Evaluation of Nifedipine on Action Potentials, Ion Currents, and Calcium Transients from Adult Human Stem Cell-Derived Cardiomyocytes

Ionic Transport Assays

John Ken Gibson, PhD, Yimei Yue, Jared Bronson

Ionic Transport Assays, Inc., Saint Louis, Missouri, USA

Ionic Transport Assays

Introduction

Cardiac safety pharmacology must be determined during non-clinical and clinical drug development to identify changes in cardiovascular function and is mandated by regulatory agencies. At present, although there is no "universal" *in vitro* pre-clinical system using human tissue for cardiac safety testing, a significant effort (e.g., CiPA) is underway with industrial, academic and government contributors to redesign the discovery and development funnel with adult hiPSC (human induced pluripotent stem cell-derived) cardiomyocytes for safer, effective drugs with decreased cost and development times.

To evaluate hiPSC cardiomyocytes (CM) as a preclinical tissue for safety pharmacology, we investigated the actions of nifedipine at different concentrations on action potentials, ion currents and calcium transients recorded from ventricular-like hiPSC-CM. We would emphasize these experiments were designed to evaluate the use of hiPSC-CM as a tissue source in safety pharmacology, not to provide detailed kinetic mechanisms of nifedipine drug action. We found that nifedipine blocked the transmembrane L-type calcium channel with an IC_{50} of 0.03 µM. Action potential duration at 60% and 90% repolarization were significantly decreased at 0.01 to 0.03 µM nifedipine. The rate and the amount of internal calcium release was decreased by 0.03 µM nifedipine. While higher doses of nifedipine inhibited internal calcium release and produced cell death, these experiments demonstrate that nifedipine selectively blocks the L-type calcium channel at lower doses. We found that hiPSC-CM can serve as a useful tool in early drug screening or cardiovascular safety front-loading and additional studies are underway with diverse agents.

Methods

Electrophysiology Experiments

Adult human heart cells (iCell® Cardiomyocytes) were created by reprogramming an adult human fibroblast cell line with retroviral expression of reprogramming factors. When delivered to Ionic Transport Assays, these cells were thawed in Cellular Dynamics International (CDI) plating media and plated onto gelatin coated coverslips for electrophysiology studies. When the cells were placed on glass coverslips precoated with 0.1% gelatin, this was defined as culture day one for the purpose of this study. After 48 hours, cell culture maintenance media was 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 use of a Thermoclamp-1 in-line heater (AutoMate Scientific, CA) with a perfusion rate of 0.8 - 1.2 ml/minute. In general, the external perfusion 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, 5 mM MgCl, 5 mM EGTA, 10 mM HEPES, pH adjusted to 7.2 with 10 N KOH. 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).

Human iPSC ventricular-like cardiomyocytes first were allowed to spontaneously fire during these experiments; pacing was then 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 cells were not used in the experiments for action potentials. Human iPSC ventricular-like cardiomyocytes were paced at 1 Hz during a four minute baseline period, and nifedipine was administered for four minutes for each of five test concentrations of 0.01, 0.03, 0.1, 0.3, or 1.0 µM. 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. Nifedipine was dissolved in DMSO to make stock solutions. Exposure to $\leq$ 0.1% DMSO had no effect on cardiac action potentials (data not shown) Statistical analysis was evaluated by ANOVA.

Voltage Clamp Experimental Protocol

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 four minute baseline control period (no rundown observed). Nifedipine was administered for four minutes for each of five test concentrations of 0.01, 0.03, 0.1, 0.3, or 1.0 µM. Calcium current peak amplitude was averaged from five voltage pulses for each nifedipine concentration as well as for the control period.

Intracellular Calcium Flux Detection

Changes in intracellular calcium flux associated with cardiomyocyte contractions were monitored on a fluorescence imaging plate reader (SpectraMax® i3 Multi-Mode Microplate Platform). A cardiac beating assay was performed using the EarlyTox™ Cardiotoxicity Kit (Assay Kit R8210) from Molecular Devices®. Cardiomyocyte beating parameters were measured using a kinetic, well-by-well fluorescence read mode and analyzed using Peak Pro Software algorithms in SoftMax® Pro Software. Analysis parameters, including peak beating frequency, peak amplitude, peak width, and decay time during a 30 second reading, were used to quantify the effects of nifedipine on cardiac beating. When thawed as described above, hiPSC-CM were plated into the central wells of a gelatin treated 96 well plate. After 10 - 15 days of growth in the incubator (7% CO_2 and 37˚C), the cells were treated with the Cardiotoxicity Dye for two hours. A baseline measurement was then recorded and test compounds were administered to the plate. The plates were maintained in the incubator for 15 minutes and then transferred to the SpectraMax i3 for fluorescence reading. Upon the completion of reading, the plates were returned to the incubator and transferred to the SpectraMax for additional readings after 30 minutes.

