Implementation of hERG assay using CiPA recommended protocol – Assay development of ICH E14/S7B Q&As best practice *in vitro* hERG assay under GLP-compliance -

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Background

The new ICH E14/S7B Q&A 2.1 provides best practice recommendations for patch-clamp assays including hERG channel. hERG current data acquisition in line with best practice is encouraged for better translation to clinical findings, supporting integrated risk assessment. In this study, we evaluated the effects of positive control drugs, moxifloxacin, ondansetron, and dofetilide on hERG currents and further assessed intra-facility reproducibility for moxifloxacin, with the aim of establishing a GLP-compliant best practice *in vitro* hERG assay.

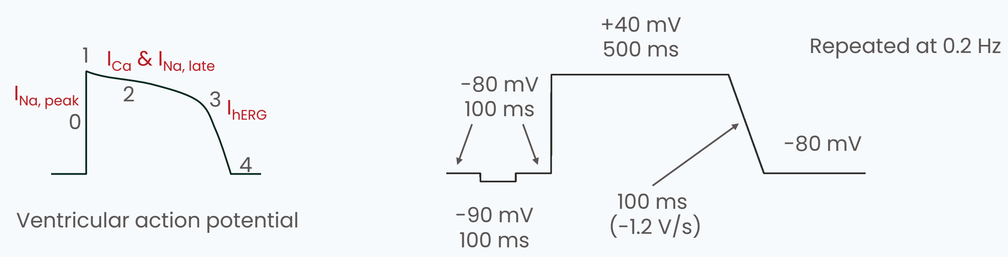
Materials & Methods

☐ **Cell line** : hERG-transfected HEK293 cells

☐ **Drugs** : Moxifloxacin, ondansetron, dofetilide, and ranolazine

☐ **Electrophysiology**

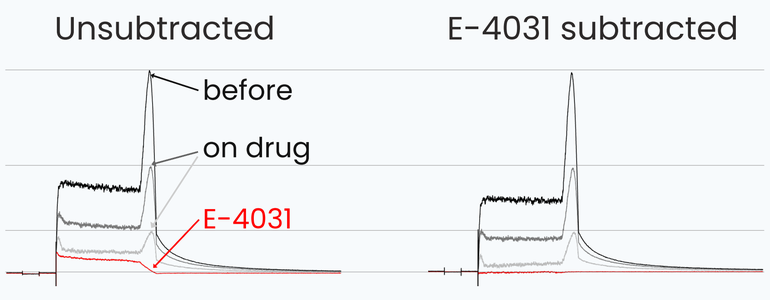
- Whole cell patch-clamp method (manual platform)
- Recording at near physiological temperature (36±1°C)
- External and internal solutions : -15 mV of junction potential; FDA best practice guidance [1)]
- Voltage protocol : FDA best practice guidance [1)]



1) : Recommended voltage protocols to study drug-cardiac ion channel interaction using recombinant cell lines

☐ **Measurment and analysis**

- After confirming a stable baseline for 2 minutes or more, individual drugs were applied to cells while giving the voltage protocol, and subsequently E-4031 was applied at a supra-saturating concentration (1 μmol/L).
- Current waveform recorded in E-4031 was subtracted from all recorded waveforms to isolate E-4031-sensitive hERG currents, and the resultant waveforms were used to calculate fractional inhibition by each drug concentration.



