Usefulness of combining multi-ion-channel assays with the Langendorff assay in an assessment of proarrhythmic risks

DSTC
Drug Safety Testing Center

○ Mao YAMAGUCHI[1], Koji NAKANO[1], Satomi TOMIZAWA[1], Rie URA[1], Taku IZUMI[1], Hironori OHSHIRO[1], Hiroshi MATSUKAWA[1], Akihiro KANNO[1]

[1]*Drug Safety Testing Center Co., Ltd., Higashimatsuyama-shi, Saitama, Japan*

Introduction

Precise evaluation of cardiac safety in an early stage is essential for developing novel drugs. In particular, it is important to predict a possible risk for proarrhythmia in safety pharmacology testing. Prolongation of the QT interval, which is caused by blockade of the hERG channel, can lead to a life-threatening arrhythmia known as torsades de pointes (TdP). Although risk predictions by *in silico* model have been promoted, the models seem to have room for further improvement. Recently, the QT prolongation associated with blockade of other ion channels has also been discussed. So, we attempted to predict the proarrhythmic risks more accurately by combining ion-channel assays with the Langendorff assay. In this study, we tested 6 drugs, which had been evaluated in clinical QT prolongation assessments by IQ-CSRC*, using the manual patch-clamp technique and the Langendorff system.

* : Innovation and Quality in Pharmaceutical Development (IQ) and the Cardiac Safety Research Consortium (CSRC)

Materials & Methods

◆Drugs : 6 drugs which were used in the IQ-CSRC study

Drug	TdP Risk**
Dofetilide	⚠
Ondansetron	⚠
Moxifloxacin	⚠
Dolasetron	?
Quinine	⚖
Levocetirizine	none

⚠ : Known Risk of TdP
? : Possible Risk of TdP
⚖ : Conditional Risk of TdP

** : TdP risk categories by CredibleMeds (https://www.crediblemeds.org/)

◆Patch Clamp—Whole cell mode

▸Cell Lines : 7 stable cell lines

Cell Line	Supplier	Current
HEK-hNa$_V$1.5	SB Drug Discovery	$I_{Na,P}$, $I_{Na,L}$***
CHO-hCa$_V$1.2/β_2/$\alpha_2\delta_1$	ChanTest	I_{Ca}
CHO-hK$_V$4.3	B'SYS	I_{to}
CHO-hK$_V$1.5	CytoBioscience	I_{Kur}
HEK-hERG	University of Wisconsin-Madison	I_{Kr}
CHO-hK$_V$LQT1/mink	CytoBioscience	I_{Ks}
CHO-hKir2.1	B'SYS	I_{K1}

*** : Late Na^+ current ($I_{Na,L}$) was evoked by 50 μmol/L-veratridine.

◆Langendorff assay

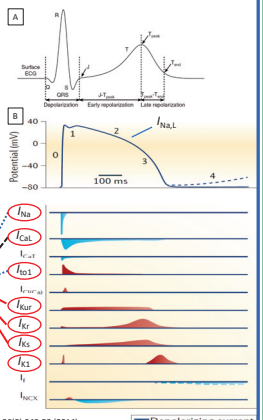
▸Animals : guinea pig

▸Measurement parameters :

PR, QRS, RR, QT, HR, etc.

QTc and J-T$_{peak}$c : Fridericia's formula

QTc or J-T$_{peak}$c = (QT or J-T$_{peak}$) / RR$^{1/3}$

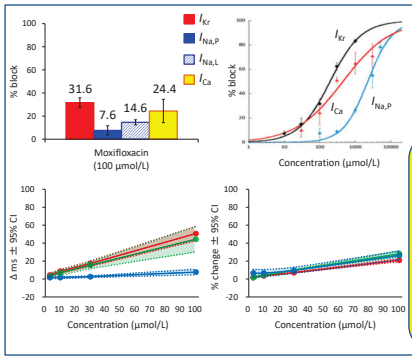


A : Johannesen *et al.*, Clin Pharmacol Ther. 96(5):549-58 (2014)
B : Gary Gintant *et al.*, Nat Rev Drug Discov. 15, 457–471 (2016)

Results

I_{Kr} inhibition : Moderate — I_{Kr} > I_{Ca} > I_{Na}

⚠ **Moxifloxacin**



: QTc with 95% CI
: J-T$_{peak}$c with 95% CI
: T$_{peak}$-T$_{end}$ with 95% CI

IC$_{50}$, μmol/L	
I_{Kr}	193.2
$I_{Na,P}$	2220.1
I_{Ca}	444.7

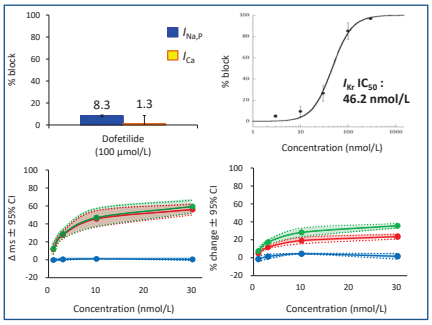
Moderate I_{Kr} blocking
+
I_{Ca}, $I_{Na,P}$ blocking
↓
QTc ↑
J-T$_{peak}$c ↑
T$_{peak-end}$ ↑
～20 %

