Validating Cardiac Ion Channel assays on the IonFlux™ System for the CiPA Paradigm

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Abstract

Drug-induced QT interval prolongation and Torsades de Pointes (TdP) arrhythmia are the leading causes for drug withdrawal from the market. For the past decade, *in vitro* hERG channel assays and *in vivo* QT measurements have been conducted as surrogates for proarrhythmic risk propensity according to ICH S7B and ICH E14 guidelines. This paradigm, although effective, suffered from lack of specificity and led to unnecessary compound attrition during drug development. The Comprehensive in vitro Proarrhythmia Assay (CiPA) is a new cardiac safety testing paradigm under development with the goal of addressing this limitation and improving the ability to predict a drug's proarrhythmic liability. This new paradigm includes a panel of *in vitro* assays that integrates the effects of test compounds on several cardiac ion channels. In this study, HEK-hERG, HEK-hNav1.5 peak and HEK-hNav1.5 late cardiac ionic currents were validated on the microfluidic-based automated IonFlux™ HT patch clamp system, using HESI-sponsored High Throughput Screening (HTS) cardiac protocols and assay solutions with a set of 12 compounds from Phase 1 to assess the variability and reproducibility of HTS platforms/sites and for the calibration and validation of the in silico action potential (AP) model. The results demonstrate suitability of the IonFlux™ HT system for high throughput screening of drug effects on cardiac ionic currents, and provide data for in silico reconstructions in the CiPA paradigm for defining proarrhythmic risk.

1. HEK-hERG assay on the IonFlux™ HT

Figure 1a

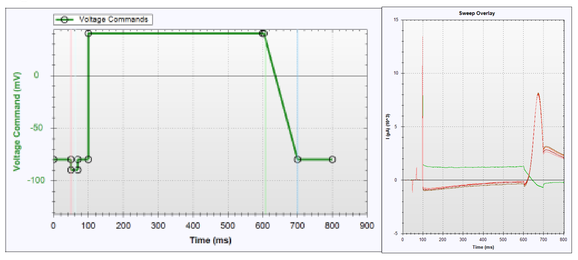

Figure 1a. CiPA hERG protocol and elicited current
Onset and steady state block of hERG current was measured using a pulse pattern (shown on the left panel), repeated every 5 sec, consisting of a depolarization to 40 mV amplitude for a 500ms duration, followed by a ramp (1.2 V/s) to -80mV for 100ms. The holding potential was -80mV. Peak tail current was measured during the ramp (shown on the right panel). Leak current was measured after applying a saturating concentration of a blocker such as Cisapride (1µM) at the end of each experiment to completely block hERG current.

Figure 1b

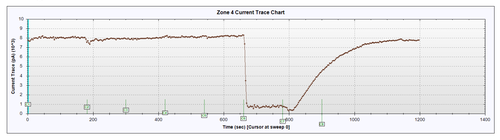

Figure 1c

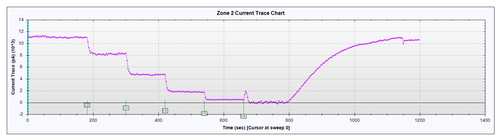

Figure 1b. hERG Current Stability for the entire assay duration
Vehicle controls that time-matched the addition of the four (4) concentrations is presented to illustrate the stability of hERG current recording without run-down during the assay.
Figure 1c. Ranolazine effect on HEK- hERG current
A representative time course of the effect of sequential addition of increasing concentrations of Ranolazine on HEK-hERG current is shown.

Figure 1d

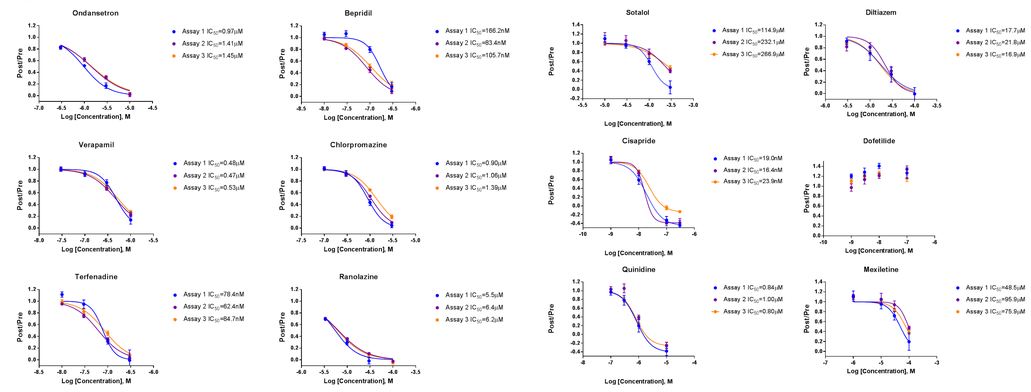

Figure 1d. HEK-hERG concentration response curves for CiPA Phase 1 compounds from three independent assays
The twelve (12) CiPA compounds were split into two groups of six (6) compounds and assayed in two separate plates with their own time-matched vehicle controls. Normalized hERG tail current (post/pre) is plotted against compound concentrations and fit with an equation to estimate the IC_{50} value.

