Verification of CiPA Recommended Voltage Protocols in Patch-Clamp Assay for hERG, Cav1.2 and Late Nav1.5 Currents

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Introduction

As revising an ICH regulatory guideline, S7B, for the Non-clinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation by Human Pharmaceuticals) was suggested, Comprehensive *In vitro* Proarrhythmia Assay (CiPA) activities are being implemented. The suggested S7B revision includes an assay of cardiac ion channels other than hERG, and CiPA-recommended voltage protocols for patch clamping was recently announced. We, Group 5 of the JSPS iSmart (investigation of *in silico* / *in vitro* model for arrhythmogenic risk prediction) play a role of wet-data collection. This time we have investigated the applicability of those protocols.

Materials & Methods

Manual patch clamp and an automated patch-clamp system, QPatch, were selected as the platforms. The manual system was used for hERG dynamic protocol (physiological temperature) and Cav1.2 (room temperature); QPatch for hERG IC_{50} only protocol and late Nav1.5 (room temperature). Test compounds were selected from each of the low-, intermediate-, and high-risk categories of the "training drugs" in the CiPA *in-silico* model. Flecainide was also selected.

- CiPA compounds used:

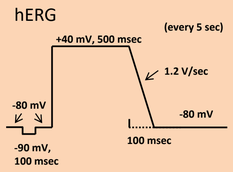
TdP risk	High	Intermediate	Low
CiPA drug	Bepridil	Astemizole	Ranolazine
	Dofetilide	Chlorpromazine	Verapamil
		Cisapride	

plus Flecainide

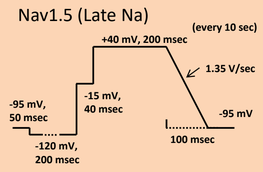
- Cell lines: hERG-HEK293/CHO-K1 cells, Nav1.5-HEK293/CHO-K1/CHL cells, Cav1.2-CHO.
- Patch-clamp conditions: identical to what the CiPA initiative announced unless otherwise stated.

➢ IC_{50} protocols

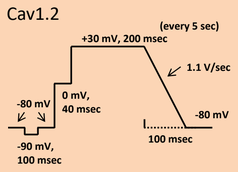
hERG



Nav1.5 (Late Na)



Cav1.2



➢ hERG dynamic model protocols

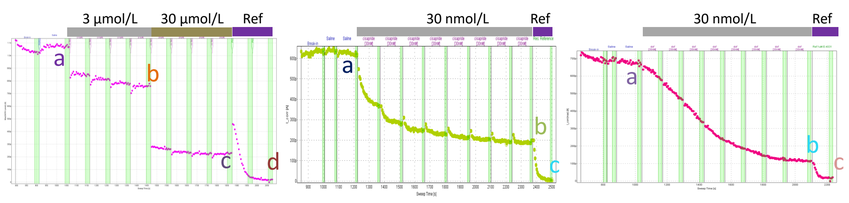
Results & Discussion

Results of hERG Assay on QPatch with IC_{50} Only Protocol

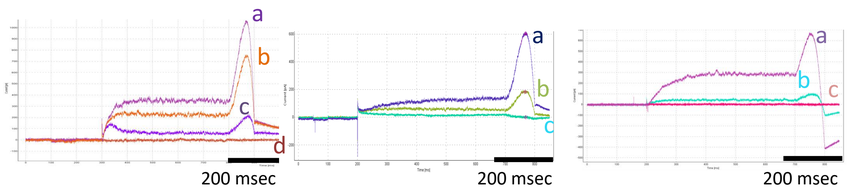
➢ I-t Plot

The compound was injected 8 times (single application) or 4 times (accumulative application) at every 20 pulses.