Results

Action Potential

Figure 1. Nifedipine significantly decreased ventricular action potential duration. As shown below, increasing concentrations of nifedipine were applied to an hiPSC-CM to produce significant reductions in APD_{60} and APD_{90}. Records are superimposed between Control (black), 0.01 (dark blue), 0.03 (green), 0.1 (orange), and 0.3 µM nifedipine (light blue).

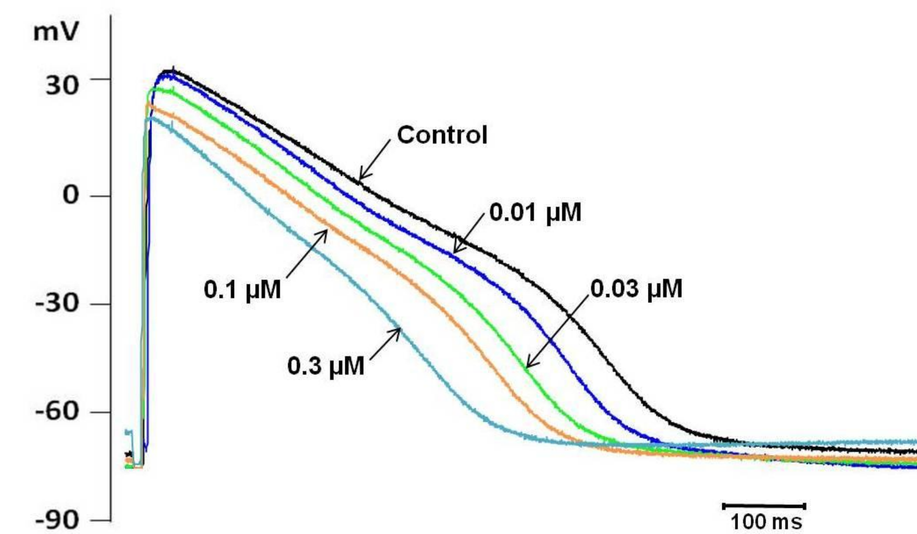


Table 1. Acute effects of nifedipine on cardiac action potentials recorded from hiPSC-CM. Nifedipine first decreased APD_{60} and APD_{90}. This was followed by a significant decrease in resting membrane potential.[a, b]

Nifedipine Concentration	n	RMP[c, f]	APD_{60}[d, h]	APD_{90}[d, g]	APA[c, i]	Rate of Rise[e, j]
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

[a] Four minutes exposure at each concentration.
[b] Data are 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 ≤ 0.05, ** p ≤ 0.01, ***p ≤ 0.001).

Voltage Clamp

Figure 2. Nifedipine blocks calcium current in an 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.

