0.003	4	0.0 ± 1.1	0.4 ± 1.3	–	–	–	–	–	–	–	–
	0.01	4	4.6 ± 2.6	8.4* ± 1.3	0.01	4	3.2 ± 2.7	1.7 ± 1.9	0.01	2	0.9 ± 1.6 ^b	0.3 ± 0.8 ^b
	0.03	4	9.0* ± 2.0	21.3* ± 2.9	0.1	4	9.9* ± 3.4	8.5* ± 2.1	0.1	2	7.1 ± 16.0 ^b	5.6 ± 11.4 ^b
	0.1	4	16.9* ± 10.5	41.2* ± 7.3	1	4	9.4* ± 3.4	18.5* ± 3.4	1	2	−0.3 ± 12.7 ^b	0.6 ± 10.1 ^b
Quinidine	Control ^a	4	215.7 ± 27.3	310.4 ± 25.8	Control	5	214.4 ± 66.2	291.2 ± 57.8	Control	4	304.0 ± 8.3	363.4 ± 6.8
	0.1	4	3.2 ± 3.1	5.2* ± 1.3	0.1	5	−2.2 ± 3.9	0.4 ± 2.4	0.1	4	0.4 ± 4.0	1.1 ± 2.1
	0.3	4	13.6* ± 3.4	25.7* ± 5.9	1	5	13.9 ± 6.2	20.4* ± 4.3	1	4	11.1* ± 3.2	12.6* ± 3.1
	1	4	29.2* ± 5.5	46.8* ± 7.1	10	5	80.2* ± 22.5	97* ± 23.9	10	4	−17.8* ± 1.1	−1.8 ± 5.2
	3	4	56.5* ± 8.6	79.9* ± 6.1	–	–	–	–	–	–	–	–
Cisapride	Control ^a	4	277.7 ± 64.7	353.9 ± 65.3	Control	4	236.7 ± 32.0	302.3 ± 23.7	Control	4	227.9 ± 17.3	276.0 ± 16.0
	0.003	4	2.7 ± 2.7	3.0 ± 2.7	0.01	4	−12.4 ± 13.3	1.6 ± 3.5	0.1	4	18.6* ± 2.3	17.6* ± 2.6
	0.01	4	7.8 ± 4.1	12.0* ± 4.8	0.1	4	36.2 ± 17	50.9* ± 16.1	1	4	33.4* ± 9.3	36.6* ± 7.8
	0.03	4	11.3 ± 5.5	30.4* ± 6.2	0.5	4	74.4 ± 34.1	124.4* ± 49.2	10	4	30.9 ± 16.3	46.1* ± 18.1
	0.1	4	−5.0 ± 17.8	21.0 ± 26.5	–	–	–	–	–	–	–	–
Sotalol	Control ^a	5	216.5 ± 21.1	300.3 ± 36.4	Control	4	161.2 ± 29.3	209.3 ± 34.3	Control	6	233.1 ± 51.7	296.1 ± 48.7
	3	4	6.5* ± 2.6	4.5* ± 0.8	1	4	3.9 ± 2.5	5.1* ± 1.9	–	–	–	–
	10	4	13.3* ± 2.2	12.2* ± 1.4	10	4	32.5* ± 3.1	30.0* ± 2.5	–	–	–	–
	30	4	20.2* ± 4.9	20.7* ± 1.4	50	380	66.0* ± 1.5	60.5* ± 1.2	100	6	51.2* ± 19.6	48.9* ± 16.7
	100	4	50.7* ± 10.5	57.2* ± 5.5	–	–	–	–	–	–	–	–
E-4031	Control ^a	4	210.6 ± 39.8	325.5 ± 36.7	–	–	–	–	Control	4	222.9 ± 9.5	280.6 ± 13.5
	0.003	4	9.8* ± 2.8	5.5* ± 0.4	–	–	–	–	0.001	4	1.8 ± 2.8	1.6 ± 2.6
	0.01	4	30.7* ± 12.3	27.4* ± 4.6	–	–	–	–	0.01	4	14.7 ± 6.6	12.9 ± 6.0
	0.03	4	48.0* ± 18.8	66.2* ± 16.8	–	–	–	–	0.1	4	49.3* ± 11.9	44.6* ± 9.4
	0.1	4	EAD	EAD	–	–	–	–	–	–	–	–

^a Control values are in ms. All other values are percent change from control.

