Evaluation of Drug Mediated Changes in Action Potentials & Calcium Transients Recorded from Adult Human Stem Cell-derived Cor.4U® Cardiomyocytes

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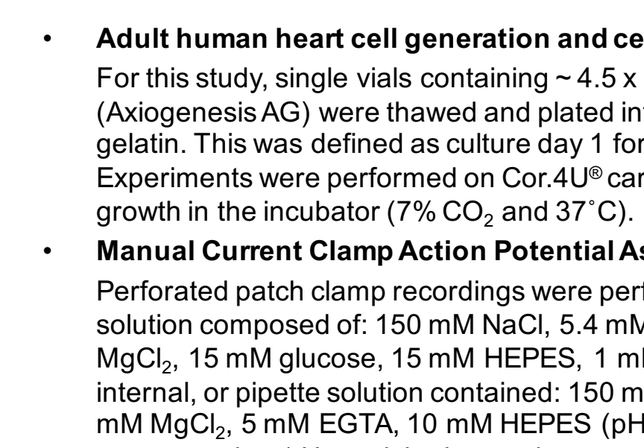
Abstract

- The FDA CiPA initiative proposes the use of adult human induced pluripotent stem cell-derived cardiomyocytes for testing proarrhythmia. This work contrasts the actions of diverse compounds on action potentials and internal calcium transients recorded from Cor.4U® Cardiomyocytes.
- Prolongation of action potential duration (APD_{90}), early after depolarizations (EADs) and decreased spontaneous beating rate of calcium transients were observed after exposure to the potassium channel blockers, E-4031, dofetilide and sotalol.
- Reduction of APD_{90}, shortened duration and increased spontaneous beating rate of internal calcium transients were observed after exposure to the calcium channel blockers, nifedipine and verapamil.
- Prolongation of action potential duration (APD_{90}) was observed after exposure to the sodium channel blocker, flecainide.
- Cor.4U® Cardiomyocytes provide a robust model for cardiac safety pharmacology.

Methods

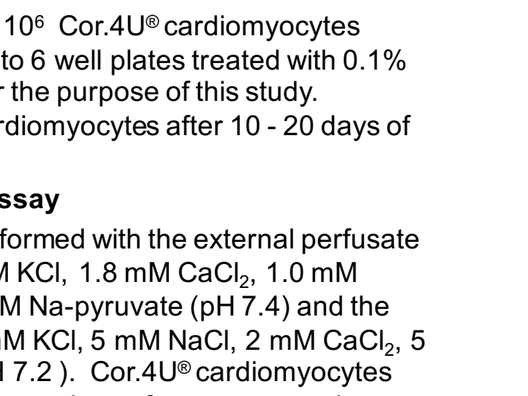
- **Adult human heart cell generation and cell culture**
  For this study, single vials containing ~ 4.5 x 10^6 Cor.4U® cardiomyocytes (Axiogenesis AG) were thawed and plated into 6 well plates treated with 0.1% gelatin. This was defined as culture day 1 for the purpose of this study. Experiments were performed on Cor.4U® cardiomyocytes after 10 - 20 days of growth in the incubator (7% CO_2 and 37˚C).
- **Manual Current Clamp Action Potential Assay**
  Perforated patch clamp recordings were performed with the external perfusate solution composed of: 150 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 1.0 mM $MgCl_2$, 15 mM glucose, 15 mM HEPES, 1 mM Na-pyruvate (pH 7.4) and the internal, or pipette solution contained: 150 mM KCl, 5 mM NaCl, 2 mM $CaCl_2$, 5 mM $MgCl_2$, 5 mM EGTA, 10 mM HEPES (pH 7.2). Cor.4U® cardiomyocytes were paced at 1 Hz and the increasing concentrations of test compounds were administered at four minute intervals. If significant increases in action potential duration were observed, the external pacing was stopped and spontaneous action potential was observed for EADs.
- **HTS Internal Calcium Transient Assay**
  On culture day 5 -7, Cor.4U® cardiomyocytes were re-suspended with trypsin and re-plated at a desired density (>10,000) in a black clear-bottom 96 well plate which were pre-coated with 0.1% gelatin. After another 10 - 15 days of growth, EarlyTox™ Cardiotoxicity Kit (Molecular Devices) was used to perform calcium transient assay. A baseline measurement was recorded and reference test compounds were administered to each plate. The plates were maintained in the incubator for 30 min and then transferred to the SpectraMax i3 for fluorescence reading at 37˚C.

