Safety Pharmacology Assessment of Cardiac Ion Channels by Manual Patch Clamp With CiPA Protocols

○ Hiroshi Matsukawa, Taku Izumi, Mao Yamaguchi, Satomi Tomizawa, Hironori Ohshiro
Higashimatsuyama Laboratory, Drug Safety Testing Center Co., Ltd., Saitama, Japan

Introduction

The ICH S7B and E14 guidelines, which focus on hERG channel block and QT prolongation, are highly sensitive but not very specific for early prediction of proarrhythmic risks. Opposed to those guidelines, the Comprehensive in vitro Proarrhythmia Assay (CiPA) was proposed to determine whether new drug candidates are proarrhythmic by integrating the drug effects on multiple human cardiac ion channels into a human cardiomyocyte model. This approach has a potential to improve sensitivity as well as specificity for proarrhythmic risks. Through the validation process of this model, the CiPA initiative has concluded that three ion channels, hERG, hNav1.5 (late current), and hCav1.2, have significant impacts on the action potential duration of cardiomyocytes. Many researchers worldwide have attempted for verification of the CiPA-recommended protocols of 2018, to our knowledge, yet few ion channel data are publicly available. The purpose of this research is to collect ion channel data with sufficient accuracy, faithfully based on the CiPA protocols, to fulfill precise proarrhythmic prediction. We performed block potency measurement with several CiPA training/validation drugs on our manual patch-clamp platform in HEK293/CHO cells expressing hERG, hNav1.5, and hCav1.2. Of the CiPA drugs, the following was selected: bepridil, dofetilide, chlorpromazine, cisapride, astemizole, ranolazine and verapamil; two or three drugs each from the high, intermediate, and low TdP risk categories. IC50 values / Hill coefficients were calculated from the results. We would like to present and discuss differences in the parameter sets from the CiPA datasets including high-throughput platform. In silico prediction will be performed with the data set obtained in the research.

Materials & Methods

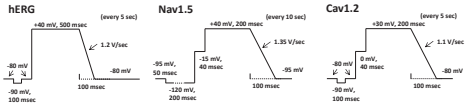
- **CiPA compounds used**:

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

plus Flecainide

- **Cell lines:** hERG-HEK293, Nav1.5-HEK293, Cav1.2-CHO.
- **Patch-clamp recording:** Voltage protocols, temperature, and ionic compositions of the solutions were fully identical to what the CiPA initiative announced. In a subset of assays, sucrose was added to the extracellular solutions for optimizing the osmotic balance.

➢ Voltage protocols

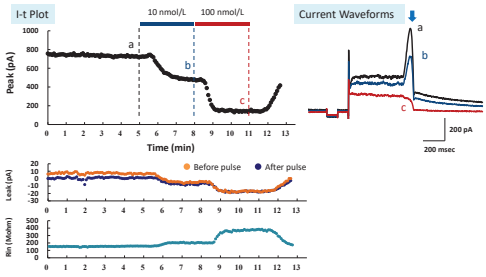


➢ hERG dynamic model protocols

Results

● hERG Assay with IC_{50} Only Protocol

➢ Cisapride



Note: Many cells showed relatively lower input resistance and outward leak currents in spite of the holding potential of -80 mV. When the cells were exposed to intensively inhibiting compounds, the input resistance was gradually increased along with decline of leak current to less than 0 pA.

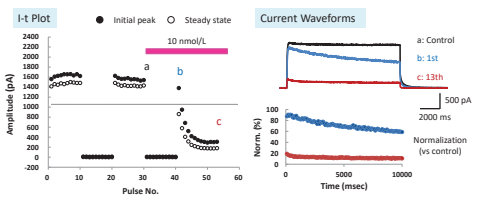
Table 1. Effects of CiPA Compounds on hERG Channel (IC_{50} only protocol)