^b Mean ± SD, all others are mean ± SEM.

* Indicates change value is statistically significant (p < 0.05).

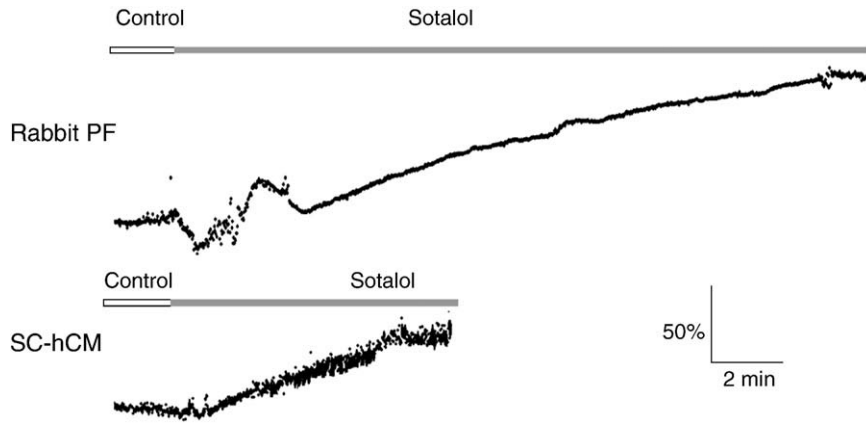


Fig. 3. Typical time course of the effect of sotalol on the AP of SC-hCM and Rabbit PF. Change of APD₉₀ during application of vehicle (control) and sotalol. The horizontal bars indicate application of the control and sotalol solutions. Scale bars define 2 min (x-axis) and 50% prolongation of the APD₉₀ (y-axis). Upper trace: rabbit PF. Lower trace: SC-hCM. Steady state response to application of 50 μM sotalol was observed after approximately 5 min in the SC-hCM assay, and after more than 15 min in the rabbit PF assay.

standard deviation values in the PF assay ranged from 7.2 to 30.8 (n=43). Representative SC-hCM and PF AP traces at various timepoints are shown in Fig. 4.

3.4. The I_{Ks} blocker chromanol 293B prolongs APD in SC-hCMs

For rabbit cardiac tissue, including PF, it is well-established that the I_{Ks} blocker chromanol 293B does not prolong the AP in the absence of adrenergic preconditioning (Lengyel et al., 2001). To test SC-hCM sensitivity to I_{Ks} inhibition, Chromanol 293B was administered at 10, 30, 100 and 300 μM. As shown in Table 5 and Fig. 5, concentration-dependent AP prolongation was observed, though the magnitude of AP prolongation was lower than observed in the presence of I_{Kr} blockers.

3.5. SC-hCMs demonstrate pharmacological sensitivity to I_{Ca,L}, I_{Na}, and multiple ion channel blockers

L-type calcium channels underlie the cardiac current I_{Ca,L} responsible for the plateau phase of the AP (Phase 2), and therefore play an important role in defining AP duration. Nifedipine, a selective blocker of I_{Ca,L}, was evaluated in the SC-hCM and canine PF assays. Shortening of the APD₆₀ and APD₉₀ was observed in SC-hCMs at all concentrations tested (0.03, 0.1, 0.3 and 1 μM, Fig. 5 and Table 5), whereas canine PFs showed >10% APD₆₀ shortening only at elevated concentrations (10 μM, Table 5).

Sodium channels are responsible for I_{Na}, the current that underlies the upstroke of the AP (Phase 0). Inhibition of I_{Na} by lidocaine, a selective Na⁺ channel blocker, and quinidine, a dual-action (Na⁺ and hERG channel) blocker, was assessed by quantifying changes in V_{max}

Table 4
AP parameters during vehicle control.