2. hNav1.5 peak current assay on the IonFlux™ HT

Figure 2a

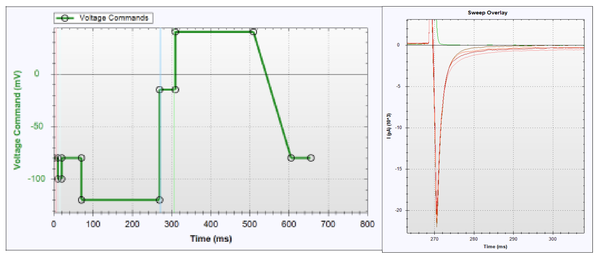

Figure 2a. CiPA hNav1.5 Peak protocol and elicited current
Onset and steady state block of peak Nav1.5 current was measured using a pulse pattern, repeated every 5 sec, consisting of a hyperpolarizing pulse to -120mV for a 200ms duration, depolarization to -15mV amplitude for a 40ms duration, followed by step to 40mV for 200ms and finally a 100ms ramp (1.2 V/s) to a holding potential of -80mV. Peak current was measured during the step to -15mV. Leak current was measured after applying a saturating concentration of a blocker such as lidocaine (2mM) at the end of each experiment to completely block hNav1.5 current.

Figure 2b

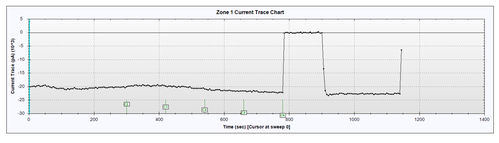

Figure 2c

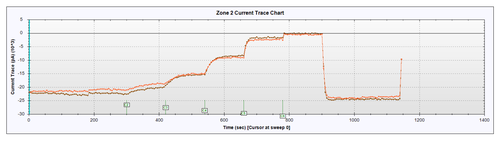

Figure 2b. hNav1.5 Peak current stability
Vehicle controls that time-matched the addition of the four (4) concentrations are presented to illustrate the stability of hNav1.5 peak current recording without run-down during the assay.
Figure 2c. Diltiazem on hNav1.5 Peak current
A representative time course of the effect of sequential addition of increasing concentrations of Diltiazem on hNav1.5 peak current is shown.

Figure 2d

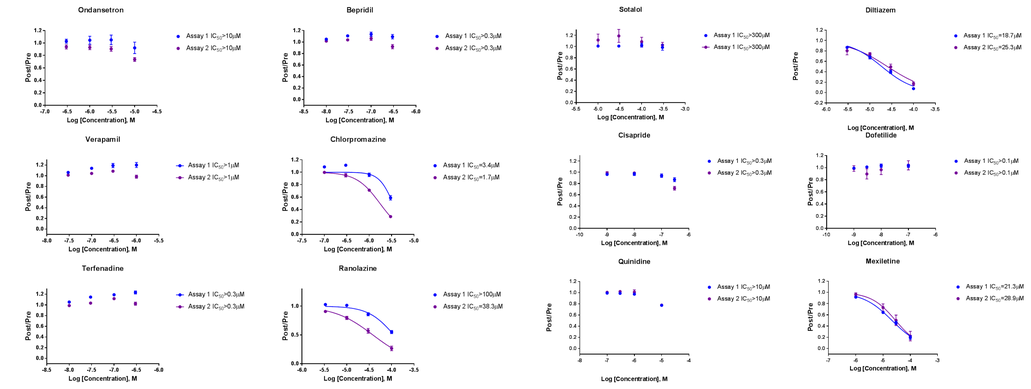

Figure 2d. Concentration response curves for CiPA Phase 1 compounds from three independent assays
The twelve (12) CiPA compounds were split into two groups of six (6) compounds and assayed in two separate plates with their own time-matched vehicle controls. Normalized hNav1.5 peak current (post/pre) is plotted against compound concentrations and fit with an equation to estimate the IC_{50} value.

3. hNav1.5 late current assay on the IonFlux™ HT

Figure 3a

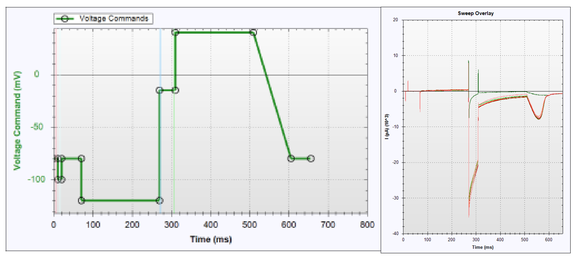

Figure 3a. CiPA hNav1.5 Late protocol and elicited current
Late current was measured using the same voltage protocol as mentioned above for hNav1.5 peak current. All external solutions contained 20 nM ATX-II to activate late currents. Late currents were measured at their maxima during the ramp.

