Safety Pharmacology Assessment of Cardiac Ion Channels by Manual Patch Clamp With CiPA Protocols and *In silico* Analysis

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Introduction

The ICH S7B and E14 regulatory guidelines, which rely solely on hERG channel blocking and QT prolongation, are highly sensitive but not very specific for early prediction of proarrhythmic risks. To overcome this limitation of the current regulations, the Comprehensive in vitro Proarrhythmia Assay (CiPA) was proposed for determination of the proarrhythmic risk of new drug candidates by integrating the drug effects on multiple human cardiac ion channels into a human cardiomyocyte model. This approach has potential to improve the TdP risk prediction from candidate drug evaluations. Through its validation of the model, the CiPA initiative has concluded that three ion channels, hERG, hNav1.5 (late current), and hCav1.2, significantly affect the action potential duration of cardiomyocytes. The peak hNav1.5 current is also important as an initiator of action potentials. Since the first announcement of the CiPA recommended protocols in June 2018, many researchers across the globe have attempted to verify the CiPA protocols. However, to our knowledge, few ion channel results, collected under the CiPA conditions without modification, are publicly available. The purposes of the present research were 1) to collect ion channel data, generated with sufficient accuracy and based faithfully on the CiPA-recommended protocols, to provide precise proarrhythmic prediction, and 2) to ensure that the TdP risk prediction for each compound performed with our datasets is comparable to the risk produced by CiPA.

Materials & Methods

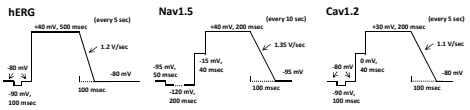
- **CiPA compounds used**:

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

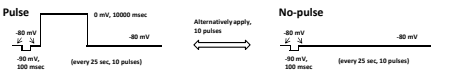
plus Flecainide

- **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.

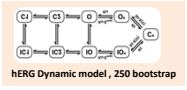
➢ Voltage protocols



➢ hERG dynamic model protocols

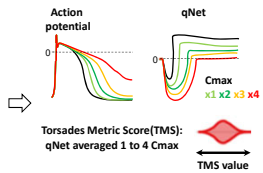


➢ In silico simulation: CiPA ORd 1.0



$$G_{(C)} = G_0 \cdot \frac{1}{1+\left(\frac{C}{IC_{50}}\right)^n}$$

Hill model: Cav1.2 & Late Nav1.5



Results

● hERG Assay with IC_{50} Only Protocol

➢ Cisapride (at room temperature)

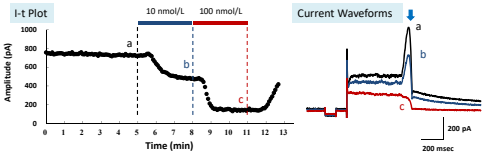
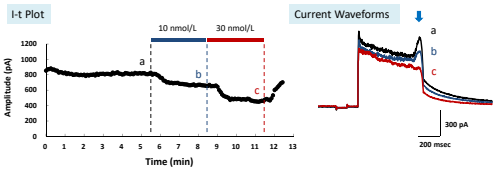


Table 1. Effects of CiPA Compounds on hERG Channel (IC_{50} only protocol)

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				

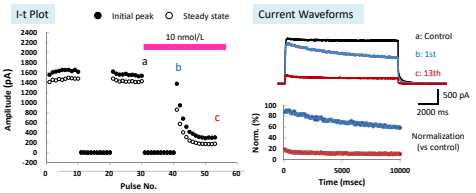
*1: At physiological temperature [Crumb et al. 2016]. *2: [Li et al. 2018]
#: Each cell was exposed to single drug concentration.

➢ Cisapride (at physiological temperature)



● hERG Assay with Dynamic Protocol

➢ Dofetilide



➢ Cisapride

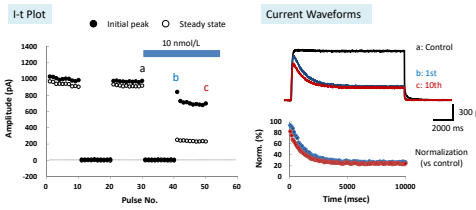
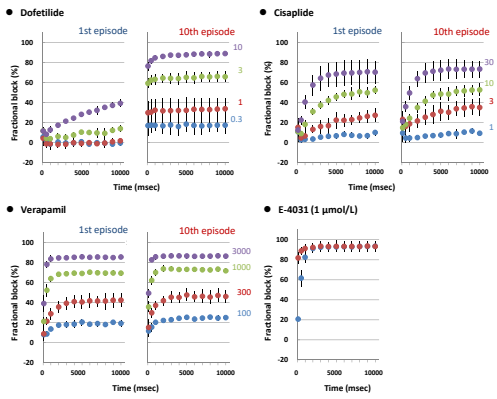


Table 2. Effects of CiPA Compounds on hERG Channel (Dynamic protocol)

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	4	74.15	0.48	17.27	0.83	50	0.9
Dofetilide	0.3, 1, 3, 10	4	2.89	0.87	2.11	1.09	4.9	0.9
Astemizole	0.1, 0.3, 1, 3	4	0.86	0.85	0.64	0.97	---	---
Chlorpromazine	100, 300, 1000, 3000	3-4	574.86	0.77	466.40	1.00	929	0.8
Cisapride	1, 3, 10, 30	4	450	0.42	8.98	0.83	10.1	0.7
Ranolazine	1000, 3000, 10000, 30000	4	1.34E+04	0.65	8.56E+03	0.86	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].