Min	N	APD ₆₀ (Δ%)	APD ₉₀ (Δ%)	RMP (ΔmV)	APA (ΔmV)	V _{max} (Δ%)
<i>SC-hCM</i>						
5	4	-0.6 ± 5.8	-3.4 ± 3.4	-0.5 ± 2.4	-4.0 ± 4.4	-0.6 ± 6.4
10	4	0.6 ± 6.4	-1.3 ± 2.6	-0.3 ± 1.4	0.6 ± 3.2	-3.4 ± 3.2
15	4	-1.0 ± 9.8	-2.5 ± 6.0	0.4 ± 1.2	-1.4 ± 2.8	1.0 ± 4.4
20	4	-0.6 ± 6.6	-0.4 ± 4.8	0.3 ± 1.0	-1.4 ± 3.8	2.3 ± 8.6
<i>Rabbit PF</i>						
20	43	3.1 ± 20.3	2.6 ± 15.7	-0.4 ± 7.2	0.3 ± 18.4	-0.5 ± 30.8
40	43	4.4 ± 30.2	3.7 ± 22.3	-0.2 ± 10.5	-0.6 ± 27.5	-0.3 ± 32.8
60	43	6.4 ± 30.8	5.1 ± 26.2	-0.1 ± 16.4	-1.5 ± 37.4	-1.7 ± 37.4

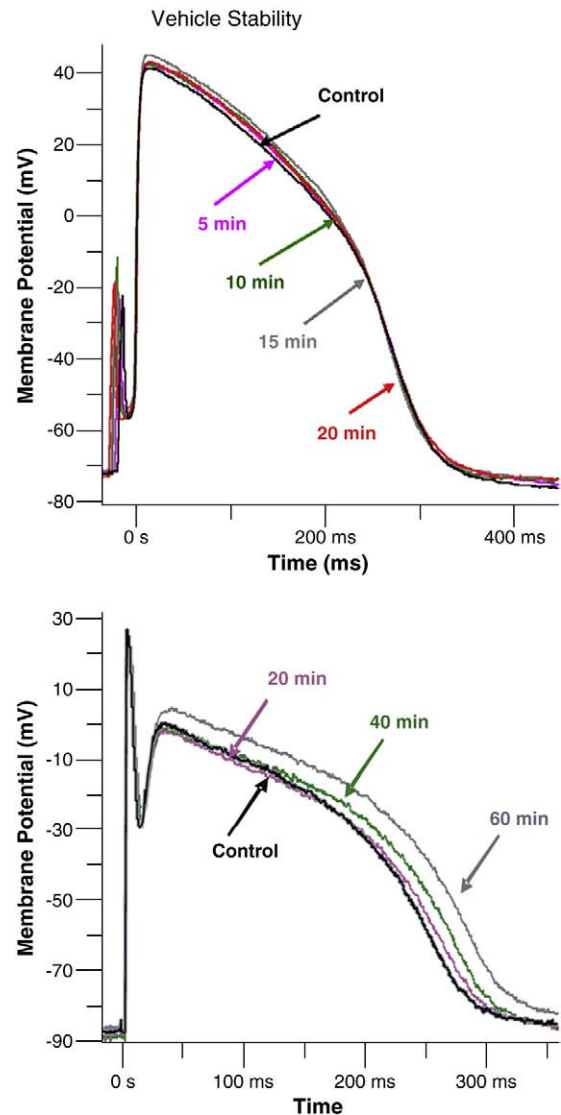


Fig. 4. Stability of SC-hCM and rabbit PF APs. Vehicle control was applied for 20 min to an SC-hCM and for 60 min to a rabbit PF. Representative traces are shown at time zero, and after 5, 10, 15 and 20 min (SC-hCM, top panel), and at time zero, and after 20, 40 and 60 min (rabbit PF, bottom panel).

Table 5AP prolongation by I_{Ks} blocker chromanol 293B, AP shortening by $I_{Ca,L}$ blocker nifedipine, and triangulation in the presence of I_{Kr} and $I_{Ca,L}$ blocker verapamil.