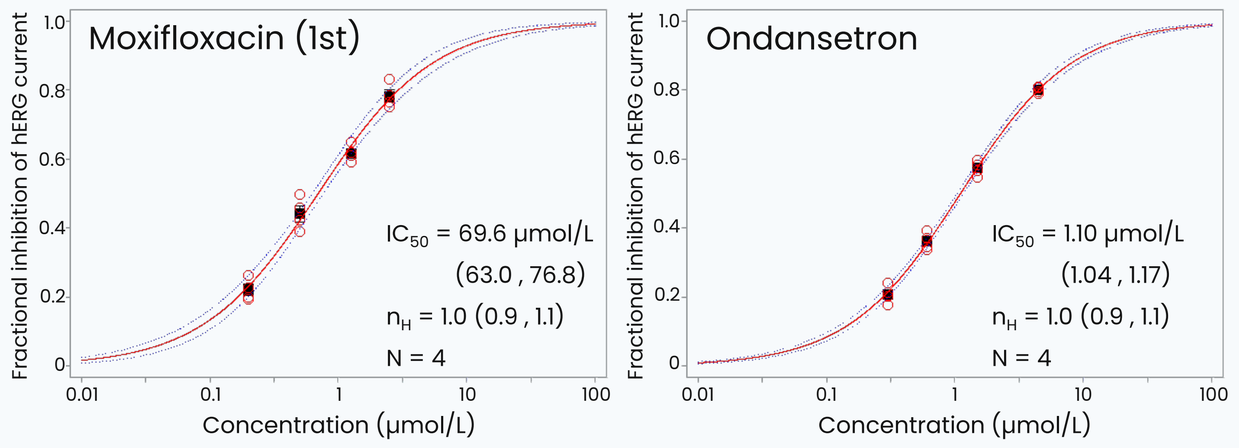
- The hERG current amplitude, input resistance, and holding current were monitored during the measurement.

Results

1. hERG current recordings on 3 positive control drugs

A. Representative hERG current waveforms from single cell. B. Time course plots of hERG current, input resistance, and holding current throughout recoding.

2. Concentration-response relationships



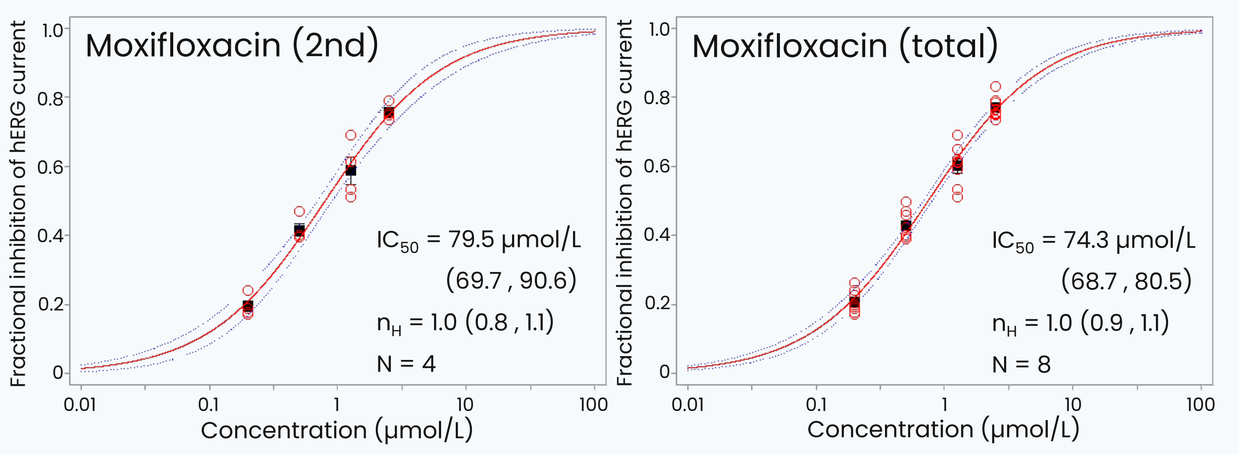
Note :
Vehicle application (N=4)

- 0.1% DMSO : % before, 93.6% at 12 min
- 0.3% DMSO : % before, 92.0% at 12 min

ICH E14/S7B Q&As Training Material Examples Supplemental File; Mar 2022	
Reference drugs	IC_{50} distribution (μM)
Moxifloxacin	62 (38, 104)
Ondansetron	1.4 (0.8, 2.6)
Dofetilide	0.01 (<0.01, 0.02)

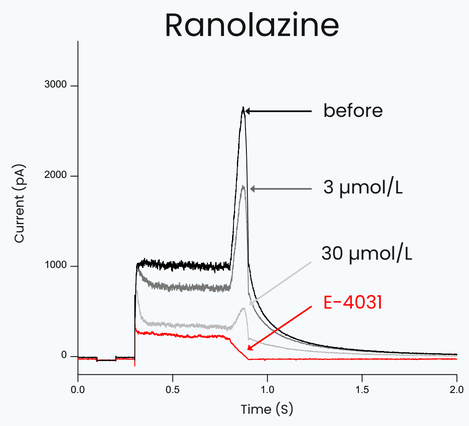
○ : individual data points — : fitted to Hill equation
■ : mean ± SEM ····· : upper / lower bound of 95% confidence interval (CI)