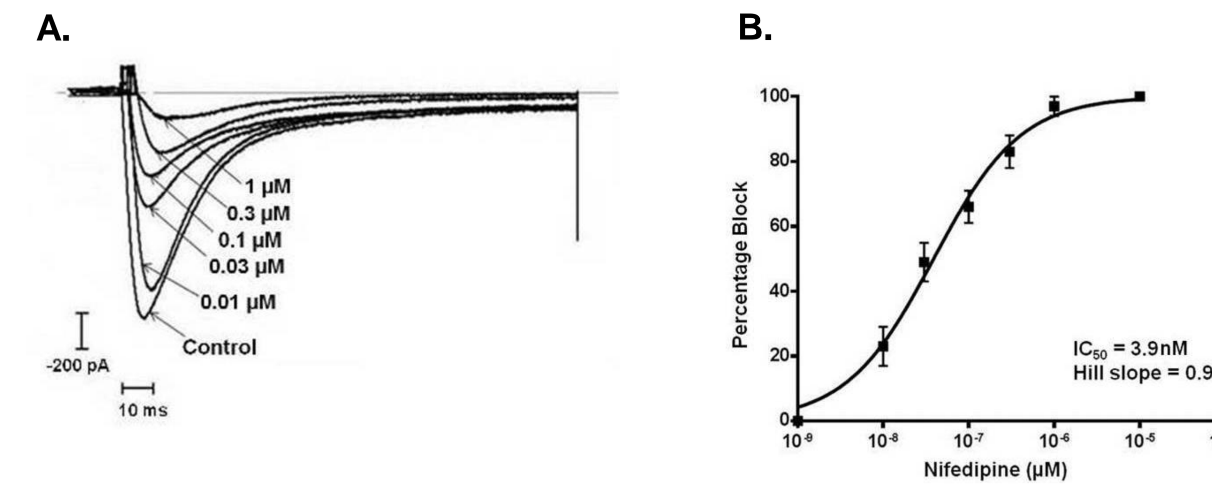


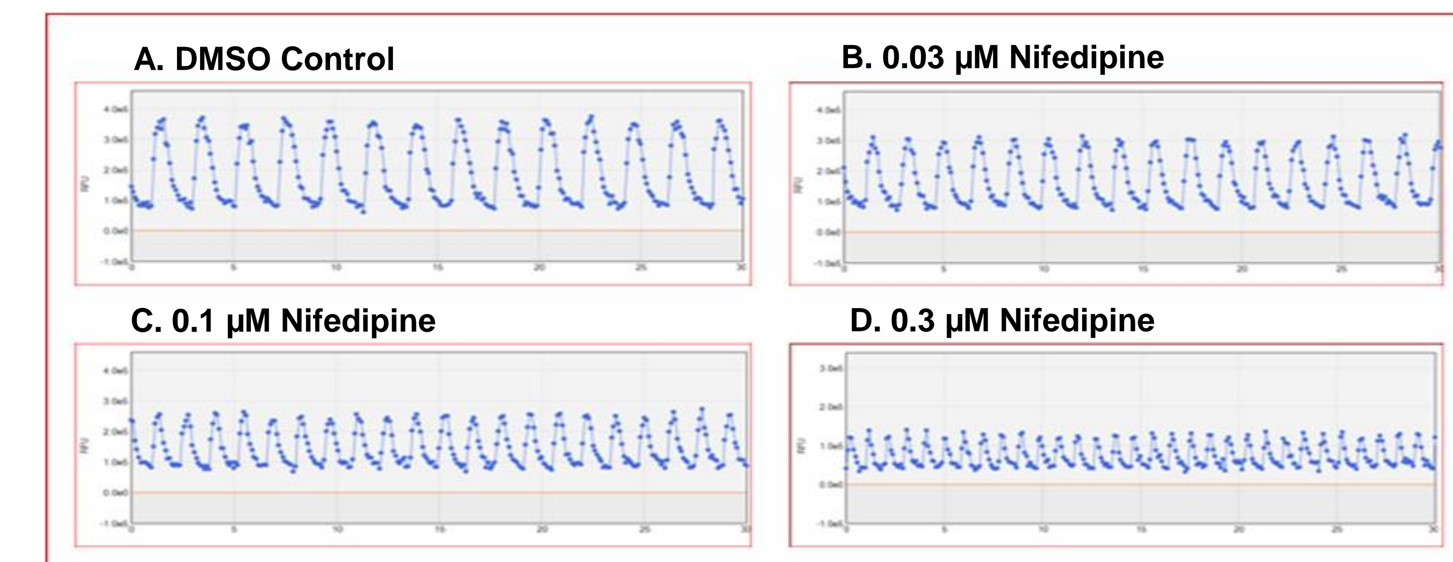
Table 2. Peak calcium current normalized to cell capacitance (pA/pF) in control and nifedipine containing solutions as well as percentage block of cardiac cell calcium current.[a]

Nifedipine Concentration	1	2	3	4	5	6	Mean ± SD (pA/pF)	Mean ± SD (%)
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
1.0 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 ≤ 0.001).

Internal Calcium Transients

Figure 3. A. Calcium transients after treatment with DMSO. **B.** 0.03 µM nifedipine: slight increase observed in Peak Frequency of beating, decrease observed in Peak Width and Peak Amplitude of calcium transient. **C.** 0.1 µM nifedipine: increase observed in Peak Frequency of beating, marked decrease observed in Peak Width and Peak Amplitude of calcium transient **D.** 0.3 µM nifedipine: marked increase observed in Peak Frequency of beating, marked decrease observed in Peak Width and Peak Amplitude of calcium transient.



Internal Calcium Transients

Figure 4. Responses to nifedipine were similar at 15 and 30 minute exposure (n = 3). **A.** Dose response inhibition of the peak amplitude of the internal calcium signal produced by nifedipine. **B.** Nifedipine also decreased the peak width of the internal calcium signal. **C.** A significant increase in spontaneous heart rate (Peak Frequency) is produced as nifedipine shortens the duration of the cardiac action potential in spontaneously beating cells.

