hERG，Na及びCa電流測定によるTdPリスク評価の検討

○吉川　公人，荒山　美波，武内　史英，松本　季男，大保　真由美，中川　宗洋　株式会社ＬＳＩＭ安全科学研究所

Investigation of TdP risk evaluation by hERG, Na, and Ca current measurement

○Kimihito Yoshikawa, Minami Arayama, Fumihide Bunai, Yoshio Matsumoto, Mayumi Obo, Munehiro Nakagawa, LSIM Safety Institute Corporation
If you have any questions, please contact the following: yoshikawa.kimihito@mr.medience.co.jp

Objective

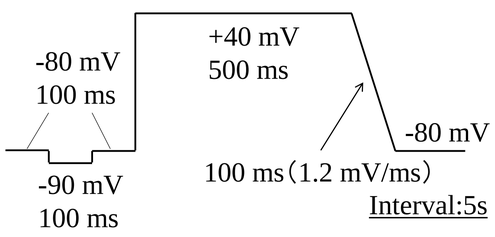
In drug development, it is essential to evaluate the risk of arrhythmia induced by drug candidate compounds. A safety pharmacological study as part of a nonclinical study evaluates the effect of QT interval prolongation, which has the risk of inducing lethal arrhythmia, Torsade de points (TdP), on an electrocardiogram. It is known that QT interval prolongation occurs when a compound inhibits the K current (hERG current) that passes through the hERG channel, which is one of the K channels expressed in the human heart. The effect of QT interval prolongation by a compound is evaluated by hERG current inhibition in a hERG study (*in vitro*) and the QT interval change in a telemetry study (*in vivo*). However, since the QT interval is affected not only by the hERG current but also other ion channel currents (Na and Ca current), hERG current inhibition does not always induce QT interval prolongation. Additionally, QT interval prolongation does not always induce TdP. Therefore, there is a concern that the development of useful compounds that do not cause TdP may have been omitted from drug development. Recently, comprehensive risk assessment, such as CiPA (Comprehensive In vitro Proarrhythmia Assay), has been proposed and discussed.
In addition to the hERG study, we have constructed a test system of Na and Ca current measurements and field potential measurement in human iPS-derived cardiomyocytes as a part of new test studies. Therefore, we attempted a multilateral analysis for TdP risk assessment using in vitro test systems based on the results of the study.

Material and Methods

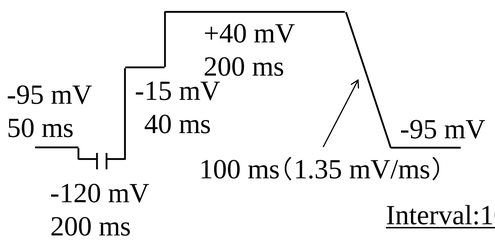
Ion current measurement

- Cells
  - hERG-HEK293 (Cytomycs)
  - Nav1.5-HEK293 (SB Drug Discovery)
  - Cav1.2-HEK293 (SB Drug Discovery)
- Whole cell patch-clamp method (manual)
- Recording temperature: ambient or physiological temperature
- External and internal solutions: followed the CiPA protocol (serum free)
- Evaluation parameter
  - hERG: peak current at ramp down phase
  - Late Nav1.5: peak current at -15 mV step and ramp down phase
  - Cav1.2: peak current at 0 mV step and ramp down phase