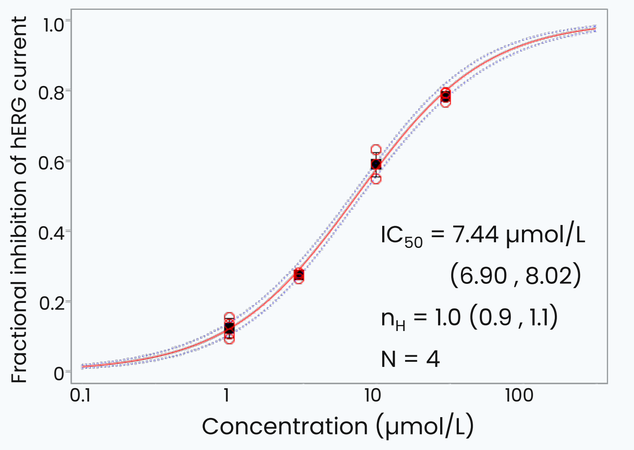
3. Repeat assay for moxifloxacin application*



* : Conducted 3 months later using different cell lot and stock solution (left). The ratio of IC_{50} values (1st/2nd) was 1.14. Data altogether (right).

4. hERG current recording on ranolazine





Research interest in ranolazine : hERG and late Na currents blocker.

5. Pooled hERG safety margin and threshold_ongoing initiative

hERG Safety Margin Threshold Defined by Reference Drugs					Across-laboratory data		Within-laboratory data	
Reference Drugs	In Vitro Assay	Critical Concentration (ng/mL) molar concentration (μM)	Protein Binding (%)	Mol Wt (g/mole)	IC_{50} Distribution (μM)	Safety Margin	IC_{50} Distribution (μM)	Safety Margin
Moxifloxacin	Protocol-001	1866 (1591, 2188) 4.65 (3.96, 5.45)	40 (37, 43)	401.44	62 (38, 104) (N=10)	23x (13, 39)	74.6 (69.8, 79.3) (N=2)	27x (20, 35)
Ondansetron		249 (152, 412) 0.849 (0.518, 1.40)	73 (71, 76)	293.37	1.4 (0.8, 2.6) (N=4)	10x (4, 27)	1.10 (N=1)	5x (3, 9)
Dofetilide		0.37 (0.24, 0.55) 0.000838 (0.000544, 0.00125)	64 (62, 66)	441.56	0.01 (<0.01, 0.02) (N=4)	44x (16, 117)	0.0117 (N=1)	39x (25, 63)
					Pooled Safety Margin for Reference Drugs	22x (9, 51)		17x (5, 60)*
					Threshold	>51x		>60x

*: A random effects meta-analysis is used to derive the pooled safety margin across trials and drugs; shown as mean (95% CI; upper, lower) not 50th (2.5th, 97.5th) percentile. It is NOT YET conclusive how to calculate our pooled safety margin. EZR (Saitama Medical Center, Jichi Medical University), a freely-available software, was used in this analysis.
ICH E14/S7B Q&As Training Material Examples Supplemental File, Table 1-C; Mar 2022 (modified).

Conclusions & Discussions

- The values of IC_{50} and Hill coefficient on 3 positive control drugs, moxifloxacin, ondansetron, and dofetilide, were comparable to the values described in ICH E14/S7B Q&As Training Materials, thereby developing the *in vitro* hERG assay in accordance with a GLP-compliant best practice in the present study.
- The ratio of IC_{50} values (1st/2nd) for moxifloxacin application was 1.14, confirming the unaltered assay sensitivity as well as high reproducibility across time.
- hERG current inhibition by ranolazine were also measured for future studies such as assessing proarrhythmic risk *in silico*.
- We are working on whether/how the pooled hERG safety margin and threshold can be determined with the limited number of assays we have conducted.