Optimization of Cardiac CiPA Targets (hCav1.2 and hKCNQ1/hminK) on the QPatch HT Automated System

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Abstract # 0029

Abstract

Drug-induced QT interval prolongation, ventricular arrhythmia and sudden death are one of the leading causes for drug withdrawal from the market or denied regulatory approval. Preclinical profiling for off-target cardiac ion channel interactions using The Comprehensive *in vitro* Proarrhythmia Assay (CiPA) paradigm has been initiated to overcome drug attrition due to hERG inhibition only. The aim is to improve the prediction of a drug's proarrhythmic liability for the pharmaceutical industry. This new paradigm includes a panel of *in vitro* assays that integrates effects of the test compounds on several cardiac ion channels. Voltage-gated ion channels in this panel, particularly hCav1.2 and hKCNQ1/hminK, exhibit rapid activities decrease when contact between membrane and cytosol is disrupted upon patch excision (run-down). The loss of channel activity without pharmacological interaction poses a challenge for developing accurate, precise and robust assay yielding high success rate, minimal current run-down and reliable pharmacological results. In the present study, hCav1.2 and hKCNQ1/hminK cardiac ionic currents were validated on QPatch HT patch clamp system with the specific focus on preventing current run-down. The results demonstrate suitability of these assays for screening and profiling of drug effects by significantly reducing run-down (<20%) by optimizing protocol parameters. Reference pharmacology was assessed for Cav1.2 (Nifedipine IC_{50} =144nM) and hKCNQ1/hminK (Chromanol 293B IC_{50} =12.3μM). The optimized assays for hCav1.2 and hKCNQ1/hminK on the QPatch are able to provide data that is comparable to manual patch clamp, which allows the assessment of a novel compounds proarrhythmic risk.

Methods

HEK-293 cell lines stably-expressing exogenous human Cav1.2 and KCNQ1/mink, were cultured according to internal protocols. Briefly, Cav1.2-HEK cells were grown in DMEM/F12 + Glutamax media supplemented with non-essential amino acids (NEA) and 10% FBS, while KCNQ1/mink-HEK cells were grown in IMDM (Iscove's Modified Dulbecco's Medium) supplemented with 10% FBS. T-150 flasks were seeded 48-72 hours (maintained at 37C, 5% CO_2) prior to the experiment to achieve a cell confluency of 80%.

Automated patch-clamp (APC) electrophysiology assays for both hCav1.2 and hKCNQ1/mink were conducted on the QPatch HT platform (Sophion, Denmark). Standard single hole 48 well plates (~2.5MΩ) were used in all experiments. Voltage protocols are described in the figure legends.

1. hCav1.2 assay on the QPatch HT

Figure 1a

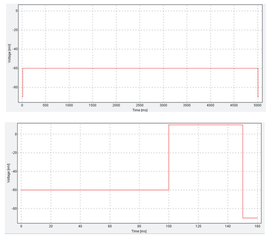

Figure 1a. hCav1.2 protocol and elicited current
Drug-induced inhibition of voltage-activated calcium channels (hCav1.2) for some compounds can be more accurately predicted upon application of a train of 4 conditioning pulses from a holding potential of -90mV to -60mV for 5s with a sweep interval of 20s (top panel). After the 4 conditioning pulses, cells were stepped from the -90mV holding potential to -60mV for 100ms before stepping to +10mV for 50ms to activate hCav1.2 currents. Cells were then stepped back to the holding potential -90mV for 20s (to allow for channels to recover from inactivation) and this pattern was repeated 3 times with a sweep interval of 20s (0.05Hz) (lower panel).

Figure 1b

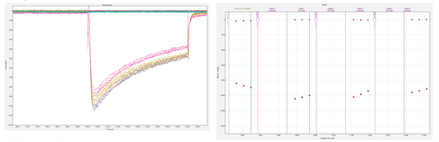

Figure 1c

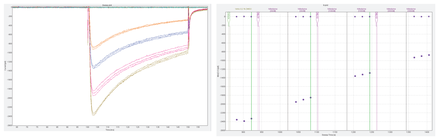

Figure 1b. hCav1.2 Current Stability for the entire assay duration
Sweep plot is presented to illustrate the stability of Cav1.2 current recording with minimal run-down during the assay. Vehicle controls (0.3% DMSO) that time-matched the addition of compounds over three (3) applications..
Figure 1c. Nifedipine effect on HEK-Cav1.2 current
A representative time course and sweep plot of the effect of sequential addition of escalating concentrations of Nifedipine on HEK-Cav1.2 current is shown.