TdP risk	Test concentration (nmol/L)	n	IC_{50}	Hill	CiPA data set Manual*1 IC_{50}	Manual*1 Hill	HTS*2 IC_{50}	HTS*2 Hill
Bepridil	3, 10, 30, 100	4	33.38	0.97	150	0.93	264	1.4
Dofetilide#	3, 10, 30, 100	4-6	21.67	1.29	1.47	0.63	13.4	1.4
Astemizole#	0.1, 0.3, 1, 3	4	1.00	0.99	9.99	0.54	23.3	5.4
Chlorpromazine#	100, 300, 1000, 3000	4	475.99	1.07	1.12E+03	0.9	850	1.7
Cisapride	3, 10, 30, 100	4	23.54	1.01	11.8	1.3	71	1.9
Ranolazine	3000, 10000, 30000, 100000	4	1.60E+04	0.81	6.52E+03	0.84	3.38E+03	1.1
Verapamil#	30, 100, 300, 1000	4-8	535.74	0.99	502	1.1	172	1.3
Flecainide	100, 300, 1000, 3000	4	871.70	1.05				

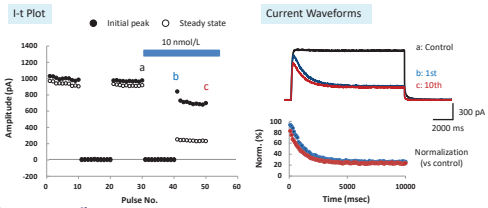
*1: At physiological temperature [Crumb et al. 2016]. *2: [Li et al. 2018]
#: Each cell was exposed to single drug concentration.

● hERG Assay with Dynamic protocol

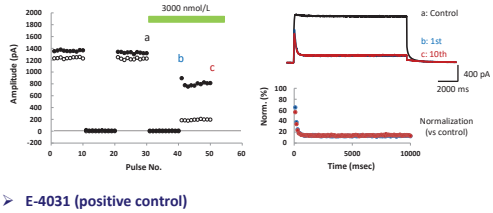
➢ Dofetilide



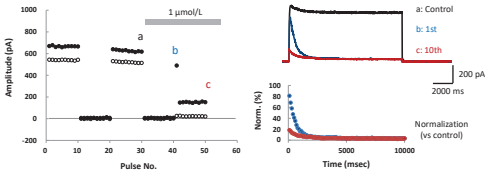
➢ Cisapride



➢ Verapamil



➢ E-4031 (positive control)



Suppression rate: 80.3% (initial peak) and 91.5% (steady state); n=3

Note: An appropriate amount of sucrose was added to the extracellular solution to adjust osmotic balance for the recording using hERG dynamic protocol.

➢ Fractional Block

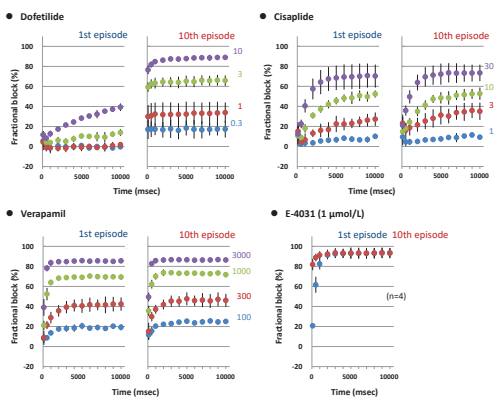


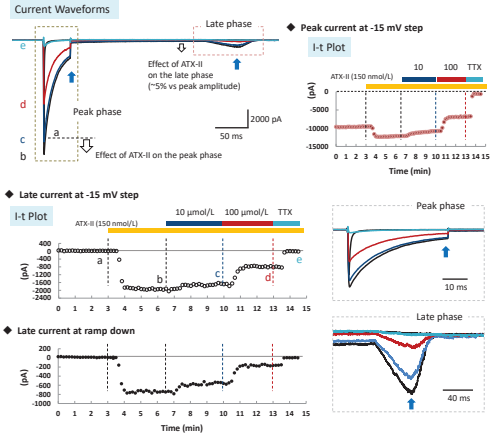
Table 2. Effects of CiPA Compounds on hERG Channel (Dynamic protocol)