Cor.4U® Cardiomyocyte Interaction of the Action Potential and Cardiac Contraction



- **An action potential is the interactive summary of transmembrane ion currents to produce electrical activation of the myocyte leading to an increase in cytoplasmic free Ca^{++}, the internal biochemical stimulation of myocyte contraction.**

Calcium Fluorescence* Indicator

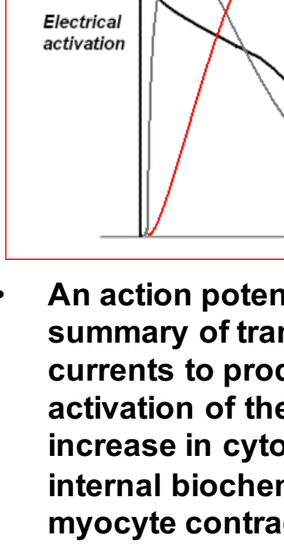


- Peak Count & Frequency
- Peak Position (time) and Amplitude
- Peak Width (FWHM)
- Rise Time (10% to 90%)
- Decay Time (90% to 10%)

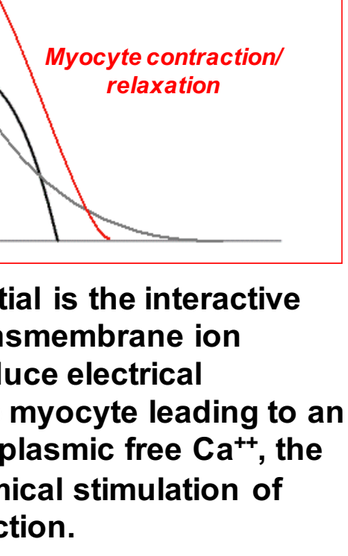
*SpectraMax i3, SoftMax Pro 6.4, Molecular Devices, Sunnyvale, CA