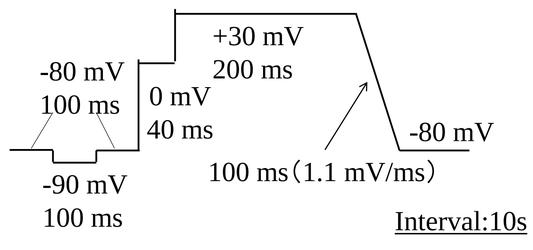
- **Voltage protocol**
  - hERG

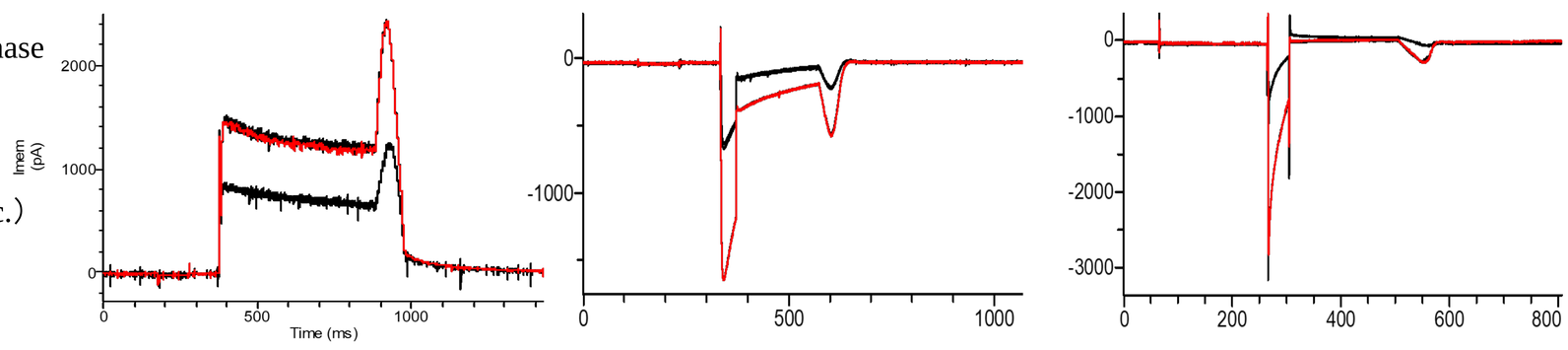


  - Late Nav1.5



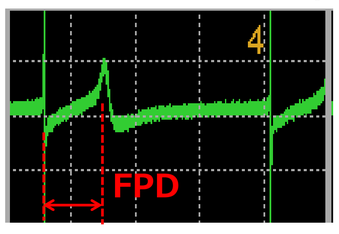
  - Cav1.2





Field potential measurement

- Cells: iCell cardiomyocyte（Cellular Dynamics International Inc.）
- Recording conditions: 37˚C and 5% CO_2
- Medium: iCell maintenance medium containing serum
- Evaluation parameter
  - Field potential duration corrected by Friderisia (FPDcF)



Test drug information in clinical

Drug		Free effective therapeutic plasma concentration (fETPC)*	TdP
Amiodarone	Antiarrhythmic drug	150 nmol/L	+ (4 cases in Japan)#
Verapamil		92 nmol/L	-
Ranolazine		2300 nmol/L	-

