Validating Multi-Ion Channel Inhibition Assays Following Best Practice Considerations

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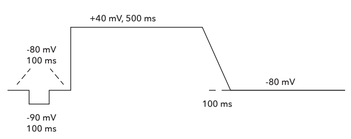
Purpose

As ICH E14/S7B Q&As was adopted in February 2022, the pharmaceutical industry paid more attention to the best practice considerations, as well as CiPA activities. When sponsors are using hERG data to support interpretation of clinical QT data in specific scenarios, and when using Cav1.2 and Nav1.5 to support a proarrhythmia assessment, best practice aspects should be considered as described in the E14/S7B Q&As. More and more sponsors request the multi-ion channel inhibition assays following best practice considerations for submission. Considering this trend and the ICH guideline adoption, we have initiated and completed the validation for hERG, Cav1.2, peak and late Nav1.5 currents following best practice considerations using manual patch-clamp technique.

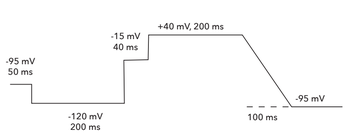
Method

Manual patch-clamp system was used as the platform. The recording temperature was near physiological temperature (35–37°C) for the multi-ion channel inhibition assays.

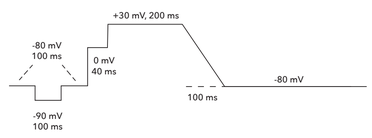
Voltage Protocol Command



Voltage protocol for hERG testing



Voltage protocol for Nav1.5 testing



Voltage protocol for Cav1.2 testing

The stimulation frequency of 0.2 Hz was applied for all ion channel inhibition assays.

Cell lines: CHO-hERG, CHO-Nav1.5, HEK293-Cav1.2.

Compounds:

hERG	Moxifloxacin	Ondansetron	Dofetilide	Verapamil	Cisapride
Peak Nav1.5	Ranolazine	Tetracaine	Flecainide		
Late Nav1.5	Ranolazine	Tetracaine	Flecainide		
Cav1.2	Verapamil	Cisapride	Quinidine		

Full Blockers:

hERG	E4031	1 μM
Peak Nav1.5	TTX	100 μM
Late Nav1.5	TTX	30 μM
Cav1.2	Verapamil	100 μM

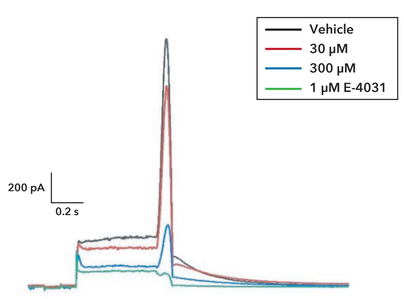
Results

hERG Assay

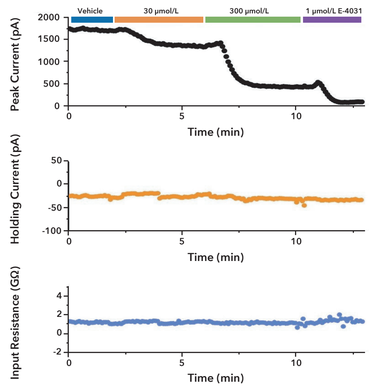
Table 1 Block of Compounds on hERG Current

Compounds	Test Voncentrations (μmol/L)	IC_{50} Values (μmol/L)	Hill Coefficient
Moxifloxacin	10, 30, 100, 300	67.40 ± 9.66	1.04 ± 0.15
Ondansetron	0.3, 1, 3, 10	1.47 ± 0.76	1.03 ± 0.56
Dofetilide	0.002, 0.006, 0.02, 0.06	0.01667 ± 0.01049	1.43 ± 1.18
Verapamil	0.03, 0.1, 0.3, 1	0.23 ± 0.13	1.33 ± 0.95
Cisapride	0.006, 0.02, 0.06, 0.2	0.02387 ± 0.00621	1.23 ± 0.38