	SC-hCM				Rabbit PF				Canine PF			
	μM	N	APD ₆₀ ($\Delta\%$)	APD ₉₀ ($\Delta\%$)	μM	N	APD ₆₀ ($\Delta\%$)	APD ₉₀ ($\Delta\%$)	μM	N	APD ₆₀ ($\Delta\%$)	APD ₉₀ ($\Delta\%$)
Chromanol 293B	Control ^a	4	156.0 ± 26.1	226.8 ± 28.5	–	–	–	–	–	–	–	–
	10	4	4.0 ± 3.2	3.4 ± 4.4	–	–	–	–	–	–	–	–
	30	4	9.0 ± 4.6	3.3 ± 3.2	–	–	–	–	–	–	–	–
	100	4	15.0* ± 4.0	9.5* ± 2.2	–	–	–	–	–	–	–	–
	300	4	15.1* ± 3.2	14.8* ± 4.6	–	–	–	–	–	–	–	–
Nifedipine	Control ^a	4	256.2 ± 40.3	379.4 ± 36.1	–	–	–	–	Control	4	212.2 ± 9.3	272.7 ± 11.6
	0.03	4	–13.6* ± 2.0	–11.1* ± 2.7	–	–	–	–	0.1	4	–2.0 ± 1.0	–1.2 ± 1.4
	0.1	4	–19.1* ± 1.3	–16.5* ± 3.1	–	–	–	–	1	4	–7.6* ± 1.9	–2.8 ± 1.5
	0.3	4	–28.9* ± 3.9	–28.6* ± 2.8	–	–	–	–	10	4	–24* ± 4.2	–8.4 ± 5.2
	1	4	–41.0* ± 3.5	–41.3* ± 1.4	–	–	–	–	–	–	–	–
Verapamil	Control ^a	7	256.1 ± 18.8	376.6 ± 19.9	Control	4	246.3 ± 21.8	296.9 ± 22.7	Control	6	198.2 ± 5.5	248.1 ± 5.4
	0.1	7	–0.2 ± 2.1	0.6 ± 1.9	0.1	4	–12.6 ± 10.3	–4.3 ± 4.2	0.1	6	2.7 ± 1.8	3.5 ± 1.8
	1	5	–0.8 ± 2.2	15.9* ± 4.1	1	4	–9.1 ± 7.8	–0.3 ± 5.8	1	6	2.7 ± 2.7	10.2* ± 2.7
	3	5	–2.7 ± 3.1	33.1* ± 9.7	10	4	–13.3* ± 1.7	17.3 ± 7.8	10	6	–18.72 ± 9.2	4.3 ± 5.4
	10	4	–3.4 ± 3.6	54.5* ± 7.2	–	–	–	–	–	–	–	–

^a Control values are in ms. All other values are percent change from control.* Indicates change value is statistically significant ($p < 0.05$).

and APA in SC-hCMs, and in rabbit or canine PFs (Table 6). Lidocaine slowed SC-hCM AP V_{max} at 30, 100 and 300 μM , and slowed canine PF AP V_{max} at 250 μM , but not at 10 or 50 μM . Lidocaine had no effect on SC-hCM APA at any concentration, and decreased canine PF APA >10% only at 250 μM . Quinidine slowed AP V_{max} in SC-hCM, rabbit PF, and canine PF at concentrations of 0.3, 0.1 and 10 μM , respectively. APA was decreased by quinidine in the SC-hCM assay at 3 μM . No effect on APA by quinidine was observed in either PF assay.

Verapamil, an inhibitor of both hERG (I_{Kr}) and L-type calcium channels ($I_{Ca,L}$), was tested in SC-hCMs at 0.1, 1, 3 and 10 μM . Significant APD₉₀ prolongation (>10%) was observed at 1, 3, and 10 μM , whereas there was no effect on APD₆₀ at any concentration (Fig. 5, Table 5). Verapamil was tested in the rabbit and dog PF assays at 0.1, 1 and 10 μM . Significant APD₉₀ prolongation was observed in the rabbit assay at 10 μM , and in the canine PF assay at 1 μM . The magnitude of APD₉₀ prolongation was significantly greater in the SC-hCM assay versus the PF assays (maximal prolongation 54.5% for SC-hCM, versus 17.3% for rabbit PF).

3.6. SC-hCMs are sensitive to adrenergic modulation by NE

Changes in SC-hCM parameters in response to NE were assessed in spontaneously beating SC-hCMs. NE prolonged APD at concentrations of 1 and 3 μM (Fig. 5, Table 7). In the example shown, AP shortening and delayed afterdepolarizations (DADs) were observed in response to 3 μM NE (Fig. 5). The AP shortening was most likely due to rate-adaptation as in this cell, 3 μM NE increased the spontaneous beating rate.