*: Ando et al. 2016, #: Nagao et al. 2011

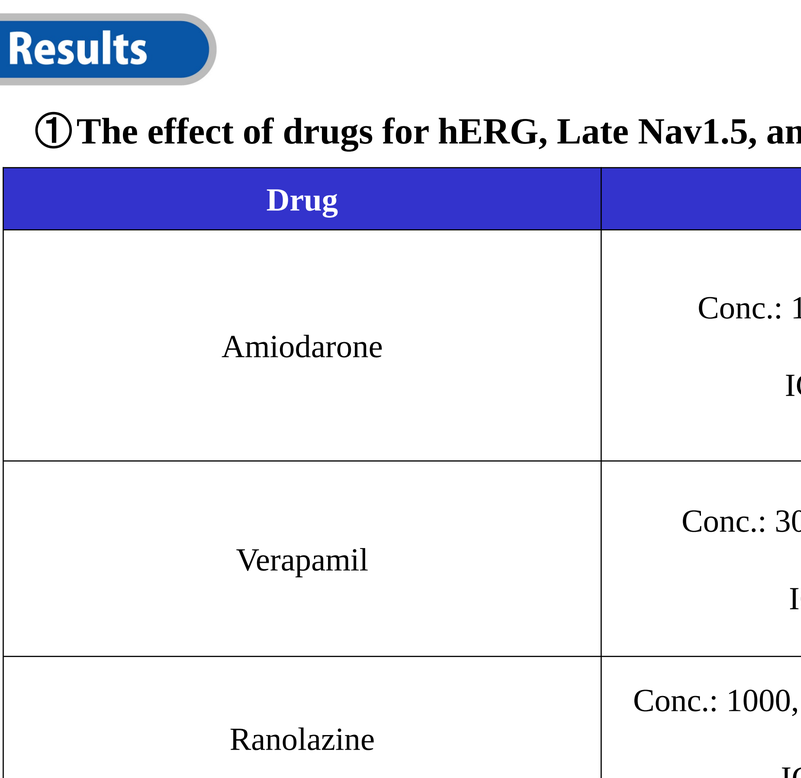
Results

①The effect of drugs for hERG, Late Nav1.5, and Cav1.2 current

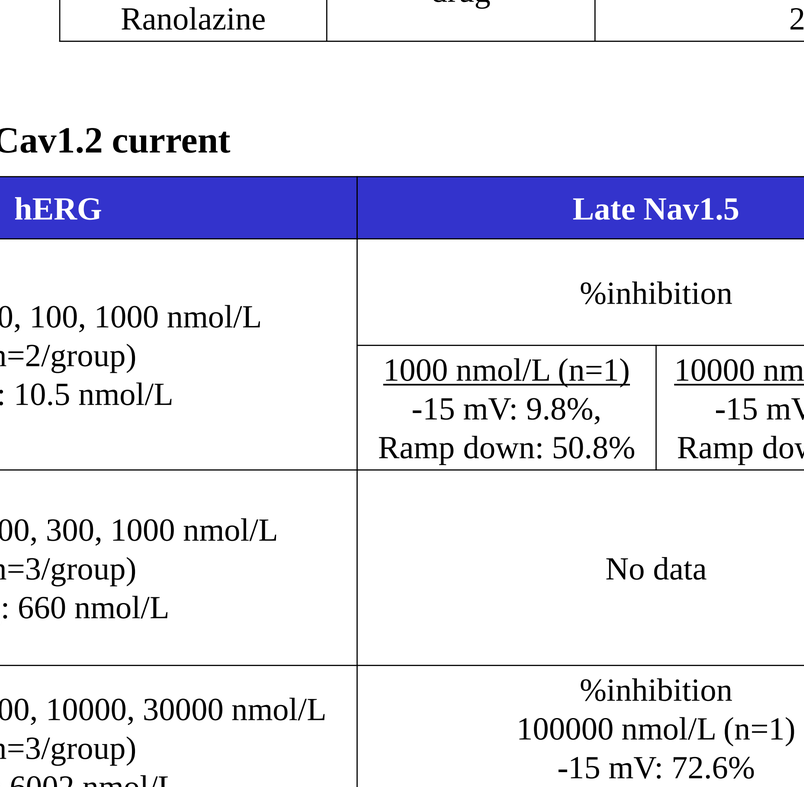
Drug	hERG	Late Nav1.5		Cav1.2	
Amiodarone	Conc.: 1, 10, 100, 1000 nmol/L (n=2/group) IC_{50}: 10.5 nmol/L	%inhibition		%inhibition (mean) 1000 nmol/L (n=3) 0 mV: 30.2% Ramp down: 63.2%	
		1000 nmol/L (n=1) -15 mV: 9.8%, Ramp down: 50.8%	10000 nmol/L (n=1) -15 mV: 35%, Ramp down: 87.8%		
Verapamil	Conc.: 30, 100, 300, 1000 nmol/L (n=3/group) IC_{50}: 660 nmol/L	No data		%inhibition (mean)	
				300 nmol/L (n=3) 0 mV: 62.2% Ramp down: 63.2%	10000 nmol/L (n=5) 0 mV: 61.4% Ramp down: 78.6%
Ranolazine	Conc.: 1000, 3000, 10000, 30000 nmol/L (n=3/group) IC_{50}: 6002 nmol/L	%inhibition 100000 nmol/L (n=1) -15 mV: 72.6% Ramp down: 75.6%		No data	