- Ranolazine
- Cisapride
- Dofetilide



➢ Current Traces



➢ Comparison with CiPA Data Set

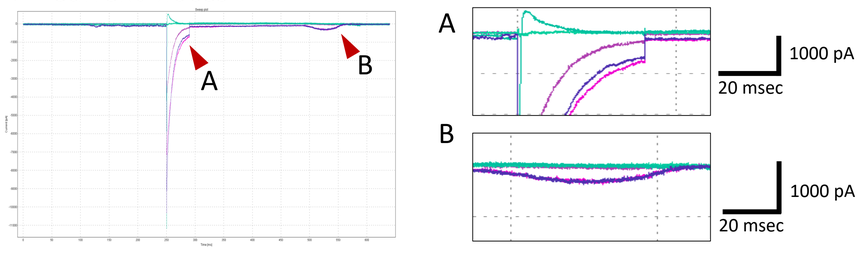
Drug	Site A IC_{50} (μmol/L)	Site A Hill	Site A n	Site B IC_{50} (μmol/L)	Site B Hill	Site B n
Bepridil	0.26	1.9	6-7	0.28	1.3	6
Dofetilide	0.015	1.5	6-8	0.011	1.8	6
Astemizole	0.026	1.4	6-9	0.026	1.3	6
Chlorpromazine	1.7	2.0	6-7	0.85	1.6	6
Cisapride	0.078	1.6	6-10	0.061	1.3	6
Ranolazine	9.0	0.92	6	9.0	0.91	6
Verapamil	0.90	2.1	7-10	0.63	1.2	6
Flecainide	1.8	1.0	7-8	1.2	0.98	6

Drug	Site C IC_{50} (μmol/L)	Site C Hill	Site C n	Site D IC_{50} (μmol/L)	Site D Hill	Site D n
Bepridil	0.031	1.3	6	0.10	1.6	7-8
Dofetilide	0.0095	1.2	6	0.014	1.8	6-8
Astemizole	0.0042	1.2	6	0.0066	1.00	6-9
Chlorpromazine	0.17	1.2	6	0.50	1.3	6-8
Cisapride	0.0080	0.93	6	0.019	0.93	6-11
Ranolazine	3.5	0.89	6	5.2	0.89	7-9
Verapamil	0.13	1.1	6	0.35	0.90	6-8
Flecainide	0.44	0.82	6	0.59	0.90	6-8

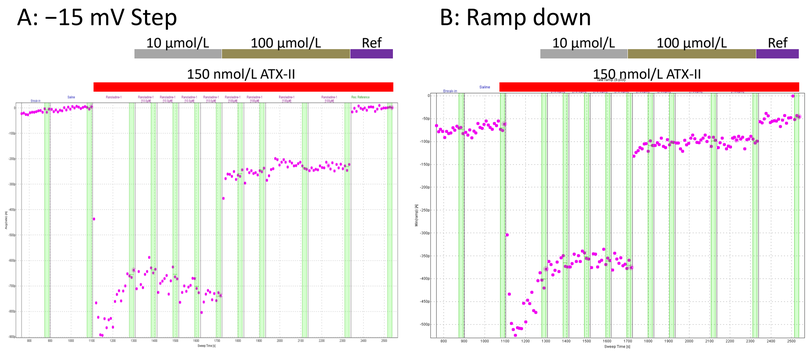
Drug	Li et al. 2018 IC_{50} (μmol/L)	Li et al. 2018 Hill	Crumb et al. 2016 IC_{50} (μmol/L)	Crumb et al. 2016 Hill
Bepridil	0.26	1.4	0.15	0.93
Dofetilide	0.013	1.4	0.0015	0.63
Astemizole	0.023	5.4	0.010	0.54
Chlorpromazine	0.85	1.7	1.12	0.90
Cisapride	0.071	1.9	0.012	1.3
Ranolazine	3.4	1.1	6.5	0.84
Verapamil	0.17	1.3	0.50	1.1
Flecainide	—	—	—	—