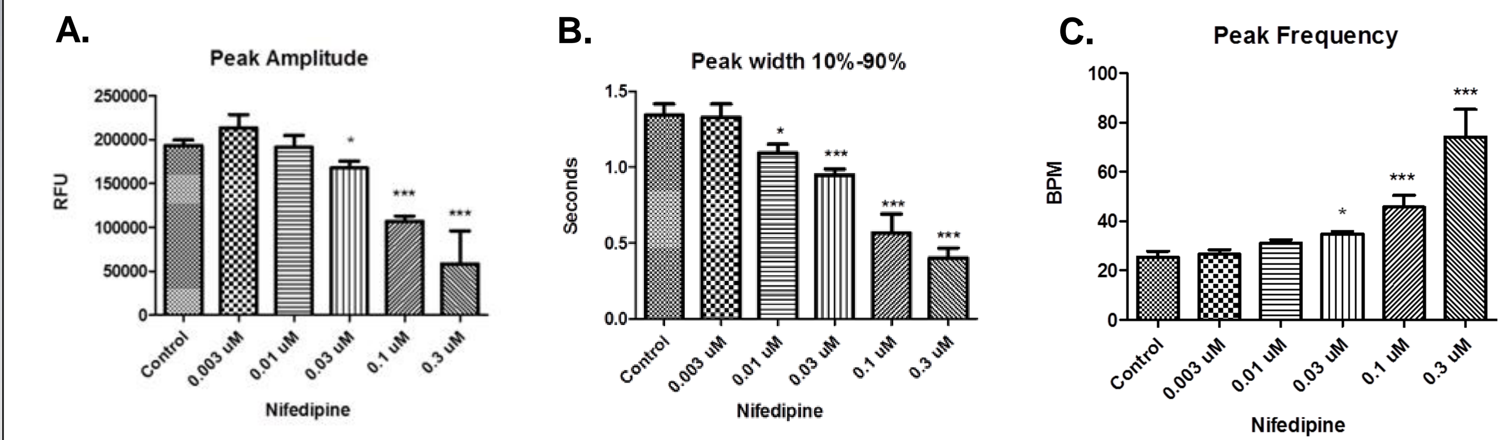


Table 3. Overall responses to nifedipine. A decrease in action potential duration was observed at 0.01 µM. An inhibition of transmembrane L-type calcium current was measured at 0.01 µM. Decreases in the amplitude and peak width of the internal calcium transients also were measured at 0.01 µM. A significant increase in the peak beating frequency was not observed until the hiPSC-CM were treated with 0.03 µM.

Nifedipine Concentration	% APD_{60} (n = 6)	% APD_{90} (n = 6)	ICa % (n = 6)	% Amplitude (n = 3)	% Peak Width (n = 3)	Peak Frequency (n = 3)
0.003 µM	NR[a]	NR	NR	100	101	27 ± 1.8
0.01 µM	-13 ± 5*	-10 ± 5*	-23 ± 6*	-0.26	-0.29*	31 ± 1.4
0.03 µM	-21 ± 12*	-23 ± 11*	-49 ± 6*	-0.37*	-0.35*	35 ± 1.0*
0.1 µM	-38 ± 14*	-34 ± 14*	-66 ± 5*	-0.72*	-0.77*	46 ± 4.8*
0.3 µM	-56 ± 15*	-50 ± 16*	-83 ± 5*	-0.78*	-0.80*	74 ± 11.3*
1.0 µM	-66 ± 13*	-64 ± 6*	-97 ± 1*	NR	NR	NR

[a] NR: Not recorded.
* Indicates change value is statistically significant (*p ≤ 0.05).

Conclusion

Nifedipine blocked the transmembrane L-type calcium channel of hIPSC cardiomyocytes with an IC_{50} of 0.03 µM. Action potential duration at 60% and 90% repolarization were significantly decreased at 0.01 µM nifedipine. The amount of internal calcium release was decreased by 0.01 µM nifedipine. A significant increase in spontaneous heart rate was produced as 0.03 µM nifedipine shortened the duration of the cardiac action potential. While higher doses of nifedipine inhibited internal calcium release and produced cell death, these experiments demonstrate that nifedipine selectively blocks the L-type calcium channel at lower doses. This results in a significant reduction in action potential duration and increase in the spontaneous beating rate of hiPSC cardiomyocytes. In summary, hiPSC cardiomyocytes can serve as a useful tool in early drug screening or cardiovascular safety front-loading to determine the diverse cardiac actions of a therapeutic agent.

Bibliography

Gibson, J.K., Yue, Y., Bronson, J., Palmer, C., Numann, R. (2014). Human stem cell-derived cardiomyocytes detect drug-mediated changes in action potentials and ion currents. *Journal of Pharmacological and Toxicological Methods, 10.1016/j.vascn.2014.09.005.*