Figure 3b

Figure 3c

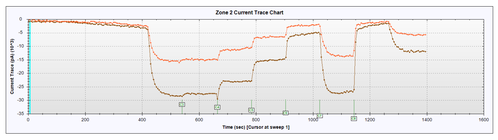

Figure 3b. hNav1.5 Late current stability
Vehicle controls with 20nM ATXII that time-matched the addition of the four (4) concentrations is presented to illustrate the stability of hNav1.5 late current recording without run-down during the assay.
Figure 3c. Diltiazem effect on hNav1.5 Late current
A representative time course of the effect of sequential addition of increasing concentrations of Diltiazem on hNav1.5 late current is shown.

Figure 3d

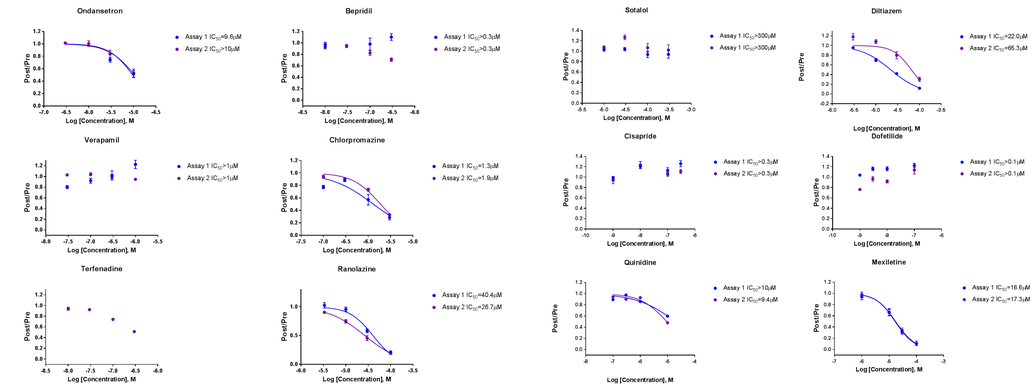

Figure 3d. Concentration response curves for CiPA Phase 1 compounds from two independent assays
The twelve (12) CiPA compounds were split into two groups of six (6) compounds and assayed in two separate plates with their own time-matched vehicle controls. Normalized hNav1.5 late current (post/pre) is plotted against compound concentrations and fit with an equation to estimate the IC_{50} value.

4. Comparison of IonFlux™ HT IC_{50} values to manual patch clamp IC_{50} values

Drug	Estimated IC50 (nM)					
	hERG		hNav1.5 Peak		hNav1.5 Late	
	MPC	IonFlux	MPC	IonFlux	MPC	IonFlux
BEPRIDIL	149.28	118.00	2929.27	>0.3µM	1813.91	>0.3µM
CHLORPROMAZINE	1118.46	1115.10	4535.60	2569.00	4559.55	1304.00
CISAPRIDE	11.82	19.80	NA	>0.3µM	NA	>0.3µM
DILTIAZEM	6568.59	18813.30	110859.80	23580.00	21868.47	21980.00
DOFETILIDE	1.46	>0.1µM	380.51	>0.1µM	753171.10	>0.1µM
MEXILETINE	24367.69	73436.67	NA	25095.00	8956.75	16590.00
ONDANSETRON	1492.45	1277.97	57666.43	>10µM	19180.80	9625.00
QUINIDINE	343.38	880.00	12329.04	>10µM	9416.98	9400.00
RANOLAZINE	6490.48	6031.30	68774.53	75575.00	7884.47	40390.00
SOTALOL	86369.31	204633.00	NA	>300µM	NA	>300µM
TERFENADINE	18.51	75.15	4803.18	>0.3µM	20056.03	>0.3µM
VERAPAMIL	498.62	494.20	NA	>1µM	7028.01	>1µM

Conclusion

1. **The twelve (12) CiPA Phase 1 compounds were screened against HEK-hERG, hNav1.5 peak and hNav1.5 late current in the IonFlux™ HT platform using HESI-sponsored High Throughput Screening (HTS) protocols and assay solutions.**
2. **The IC_{50} values were generated from HEK-hERG, hNav1.5 peak and hNav1.5 late current inhibition and compared to the IC_{50} values generated for these compounds using manual patch clamp.**
3. **IonFlux HT hERG IC_{50} values for all compounds, other than Dofetilide, correlated well with the hERG IC_{50} values generated using manual patch clamp.**
4. **IonFlux HT Nav1.5 peak and late current IC_{50} values for all compounds, correlated well with the IC_{50} values generated using manual patch clamp.**

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