➢ Fractional Block



● Late Nav1.5 Assay Using ATX-II

➢ Ranolazine

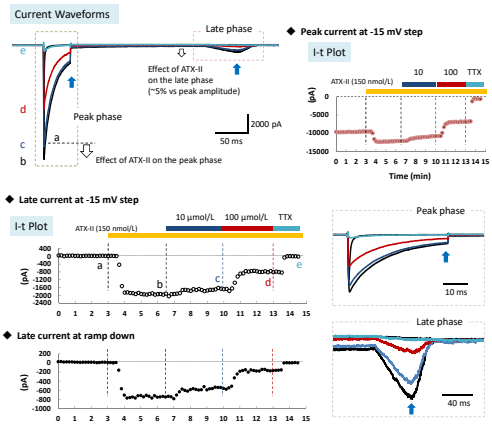
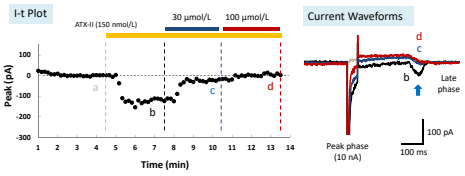


Table 3. Effects of CiPA Compounds on Late Current of Nav1.5 Channel

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	4	>> 100*3	NA	>> 100*3	NA	126	1.1	837	4.6
Astemizole	0.1, 0.3, 1, 3	4	0.67	1.22	0.41	1.19	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	4	5.51	1.07	2.59	1.15	9.26E+03	6.3	0.421	2.2
Ranolazine	3, 10, 30, 100	4-5	83.06	1.41	41.50	1.09	7.94	0.95	5.95	0.99
Verapamil	1, 3, 10, 30	4	17.77	0.97	7.81	0.96	24.1	2	0.982	1.2
Flecainide	0.3, 1, 3, 10	4	2.37	1.10	1.68	1.12				

*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.

➢ Ranolazine (at physiological temperature)



● Cav1.2 Assay

➢ Verapamil

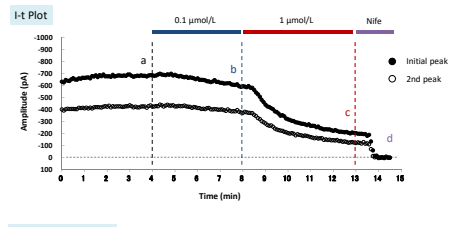
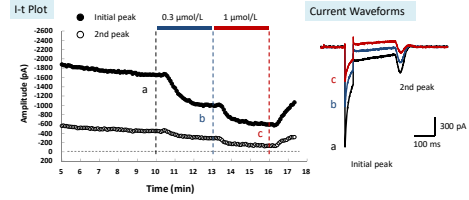


Table 4. Effects of CiPA Compounds on Cav1.2 Channel

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	0.63	638	4.6
Dofetilide#	100	4	>> 100*3	NA	>> 100*3	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				

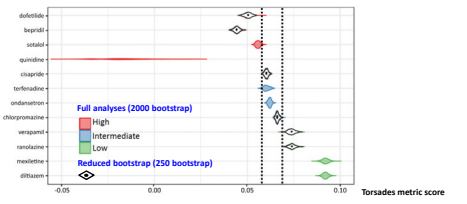
*1: Action potential protocol, Ba2+ as charge carrier, physiological temperature [Crumb et al. 2016]. *2: [Li et al. 2018].
*3: 8.6% at 100 μmol/L. *4: 5.7% at 100 μmol/L.
#: Each cell was exposed to single drug concentration. NA: Not applicable.

➢ Verapamil (at physiological temperature)

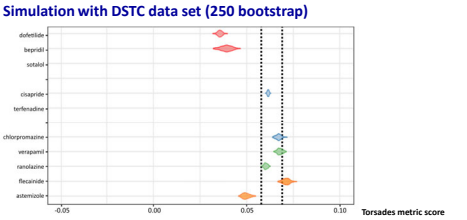


● *In silico* Analysis of Proarrythmic Risk (CiPA Ordv1.0)

➢ Simulation with CiPA data set (2000 bootstrap vs 250 bootstrap)



➢ Simulation with DSTC data set (250 bootstrap)



Conclusions

- IC_{50} only and dynamic hERG protocols showed similar results to the ones obtained by CiPA.
- Cav1.2 and Late Nav1.5 protocols for dofetilide and cisapride resulted in IC_{50} values that deviated far from CiPA's results.
- Our analyses proved that a reduced approach with CiPA Ordv1.0 could be enough to give satisfactory outcome. *In silico* prediction was performed with the data set obtained in the research, and our data were successfully integrated to run uncertainty propagation using the validated CiPA Ordv1.0 model for 8 compounds and 3 ion channels, showing the generated TMS values were useful *in silico* risk prediction.
- Differences in astemizole are most likely due to our hERG IC_{50} being ten times smaller than the one used in the CiPA Ordv1.0. Likewise, the deviated IC_{50} values of dofetilide/cisapride for late Nav1.5 and Cav1.2 could account for the differences with CiPA's results.
- With the data set that we generated in the research, a collaboration with the FDA/CiPA *in silico* working group was initiated, hoping to contribute to safety pharmacology.

References

- Recommended voltage protocols to study drug-cardiac ion channel interactions using recombinant cell lines (2018, 2019)
- 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

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