Figure 1d

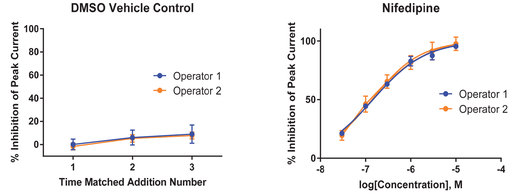

Figure 1d. hCav1.2 concentration response curve for reference compound Nifedipine and DMSO vehicle control from two independent assays run by different operators
Time-matched vehicle (0.3% DMSO) controls plotted for three additions at 3 minute intervals (n=3 for both Operator 1 and 2). Normalized Cav1.2 peak current (post/pre) is plotted against compound concentrations and fit with an equation to estimate the IC_{50} of 145nM(n=3) and 142nM(n=3) for Operator 1 and 2, respectively.

2. hKCNQ1/hminK assay on the QPatch HT

Figure 2a

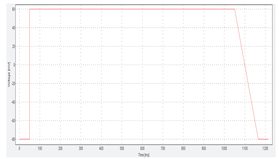

Figure 2a. CiPA hKCNQ1/hmink Peak protocol and elicited current
Onset block of peak hKCNQ1/hmink current was measured using a pulse pattern, repeated every 15 sec, consisting of pulse from a holding potential of -80mV to 60mV for a 1000ms duration, followed by a 115ms ramp (1.2V/s back to -80mV holding potential. Peak current was measured during the step to 60mV.

Figure 2b

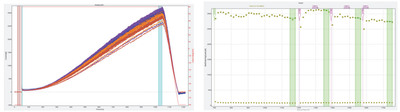

Figure 2c

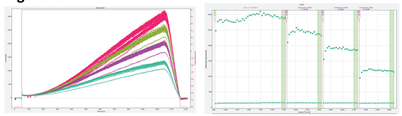

Figure 2b. hKCNQ1/hmink Peak current stability
Sweep plot is presented to illustrate the stability of KCNQ1/mink current recording with minimal run-down during the assay. Vehicle controls (0.3% DMSO) that time-matched the addition of compounds over three (3) applications..
Figure 2c. Chromanol 293B on hKCNQ1/hmink Peak current
A representative time course and sweep plot of the effect of sequential addition of escalating concentrations of Chromanol 293B on hKCNQ1/hmink peak current is shown.

Figure 2d

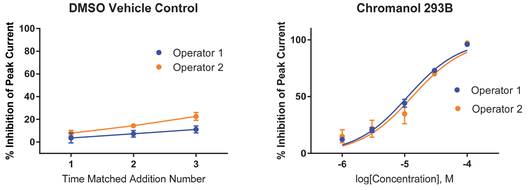

Figure 2d. hKCNQ1/hmink concentration response curves for reference compound Chromanol 293B and DMSO vehicle control from two independent assays each run by different operators
Time-matched vehicle (0.3% DMSO) controls plotted for three additions at 3 minute intervals (n=3 for both Operator 1 and 2). Normalized hKCNQ1/hmink peak current (post/pre) is plotted against compound concentrations and fit with an equation to estimate the IC_{50} of 11.2μM(n=3) and 13.3μM(n=3) for Operator 1 and 2, respectively.. .

3. Comparison of QPatch HT IC_{50} values to manual patch clamp IC_{50} values

Compound	Estimated IC50 value		
	Channel	QP-Eurofins	MPC
Nifedipine	Cav1.2	144μM	0.3-120μM
Chromanol 293B	KCNQ1/mink	12.3μM	1.2-6.9μM

Conclusion

1. The IC_{50} values were generated from HEK-hCav1.2 and hKCNQ1/mink inhibition on the QPatch compared (<2-fold) to the IC_{50} values generated for these compounds using manual patch clamp.
2. Rundown was minimized (<20%) for both ion channels by optimized protocols and assays parameters.
3. Reproducibility and robustness was observed in both assays verified by multiple runs with different operators.

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