4. Discussion

The commercial availability of SC-hCMs provides an exciting opportunity to assess the cardiac liability of drug candidates in human cardiomyocytes. However, before widespread adoption of the assay, there is a clear need for validation studies that benchmark the utility of SC-hCMs versus conventional safety pharmacology assays. While the sensitivity of SC-hCMs to hERG blockers and beta-adrenergic modulators has been established (He, Ma, Lee, Thomson, & Kamp, 2003; Mummery et al., 2003; Sartiani et al., 2007; Zhang et al., 2009), there has not been a thorough profiling of AP modulators using these cells. Moreover, the published data on SC-hCMs were obtained from various cell lines (e.g., H1, H7, and H9), and there is considerable variability in the AP profiles between these cell lines (Zhang et al., 2009). In addition to cell line-dependent and culture condition-

dependent variability in SC-hCMs, there are clear time-dependent changes in ion channel expression, which lead to changing AP profiles depending on the duration of cell culture under differentiating conditions (Sartiani et al., 2007). Therefore, we sought to characterize the H7 cell line, which will be commercially available under an agreement between Geron Corporation and GE Healthcare (Piscataway, NJ, USA). This cell line is prepared using standardized methodologies and therefore should provide consistently uniform cell populations for cardiac safety testing.

4.1. Overall pharmacological sensitivity of SC-hCMs is higher than in PF assays

The primary goal of any cardiac risk assessment assay is to detect, with the highest possible sensitivity, proarrhythmic potential of drug candidates. We have demonstrated for the first time that the SC-hCM AP assay shows a pharmacological sensitivity that is greater than conventional rabbit or canine PF assays. We have demonstrated that the SC-hCM AP assay readily detects blockers of various phases of the CAP. Phase 0 (I_{Na}) blockers lidocaine and quinidine predictably slow the AP upstroke velocity (V_{max}), the specific $I_{Ca,L}$ blocker nifedipine dramatically shortens the AP plateau (Phase 2), and repolarization (Phase 3) is delayed by each of the six I_{Kr} inhibitors we evaluated. Of particular interest, the torsadogenic compounds quinidine, terfenadine and cisapride prolong the SC-hCM AP at concentrations at least a half-log lower than the most sensitive PF assay. Moreover, blockers of I_{Ks} can be detected in the SC-hCM assay, whereas I_{Ks} blockers are not detected in rabbit cardiac tissue, including PF assays (Lengyel et al., 2001; Lu, Vlamincx, Van De Water, & Gallacher, 2005). The relatively high sensitivity may be explained by the lack of a diffusion barrier in SC-hCMs, however an alternative explanation for the difference in equilibration times is that compounds could be taken up by cytoplasmic structures that are present in PF tissue, but absent in SC-hCMs. For example, hERG IC_{50} values measured in *Xenopus* oocytes are often approximately 10-fold higher than IC_{50} values measured from mammalian cells, presumably due to differences in cellular structure (see for example Thomas et al., 2001).

Finally, SC-hCMs are good detectors of proarrhythmic events; EADs were induced by I_{Kr} blockers terfenadine, sotalol, cisapride, and E-4031, and DADs were induced by NE. Moreover, triangulation of the AP waveform was readily induced by verapamil, a blocker of I_{Kr} and $I_{Ca,L}$. Overall, the SC-hCM AP assay shows predictable sensitivity to important modulators of the CAP.