Results

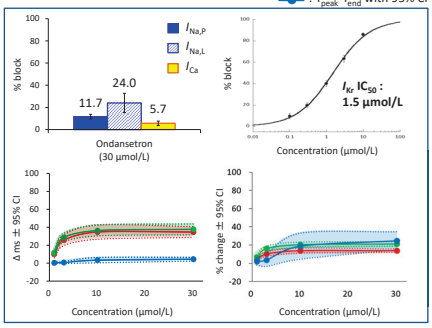
I_{Kr} inhibition : Strong — I_{Kr} >>> I_{Na}, I_{Ca}

: QTc with 95% CI
: J-T$_{peak}$c with 95% CI
: T$_{peak}$-T$_{end}$ with 95% CI

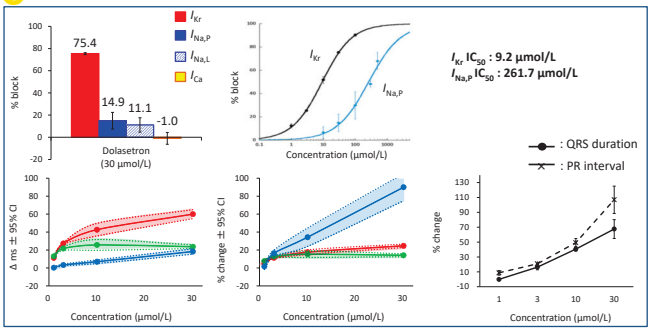
⚠ **Dofetilide**



⚠ **Ondansetron**



? **Dolasetron**

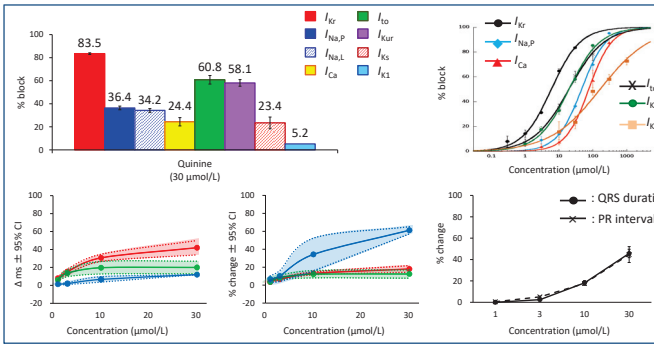


Strong I_{Kr} blocking
↓
QTc ↑
J-T$_{peak}$c ↑ 20～40 %
QRS ↑↑
PR ↑↑
↓
T$_{peak-end}$ ↑↑ ～80 %

I_{Kr} inhibition : Strong + Multichannel blocking

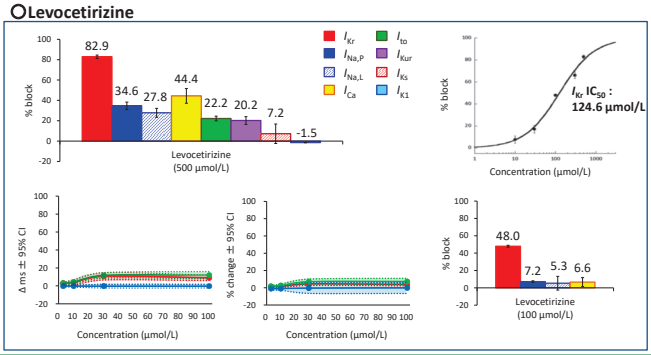
: QTc with 95% CI
: J-T$_{peak}$c with 95% CI
: T$_{peak}$-T$_{end}$ with 95% CI

Quinine



IC$_{50}$, μmol/L	
I_{Kr}	5.9
$I_{Na,P}$	46.1
I_{Ca}	75.2
I_{to}	19.6
I_{Kur}	19.7
I_{Ks}	162.8

○**Levocetirizine**



Strong I_{Kr} blocking
+
Multichannel blocking
↓
QTc ↑
J-T$_{peak}$c ↑ 10～20 %
QRS ↑↑
PR ↑↑
↓
T$_{peak-end}$ ↑↑ ～60 %

In the case of Levocetirizine...
I_{Kr} : block
↓
QTc ↑
J-T$_{peak}$c↑
T$_{peak-end}$ ↑

Conclusion

These results revealed that the proarrhythmic risks are highly predictable by combining ECG analysis in the Langendorff system with multi-ion-channel profiling. The present study provided an insight into usefulness of combining multi-ion-channel assay with Langendorff assay for an assessment of proarrhythmic risks. We concluded that this combination assays will greatly contribute to the drug discoveries and developments.

2017 Safety Pharmacology Society (SPS) Annual Meeting, September 2017, Berlin, Germany