Results of Late Nav1.5 Assay on QPatch

➢ Current Traces



➢ Effect of Ranolazine on Late Nav1.5 Current

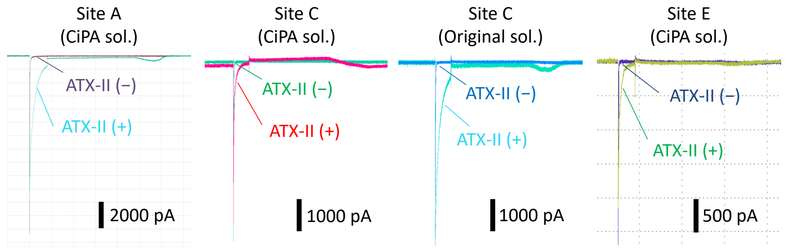


➢ Comparison with CiPA Data Set

Drug	Site A −15 mV Step IC_{50} (μmol/L)	Site A −15 mV Step Hill	Site A Ramp down IC_{50} (μmol/L)	Site A Ramp down Hill	n
Bepridil	1.9	1.5	1.2	0.5	3-5
Astemizole	1.7	1.8	1.1	1.3	3-6
Chlorpromazine	1.9	2.6	1.3	1.9	3-4
Ranolazine	52	1.7	29	1.7	7-8
Verapamil	14	1.4	6.3	1.3	5
Flecainide	1.5	1.4	1.3	1.0	3

Drug	Li et al. 2018 IC_{50} (μmol/L)	Li et al. 2018 Hill	Crumb et al. 2016 IC_{50} (μmol/L)	Crumb et al. 2016 Hill
Bepridil	0.34	1.9	1.8	1.4
Astemizole	0.60	3.1	10	2.3
Chlorpromazine	0.67	1.8	4.6	0.94
Ranolazine	6.0	0.99	7.9	0.95
Verapamil	0.98	1.2	24	2.0
Flecainide	—	—	—	—

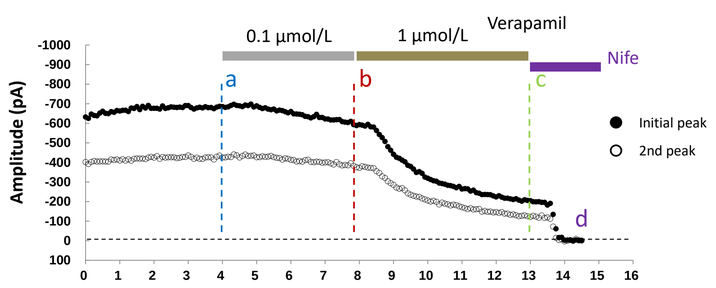
➢ Inter-facility Difference in Enhancement by ATX-II



The results show that enhancement of late Nav1.5 current by ATX-II differs between facilities and that a distinct combination of intra- or extra-cellular solutions (see below) sufficiently induced late Na current in an identical platform (site C) where late Na current could be hardly detected with CiPA conditions in the presence of ATX-II.
Note: Solutions (in mmol/L): EC, NaCl 145, KCl 4, $CaCl_2$ 2, $MgCl_2$ 1, glucose 10, HEPES 10, pH 7.4 with NaOH; IC, NaCl 10, CsF 135, EGTA 1, HEPES 10, pH 7.3 with CsOH.

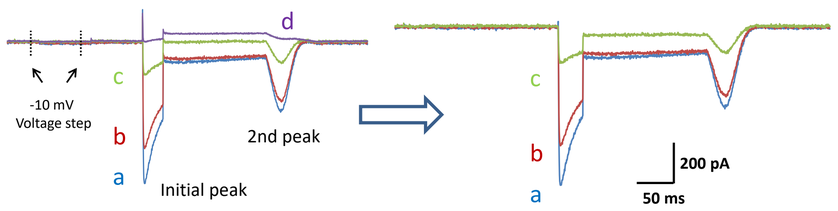
Results of Cav1.2 Assay in Manual Patch-clamp