② The effect of drugs for FPDcF and BPM in iCell cardiomyocytes

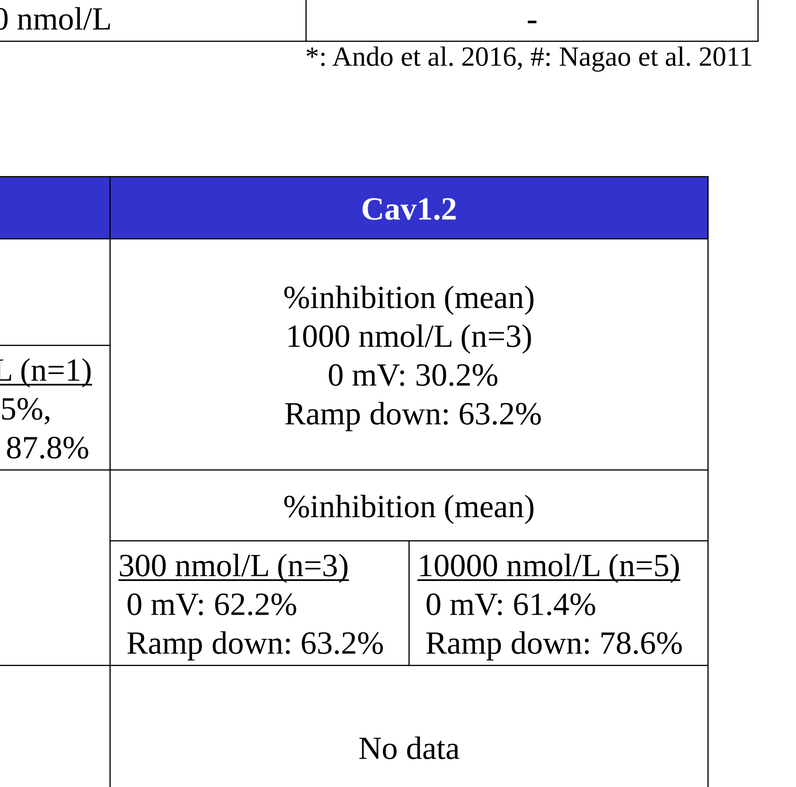
- Amiodarone

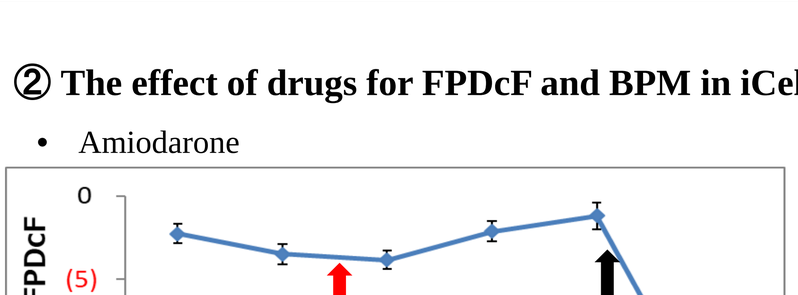


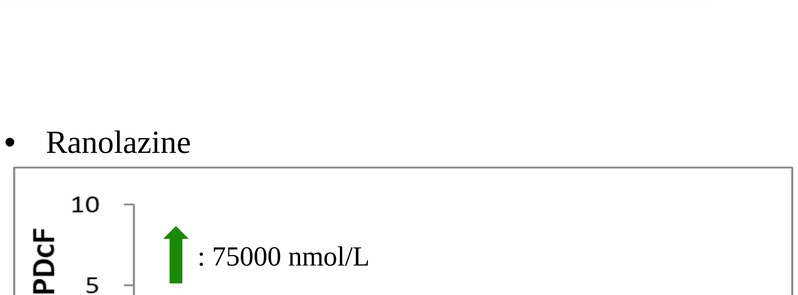
- Verapamil



- Ranolazine







:estimated free drug concentration in iCell medium corresponding to hERG IC_{50}

:estimated free drug concentration in iCell medium corresponding to Late Nav1.5 IC_{50} at ramp down (estimated)

:estimated free drug concentration in iCell medium corresponding to Cav1.2 IC_{50} at ramp down (estimated)

:estimated free drug concentration in iCell medium corresponding to fETPC

Conclusion

- The each ratio of hERG IC_{50} and Late Nav1.5 or Cav1.2 IC_{50} was about 100, 2, and 5 in Amiodarone, Verapamil, and Ranolazine, respectively.
- From each result of the IC50 ratio (above) and field potential measurement, the drug which similarly inhibited hERG and Late Nav1.5 or Cav1.2 current did not induce EAD corresponding to TdP in human in iCell.
- In field potential measurement with iCell, it is possible that proarrhythmic risk assessment can not be performed suitably in higher concentration due to arrest (ex: Ranolazine) or significant increase of BPM (ex: Verapamil).
- Therefore, it is considered that the proarrhythmic risk of the drug can be evaluated first by field potential measurement and additionally by ion channel current measurement if arrest or significant increase of BPM occurs in field potential measurement.