Potassium Channel Blockers Increase APD_{90} in Ventricular-like Cor.4U® Cells

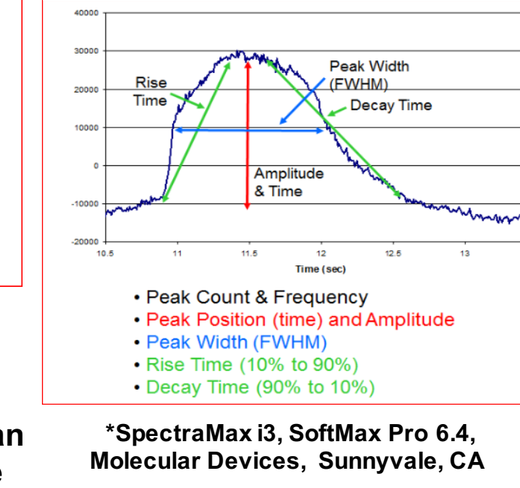
E-4031



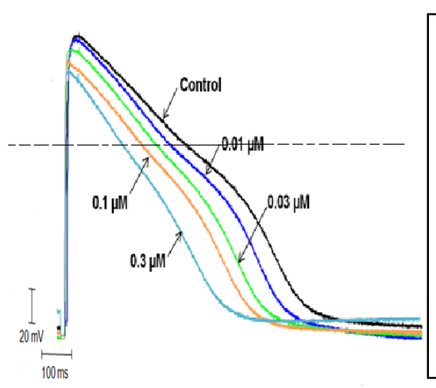
Dofetilide

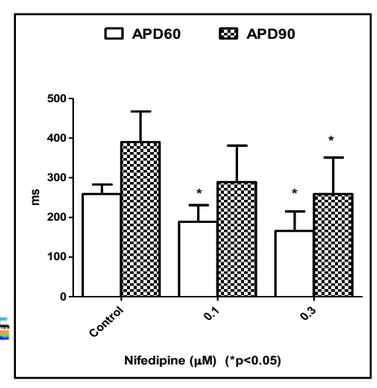


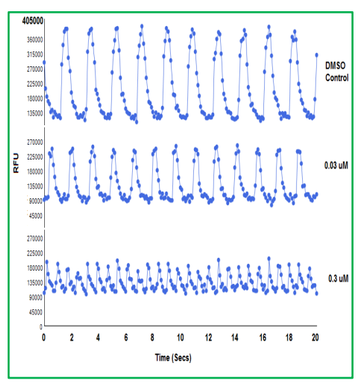
Sotalol



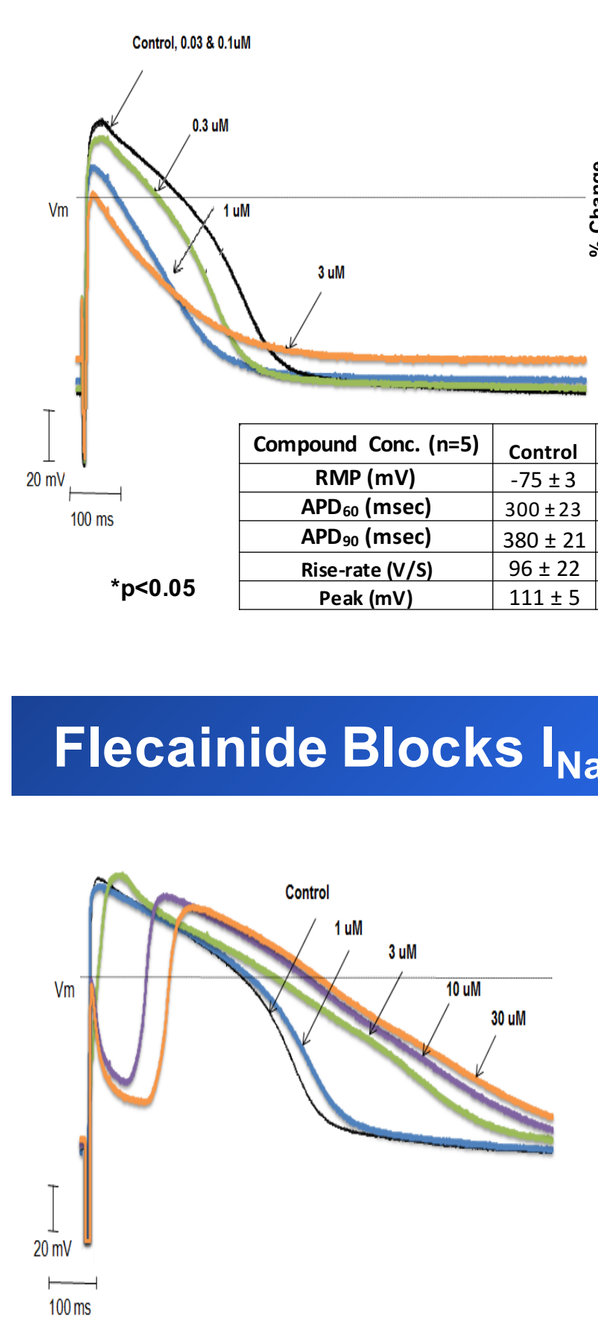
Nifedipine Blocks I_{Ca++}, Shortens APD & Increases Spontaneous Beating Rate