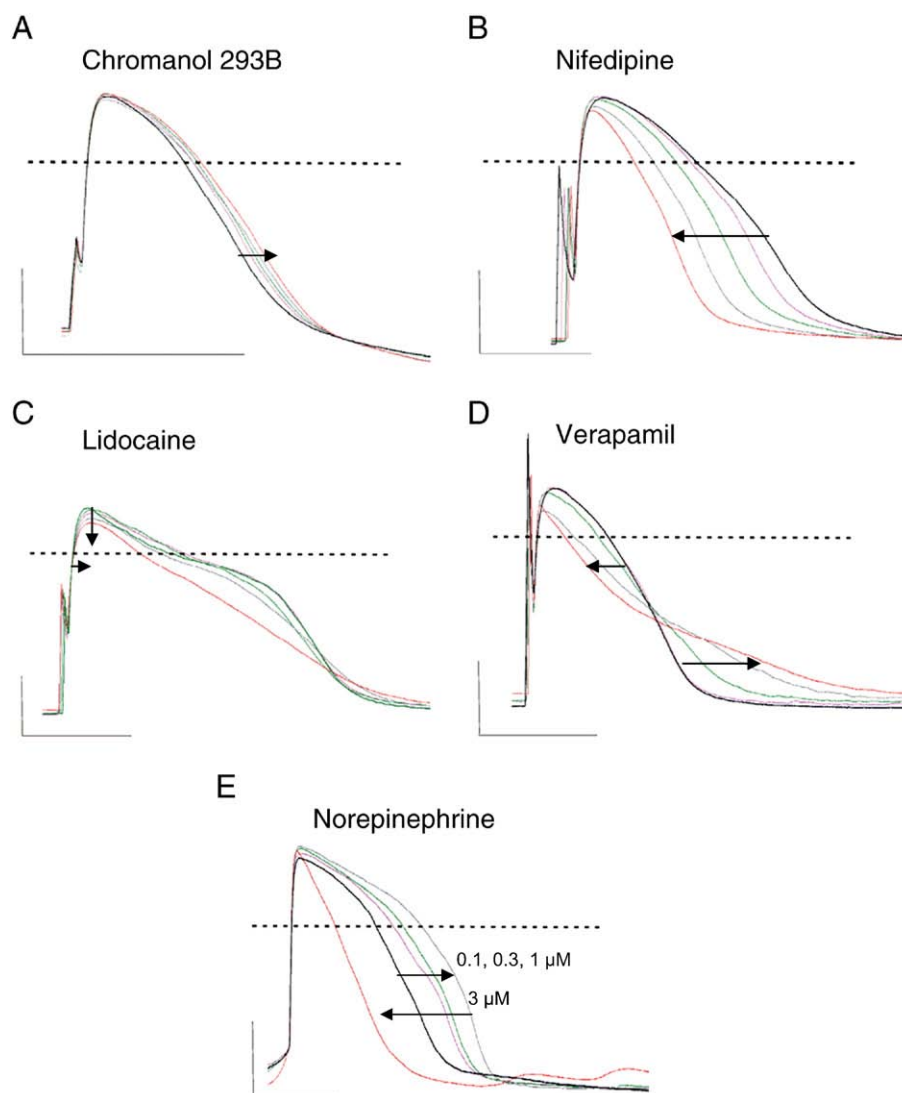


Fig. 5. Effects on the SC-hCM AP by pharmacological modulators of the CAP. Scale bars represent 200 ms (x-axis) and 40 mV (y-axis). Dashed lines indicate the 0 mV level. Black arrows indicate the direction of change induced by each compound. (A) Representative traces showing the effect of the I_{Ks} blocker Chromanol 293B on the SC-hCM AP in vehicle control and at concentrations of 10, 30, 100 and 300 μM (black, purple, green, gray and red, respectively). Concentration-dependent AP prolongation was observed. (B) Representative traces showing the effect of the L-type calcium channel blocker nifedipine on the SC-hCM AP in vehicle control and at concentrations of 0.03, 0.1, 0.3 and 1 μM (black, purple, green, gray and red, respectively). Concentration-dependent AP shortening was observed. (C) Representative traces showing the effect of the sodium channel blocker lidocaine on the SC-hCM AP in vehicle control and at concentrations of 10, 30, 100 and 300 μM (black, purple, green, gray and red, respectively). A concentration-dependent decrease in APA and V_{max} were observed. (D) Representative traces showing the effect of verapamil, a hERG and L-type calcium channel blocker, on the SC-hCM AP in vehicle control and at concentrations of 0.1, 1, 3, and 10 μM (black, purple, green, gray and red, respectively). Concentration-dependent prolongation of the APD₉₀ value was observed, demonstrating block of the hERG channel. No effect on the APD₆₀ value was observed, indicating that the AP prolongation induced by hERG block was offset by AP shortening induced by inhibition of the L-type calcium channel. (E) Representative traces showing the effect of norepinephrine (NE) on the SC-hCM AP in vehicle control and at concentrations of 0.1, 0.3, 1, and 3 μM (black, purple, green, gray and red, respectively). Changes in AP parameters were measured after application of NE to spontaneously beating SC-hCMs. In this example, NE prolonged the APD at concentrations of 0.1, 0.3 and 1 μM . AP shortening and DADs were induced by 3 μM NE.