TdP risk	Test concentration (nmol/L)	n	Initial peak IC_{50}	Initial peak Hill	Steady state IC_{50}	Steady state Hill	CiPA data set Manual*1 IC_{50}	Manual*1 Hill
Bepridil	3, 10, 30, 100	3	68.45	0.46	15.75	0.80	50	0.5
Dofetilide	0.3, 1, 3, 10	3	2.53	0.86	1.85	1.06	4.9	0.9
Astemizole	0.1, 0.3, 1, 3	3	0.77	0.84	0.57	0.95	---	---
Chlorpromazine	100, 300, 1000, 3000	3-4	574.86	0.77	466.40	1.00	929	0.8
Cisapride	1, 3, 10, 30	3-4	NA	NA	8.39	0.80	10.1	0.7
Ranolazine	1000, 3000, 10000, 30000	3-4	1.35E+04	0.67	8.50E+03	0.89	8.27E+03	0.9
Verapamil	100, 300, 1000, 3000	4	2.48E+03	0.60	369.12	0.85	288	1
Flecainide	300, 1000, 3000, 10000	4	2.47E+03	0.65	1.16E+03	0.86		

*1: [Li et al. 2017, 2018]. NA: Not applicable.

● Late Nav1.5 Assay Using ATX-II

➢ Ranolazine



Note: An appropriate amount of sucrose was added to the extracellular solution to adjust osmotic balance for the stable recording of late and peak Nav1.5 currents.

Table 3. Effects of CiPA Compounds on Late Current of Nav1.5 Channel

TdP risk	Test concentration (μmol/L)	n	-15 mV step IC_{50}	-15 mV step Hill	Ramp down IC_{50}	Ramp down Hill	CiPA data set Manual*1 IC_{50}	Manual*1 Hill	HTS*2 IC_{50}	HTS*2 Hill
Bepridil	0.1, 0.3, 1, 3	4	1.32	1.47	0.67	1.31	1.82	1.4	0.339	1.9
Dofetilide#	100	3	>> 100*3	NA	>> 100*3	NA	126	1.1	837	4.6
Astemizole#	0.1, 0.3, 1, 3	3	0.67	1.22	0.39	1.13	10.3	2.3	0.596	3.1
Chlorpromazine#	0.1, 0.3, 1, 3	4	2.35	1.67	0.76	1.30	4.59	0.94	0.673	1.8
Cisapride	0.3, 1, 3, 10	3	4.95	1.02	2.55	1.12	9.26E+03	6.3	0.421	2.2
Ranolazine	3, 10, 30, 100	3-4	82.54	1.31	38.32	1.05	7.94	0.95	5.95	0.99
Verapamil#	1, 3, 10, 30	3	18.90	0.94	8.47	0.95	24.1	2	0.982	1.2
Flecainide	0.3, 1, 3, 10	3-4	2.37	1.10	1.70	1.11				

*1: At physiological temperature [Crumb et al. 2016]. *2: [Li et al. 2018]. *3: 0.6% (-15 mV step) and 5.8% (ramp down) at 100 μmol/L.
#: Each cell was exposed to single drug concentration. NA: Not applicable.

● Peak Nav1.5 Assay

➢ Chlorpromazine

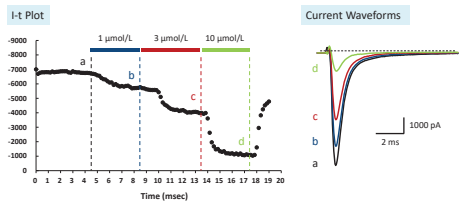


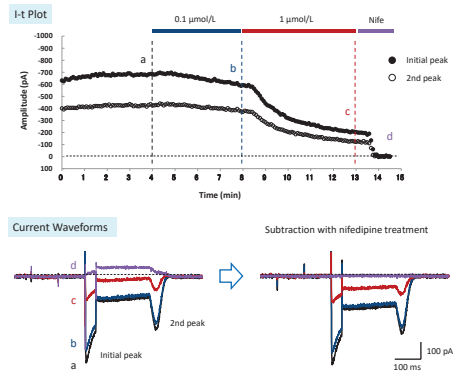
Table 4. Effects of CiPA Compounds on Peak Current of Nav1.5 Channel