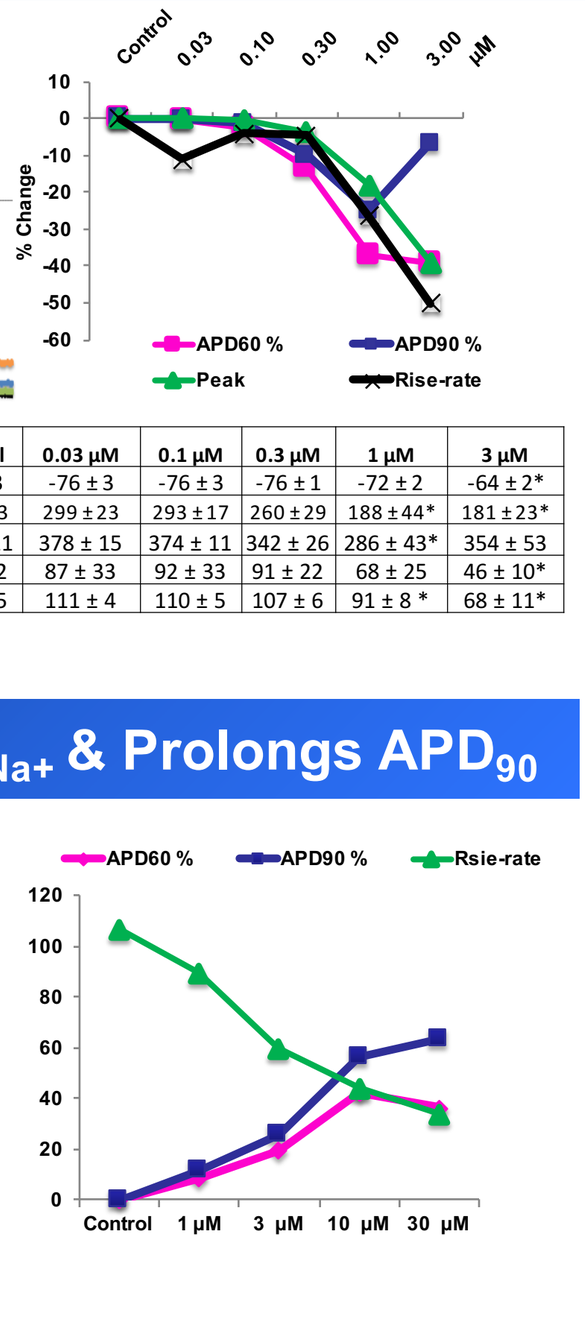






Verapamil Shortens, then Increases APD_{90}





Compound Conc. (n=5)	Control	0.03 µM	0.1 µM	0.3 µM	1 µM	3 µM
RMP (mV)	-75 ± 3	-76 ± 3	-76 ± 3	-76 ± 1	-72 ± 2	-64 ± 2*
APD_{60} (msec)	300 ± 23	299 ± 23	293 ± 17	260 ± 29	188 ± 44*	181 ± 23*
APD_{90} (msec)	380 ± 21	378 ± 15	374 ± 11	342 ± 26	286 ± 43*	354 ± 53
Rise-rate (V/S)	96 ± 22	87 ± 33	92 ± 33	91 ± 22	68 ± 25	46 ± 10*
Peak (mV)	111 ± 5	111 ± 4	110 ± 5	107 ± 6	91 ± 8 *	68 ± 11*

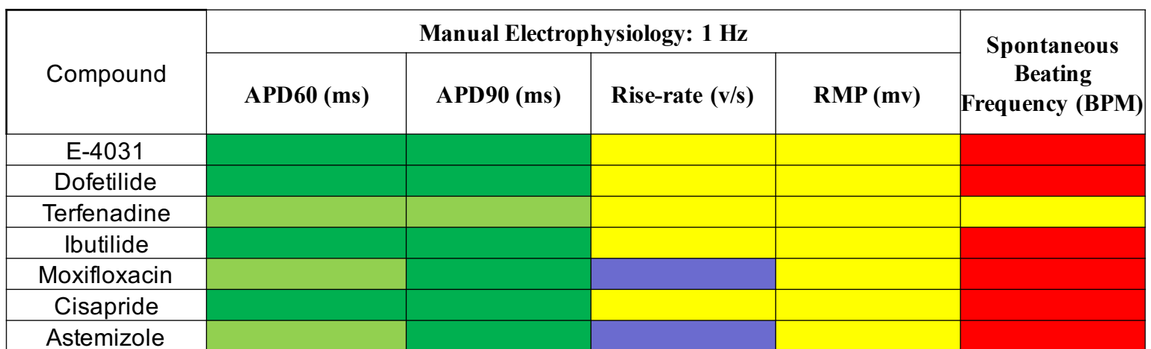
*p<0.05

Flecainide Blocks I_{Na+} & Prolongs APD_{90}

Summary of Observed Effects

Compound	Manual Electrophysiology: 1 Hz				Spontaneous Beating Frequency (BPM)
	APD60 (ms)	APD90 (ms)	Rise-rate (v/s)	RMP (mv)	
E-4031					
Dofetilide					
Terfenadine					
Ibutilide					
Moxifloxacin					
Cisapride					
Astemizole					
Sotalol					
Nifedipine					
Nitrendipine					
Verapamil					
Flecainide					
Mexiletine					
Quinidine					
Risperidone					Not Tested
Staurosprine					
Chlorpromazine					Not Tested
ATX-II					Not Tested

Decrease ≥ 50%	Decrease > 20-49%	No change	Increase > 20-49%	Increase ≥ 50%



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