➢ I-t Plot: Accumulative Application of Verapamil



➢ Analysis

- Cav1.2 current waveforms
- Subtraction with nifedipine treatment



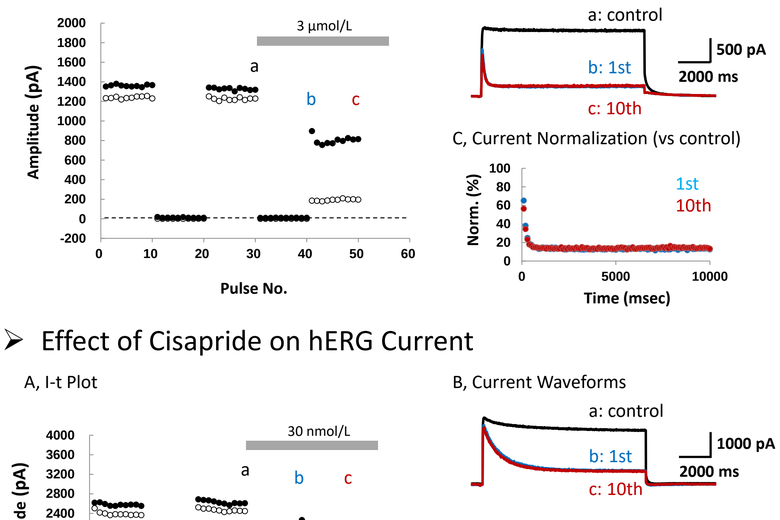
➢ Comparison with CiPA Data Set

Drug	Test conc. (μmol/L)	n	Initial peak IC_{50}	Initial peak Hill	2nd peak IC_{50}	2nd peak Hill	Manual*3 IC_{50}	Manual*3 Hill	HTS IC_{50}	HTS Hill
Bepridil	0.1, 0.3, 1, 3	4	0.392	1.08	0.365	1.22	2.82	0.65	638	4.6
Dofetilide#	100	4	>> 100*1	—	>> 100*2	—	44.5	3.6	2.30E+03	5.4
Astemizole#	0.03, 0.1, 0.3, 1	4	0.192	1.20	0.217	1.18	0.553	1.2	1.08	5.9
Chlorpromazine#	0.1, 0.3, 1, 3	4	0.741	1.59	0.728	1.98	8.32	0.85	6.35	2
Cisapride	0.3, 1, 3, 10	4	2.65	1.08	1.49	1.24	1.03E+03	4.8	4.05E+03	5.6
Ranolazine	30, 100, 300, 1000	4	514	0.99	324	0.92	900	3.9	6.54E+03	3.8
Verapamil	0.1, 0.3, 1, 3	4	0.387	0.98	0.509	1.02	0.204	1.1	11.2	0.8
Flecainide	3, 6, 30, 60	4	23.8	0.88	21.5	1.00	Crumb et al. 2016		Li et al. 2018	

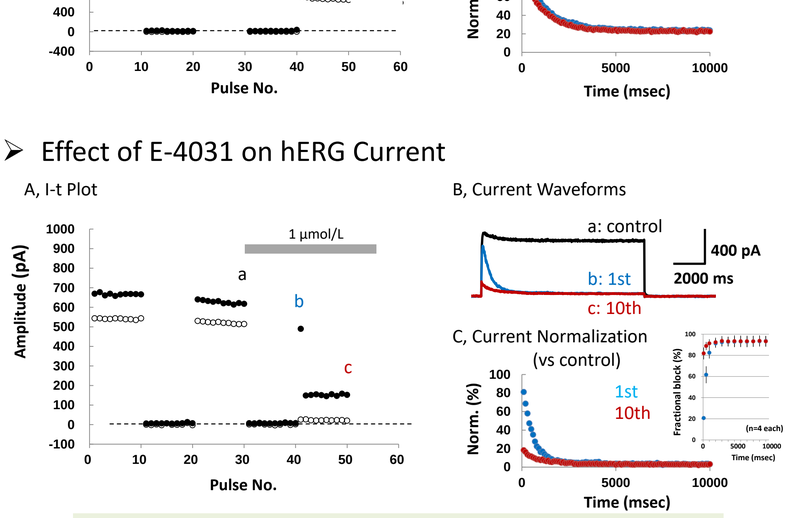
*1: 8.6% at 100 μmol/L. *2: 5.7% at 100 μmol/L. *3: Action potential protocol, Ba^{2+} as charge carrier, at physiological temperature.
#: Each cell was exposed to single drug concentration.