Moxifloxacin in hERG Assay Current trace



I-t Plot

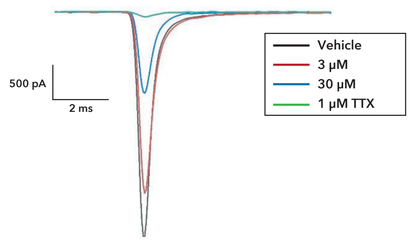


Peak Nav1.5 Assay

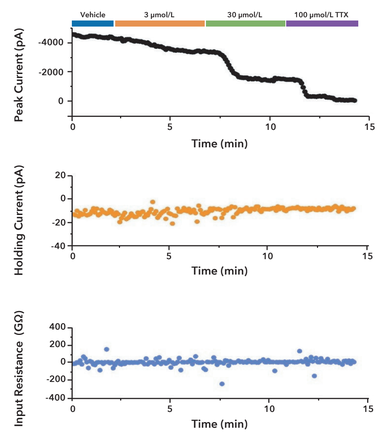
Table 2 Block of Compounds on Peak Nav1.5 Current

Compounds	Test Voncentrations (μmol/L)	IC_{50} Values (μmol/L)	Hill Coefficient
Ranolazine	30, 100, 300, 1000	334.10 ± 247.42	1.09 ± 0.90
Tetracaine	1, 3, 10, 30, 100	33.16 ± 13.47	0.81 ± 0.29
Flecainide	3, 10, 30, 100	15.95 ± 9.58	0.96 ± 0.59

Flecainide in Peak Nav1.5 Assay Current trace



I-t Plot

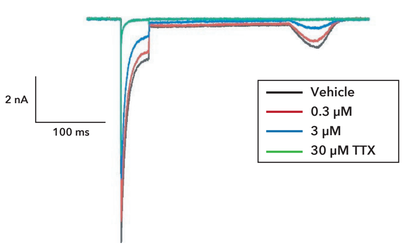


Late Nav1.5 Assay

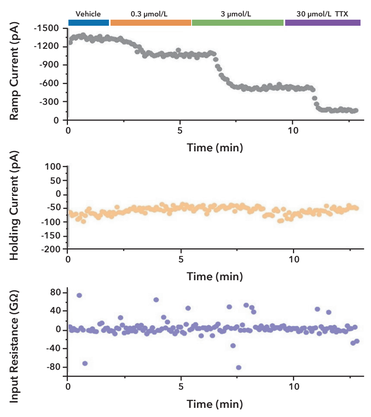
Table 3 Block of Compounds on Late Nav1.5 Current

Compounds	Test Voncentrations (μmol/L)	IC_{50} Values (μmol/L)	Hill Coefficient
Ranolazine	3, 10, 30, 100	22.83 ± 6.52	1.09 ± 0.34
Tetracaine	0.03, 0.1, 0.3, 1, 3	0.54 ± 0.41	1.65 ± 1.71
Flecainide	0.3, 1, 3, 10	1.37 ± 0.34	0.98 ± 0.25

Flecainide in Late Nav1.5 Assay Current trace



I-t Plot

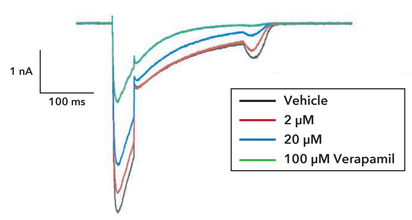


Cav1.2 Assay

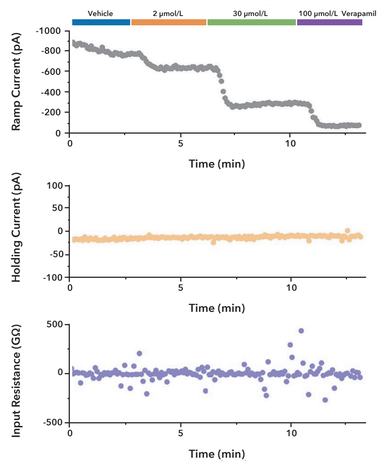
Table 4 Block of Compounds on Cav1.2 Current

Compounds	Test Voncentrations (μmol/L)	IC_{50} Values (μmol/L)	Hill Coefficient
Verapamil	2, 6, 20, 60	3.69 ± 2.55	0.97 ± 0.72
Cisapride	0.6, 2, 6, 20	2.08 ± 0.76	0.79 ± 0.27
Quinidine	6, 20, 60, 200	22.57 ± 21.27	0.64 ±0.52

Verapamil in Cav1.2 Assay Current trace



I-t Plot



Conclusion

All the results were comparable with the data reported in the literatures, which indicates these multi-ion channel inhibition assays were well validated. The CiPA multi-ion channel inhibition assays following best practice considerations can minimize the inter-lab variations, help evaluate potential effects on the cardiac action potential and estimate the proarrhythmic risk in patients more accurately.