4.2. Advantages of the SC-hCM AP assay versus PF assays for assessing cardiac risk

In addition to excellent pharmacological sensitivity, there are several advantages to using SC-hCMs that are immediately apparent. First, the SC-hCM cells are human cells, and therefore express human ion channels. This is important because species variability in pharmacological sensitivity is well-established (Lu, Marien, Saels, & De Clerk, 2001). Terfenadine, for example, does not prolong APD in canine PF assays, but does prolong APD in rabbit PF assays. Sensitivity of the SC-hCM AP to terfenadine is greater than 10-fold higher than the sensitivity of the rabbit PF AP. The reason underlying the greater sensitivity of the SC-hCM assay is not known, however as previously discussed, species and diffusion barrier differences may be important. Additionally, there is evidence that buffer composition may contrib-

ute to the pharmacological sensitivity of mammalian cardiac preparations used for preclinical safety evaluations (Fülöp et al., 2003). Since we performed our assays in different recording buffers (i.e., bicarbonate-buffered Tyrode's solution for PF assays, HEPES-buffered physiological saline for SC-hCM), it is possible that buffer composition may have contributed to the differences in pharmacological sensitivity between the preparations.

Second, the amount of test article required to evaluate effects on AP parameters is significantly less for the SC-hCM assay compared to PF assays. As demonstrated in Fig. 3, the latency for sotalolol prolongation of the AP is much shorter (~2–3 fold) in SC-hCMs compared with PF assays. Together with the slower compound perfusion rate in the SC-hCM assay (~1 mL/min for SC-hCM versus ~5 mL/min for PF), there is an expected 10- to 15-fold reduction in test article consumption for the SC-hCM assay versus PF assays.

Table 6
AP V_{\max} and amplitude modulation by I_{Na} blockers lidocaine and quinidine.

	SC-hCM				Rabbit PF				Canine PF			
	μM	N	APA ($\Delta\%$)	V_{\max} ($\Delta\%$)	μM	N	APA ($\Delta\%$)	V_{\max} ($\Delta\%$)	μM	N	APA ($\Delta\%$)	V_{\max} ($\Delta\%$)
Lidocaine	Control ^a	5	113.7 ± 6.2	14.1 ± 2.3	–	–	–	–	Control	4	117.7 ± 4.9	313.7 ± 63.0
	10	5	–1.4 ± 1.1	–8.8* ± 2.4	–	–	–	–	10	4	–5.9* ± 2.2	–7.9 ± 6.3
	30	5	–1.5 ± 1.3	–16.6* ± 2.3	–	–	–	–	50	4	–3.2 ± 2.1	–2.7 ± 14.2
	100	5	–3.6 ± 2.3	–22.6* ± 5.2	–	–	–	–	250	4	–12.3* ± 1.6	–29.9* ± 7.4
	300	4	–7.5* ± 3.5	–36.1* ± 6.6	–	–	–	–	–	–	–	–
Quinidine	Control ^a	4	109.6 ± 3.3	71.8 ± 18.9	Control	5	116.9 ± 3.9	356.4 ± 45.2	Control	4	102.8 ± 4.3	260.0 ± 38.4
	0.1	4	–1.8 ± 1.5	–3.0 ± 2.2	0.1	5	–6.7 ± 4.1	–11.4* ± 2.1	0.1	4	–1.4 ± 3.1	–6.7 ± 6.3
	0.3	4	–3.7* ± 1.2	–14.2 ± 7.5	1	5	–3.4 ± 1.7	–13 ± 5.7	1	4	–2.0 ± 2.9	–8.4 ± 6.0
	1	4	–6.5 ± 2.9	–26.3* ± 6.3	10	5	–8.8* ± 3.3	–29.2* ± 9.0	10	4	–5.6 ± 4.1	–28.0* ± 4.2
	3	4	–16.8* ± 6.5	–49.3* ± 13.0	–	–	–	–	–	–	–	–

^a Control values are in mV for APA and mV/ms for V_{\max} . All other values are percent change from control.

* Indicates change value is statistically significant ($p < 0.05$).

Table 7
Norepinephrine modulation of AP parameters.