Results of hERG Dynamic Protocol

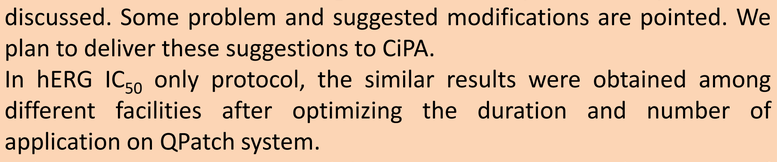
➢ Effect of Verapamil on hERG Current



➢ Effect of Cisapride on hERG Current

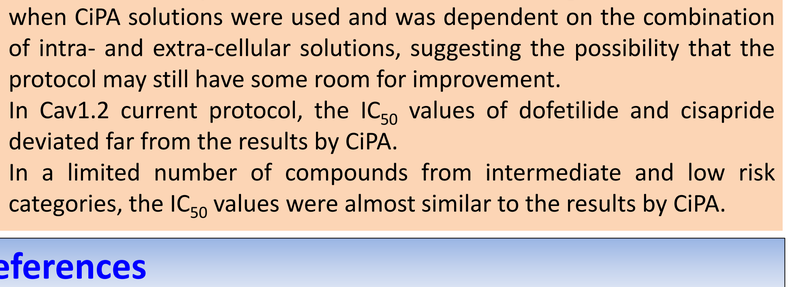


➢ Effect of E-4031 on hERG Current



Suppression rate (%) : 80.3 ± 4.8 (for initial peak), 91.5 ± 5.3 (for steady state)

➢ Concentration-dependent Progressive Block



➢ Comparison with CiPA Data Set

Drug	Test concentration (μmol/L)	n	Initial peak IC_{50} (μmol/L)	Initial peak Hill	Steady state IC_{50} (μmol/L)	Steady state Hill	IC_{50} (μmol/L)	Hill
Cisapride	1, 3, 10, 30*	3 or 4	NA	NA	10.8*	1.11	10.1*	0.7
Ranolazine	1, 3, 10, 30	3 or 4	13.5	0.67	8.50	0.89	8.27	0.9
Verapamil	0.1, 0.3, 1, 3	4	2.48	0.60	0.369	0.85	0.288	1
Flecainide	0.3, 1, 3, 10	4	2.47	0.65	1.16	0.86	Li et al. 2017, 2018	

*: Cisapride concentrations are shown in nmol/L. NA: Not applicable.

Conclusions

- Some of our data which disagree with those presented by CiPA are discussed. Some problem and suggested modifications are pointed. We plan to deliver these suggestions to CiPA.
- In hERG IC_{50} only protocol, the similar results were obtained among different facilities after optimizing the duration and number of application on QPatch system.
- In late Nav1.5 current protocol, it was turned out that enhancement of late Na current by ATX-II was not consistent among different facilities when CiPA solutions were used and was dependent on the combination of intra- and extra-cellular solutions, suggesting the possibility that the protocol may still have some room for improvement.
- In Cav1.2 current protocol, the IC_{50} values of dofetilide and cisapride deviated far from the results by CiPA.
- In a limited number of compounds from intermediate and low risk categories, the IC_{50} values were almost similar to the results by CiPA.

References

- Recommended voltage protocols to study drug-cardiac ion channel interactions using recombinant cell lines
- Journal of Pharmacological and Toxicological Methods, Crunb etal., 2016
- Circ Arrhythm Electrophysiol, Li et al., 2017
- CLINICAL PHARMACOLOGY & THERAPEUTICS, Li et al., 2018

Acknowledgement

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