TdP risk	Test concentration (μmol/L)	n	IC_{50}	Hill	CiPA data set Manual*1 IC_{50}	Manual*1 Hill	HTS*2 IC_{50}	HTS*2 Hill
Bepridil	0.3, 3, 10	4	2.07	1.08	2.96	1.2	1.61E+03	5.4
Dofetilide#	100	2	>> 100*3	NA	1.36	1.1	1.46E+03	5.1
Astemizole#	1, 3, 10	4	4.76	1.45	5.41	0.76	2.43	4.8
Chlorpromazine#	1, 3, 10	5	3.99	1.56	4.58	2.1	21.2	2.5
Cisapride	3, 10, 50	4	13.05	1.35	1.79E+03	0.67	16.8	2.3
Ranolazine	10, 100, 300, 1000	4-5	239.52	1.23	53.3	1.9	83.7	1.1
Verapamil#	10, 30, 100	4	41.43	1.19	2.59E+03	3.5	2.48E+03	5.1
Flecainide	3, 30, 100	4	24.28	1.12				

*1: At physiological temperature [Crumb et al. 2016]. *2: [Li et al. 2018]. *3: 4.4% at 100 μmol/L
#: Each cell was exposed to single drug concentration. NA: Not applicable.

● Cav1.2 Assay

➢ Verapamil



Note: In Cav1.2 current protocol, subtraction with a trace recorded in the presence of nifedipine was conducted for more accurate analyses.

Table 5. Effects of CiPA Compounds on Cav1.2 Channel

TdP risk	Test concentration (μmol/L)	n	Initial peak IC_{50}	Initial peak Hill	2nd peak IC_{50}	2nd peak Hill	CiPA data set Manual*1 IC_{50}	Manual*1 Hill	HTS*2 IC_{50}	HTS*2 Hill
Bepridil	0.1, 0.3, 1, 3	4	0.39	1.09	0.36	1.22	2.82	0.65	638	4.6
Dofetilide#	100	4	>> 100*4	NA	>> 100*4	NA	44.5	3.6	2.30E+03	5.4
Astemizole#	0.03, 0.1, 0.3, 1	4	0.19	1.20	0.22	1.18	0.553	1.2	1.08	5.9
Chlorpromazine#	0.1, 0.3, 1, 3	4-5	0.74	1.59	0.73	1.98	8.32	0.85	6.35	2
Cisapride	0.3, 1, 3, 10	4-5	2.65	1.08	1.49	1.24	1.03E+03	4.8	4.05E+03	5.6
Ranolazine	30, 100, 300, 1000	4-5	513.65	0.99	324.19	0.92	900	3.9	6.54E+03	3.8
Verapamil#	0.1, 0.3, 1, 3	4	0.39	0.98	0.51	1.02	0.204	1.1	11.2	0.8
Flecainide	3, 6, 30, 60	4	23.76	0.88	21.46	1.00				

*1: Action potential protocol, Ba2+ as charge carrier, physiological temperature [Crumb et al. 2016]. *2: [Li et al. 2018].
*3: 8.6% at 100 μmol/L. *4: 5.7% at 100 μmol/L.
#: Each cell was exposed to single drug concentration. NA: Not applicable.

Conclusions

- In hERG IC_{50} only and dynamic model protocols, the IC_{50} values were almost similar to the results by CiPA.
- In late and peak Nav1.5 current protocols, the IC_{50} values of dofetilide and cisapride deviated far from the results by CiPA.
- Also in Cav1.2 current protocol, the IC_{50} values of dofetilide and cisapride deviated far from the results by CiPA.
- In silico prediction will be performed with the data set obtained in the research, and the parameters in the analyses will be compared to CiPA work. In addition to CiPA drugs, the TdP risk of flecainide will also be estimated.

References

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

Acknowledgement

We would like to express our gratitude to iSmart C-Quest for their generous technical advices and constant discussions with respect to data acquisition and analyses.