	μM	N	APD ₆₀ ($\Delta\%$)	APD ₉₀ ($\Delta\%$)	RMP (ΔmV)	APA (ΔmV)	V_{\max} ($\Delta\%$)	Beat interval ($\Delta\%$)
Norepinephrine	Control ^a	4	212.3 ± 50.2	285.0 ± 77.1	–64.6 ± 1.2	96.1 ± 7.6	34.7 ± 7.7	991.0 ± 315.6
	0.1	4	7.5* ± 2.2	7.0* ± 2.4	–1.2 ± 0.8	3.7 ± 2.6	–1.2 ± 9.9	–1.6 ± 3.8
	0.3	4	9.5* ± 2.9	7.9 ± 3.7	–2.7* ± 0.7	6.9* ± 2.8	–0.4 ± 12.8	–8.2 ± 6.2
	1	4	18.4* ± 3.3	14.7* ± 4.0	–3.8* ± 0.8	10.9* ± 3.5	8.7 ± 16.7	–10.4 ± 11.0
	3	4	11.8 ± 14.5	11.4 ± 13.4	–4.7* ± 1.9	11.5* ± 4.1	5.9 ± 21.6	–13.5 ± 16.0

^a Control values are in ms for APD₆₀ and APD₉₀, mV for RMP and APA, and mV/ms for V_{\max} . All other values are percent change from control.

* Indicates change value is statistically significant ($p < 0.05$).

Third, we have demonstrated that the SC-hCM assay is very stable, and because drug effects are relatively rapid, the duration of the assay is much shorter than a PF assay. Therefore, the SC-hCM AP assay provides a relatively cost- and time-effective alternative to PF assays, with set-up and run-times similar to other single-cell electrophysiology assays, such as the hERG assay. Additionally, using SC-hCMs in an *in vitro* assay instead of using conventional animal models to assess cardiac risk has the advantage of reducing the use of purpose-bred laboratory animals.

4.3. Predictive value of the SC-hCM assay

Our results provide evidence for the usefulness of the SC-hCM APD assay in prediction of QT-prolongation, which serves as a surrogate biomarker for potential drug-induced TdP. Redfern et al. (2003) have shown that torsadogenic compounds typically have a margin of <30-fold difference between concentrations that produce 10–20% APD prolongation *in vitro* versus the effective therapeutic plasma concentration of the unbound (ETPC_{free}) drug. Table 3 APD₆₀ values, referenced against ETPC_{free} values in Redfern et al. (2003, on-line supplement Table 1), gave margins of 0.09, 0.68, 11.11, and 6.12, respectively, for quinidine, sotalol, terfenadine, and cisapride. Thus, all four known torsadogenic drugs in the SC-hCM assay would be predicted to have strong cardiac risk. By contrast, nifedipine, a selective Ca²⁺ channel blocker, shortened both APD₆₀ and APD₉₀ at therapeutic concentrations, whereas verapamil, a mixed hERG and Ca²⁺ blocker, shortened APD₆₀, but lengthened APD₉₀. Since both drugs are non-torsadogenic, the APD₆₀ parameter was uniformly predictive, whereas the APD₉₀ value would tag verapamil as potentially torsadogenic.

4.4. Limitations of the SC-hCM assay

While SC-hCMs showed expected pharmacological responses to all of the evaluated compounds, the SC-hCM electrophysiological phenotype is closer to embryonic than adult. The average upstroke velocity

was ~12 mV/ms (Table 2) versus 150–350 mV/ms in adult ventricular CMs (Schram, Pourrier, Melnyk, & Nattel, 2002). In addition, the RMP was relatively depolarized (–72 mV, Table 2, versus ~–80 to –85 mV), and the spike and dome shape was not observed.

Understanding the molecular and cellular determinants of the differences between SC-hCM and adult cardiomyocyte APs will require further investigation, however it seems likely the SC-hCMs have a relatively low expression of ion channels such as Kir2.1 and Nav1.5. In any case, AP parameters should be taken into consideration when evaluating pharmacological responses. For example, due to the relatively depolarized RMP of SC-hCMs, the sodium channel population is likely partially inactivated, which would lead to higher apparent potency for use-dependent compounds such as lidocaine. A final limitation of the SC-hCM assay is that, due to spontaneous beating of the SC-hCMs, studies requiring evaluation of the rate-dependence of AP modulation will be limited.

Due to the human origin of the SC-hCMs and the sensitive detection of multiple ion channel effects, it is expected that the occurrence of false negative results will be reduced. However, as is the case with other repolarization assays, the relationship between prolongation of the SC-hCM AP and arrhythmia in the clinic remains to be determined.

5. Conclusions

The SC-hCM-based AP assay offers exceptional pharmacological sensitivity, reduced compound consumption, and cost and time-savings compared with conventional PF assays. This assay should be strongly considered when performing follow-on studies per S7B guidance.

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