Applicability of Ca transient measurements in cardiotoxicity screening

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Background and Objective

In drug development, predicting QT prolongation to minimize proarrhythmic risk, including Torsade de Pointes (TdP), is of critical importance. The CiPA initiative recommends QT/TdP risk assessment using multi-ion channel current assays and MEA analysis with iPSC-derived cardiomyocytes, since the hERG assay utilized alone is not fully predictive of QT and/or TdP risk. However, application of MEA assays in the drug discovery phase is limited by the long assay duration and the amount of compound required. Therefore, this study aimed to evaluate the applicability of Ca transient measurements as an alternative screening method to MEA. In Experiment 1, we evaluated the applicability of Ca transient measurements as an alternative screening method to MEA for assessing QT/TdP risk using the 28 CiPA validation compounds. In Experiment 2, we analyzed compounds other than the 28 CiPA and compared the in vitro Ca transient results with available in vivo and in vitro findings.

Materials and Methods

Table 1 Measurement Conditions

	Ca Transient	MEA
Cell Line	iCell® Cardiomyocytes 2 (FCDI, WI)	iCell® Cardiomyocytes (FCDI, WI)
Seeding Density	16,000 cells/well	30,000 cells/probe
Culture Format	384-well plate	MED-P515 probe
Measurement System	FDSS/µCELL (Hamamatsu Photonics, Japan)	MED64-Quad I (Alpha MED Scientific, Japan)
Analytical Parameter	PWD80cF (% change)	FPDcF (% change)
Detection Target	QT prolongation & EADs	QT prolongation & EADs

Experiment 1
The 28 CiPA compounds were evaluated in Ca transient and MEA assays under the conditions shown in Table 1.
The percent changes in each assay at concentrations near the hERG IC50s values were calculated. These concentrations were set at levels at which apparent QT prolongation and arrhythmias (EAD) occur.
The sensitivity and specificity for QT prolongation and EAD occurrence were also calculated.
The hERG IC50s values were measured using CHO-hERG Duo cells (B'SYS, Switzerland) with the SyncroPatch system (Nanion Technologies, Germany).

Experiment 2
E-4031, chromanol 293B, veratridine, (±)-bay K 8644, and mallotoxin were evaluated in Ca transient and MEA assays, and the results were compared with in vivo and in vitro findings.

Results 1

In Ca transient measurements, 15 of 19 high and intermediate-risk compounds showed 10% or greater PWD80cF prolongation, and EADs were observed in 17 of 19 compounds around the hERG IC50s. In contrast, 6 of 9 low-risk compounds showed no effect or shortening of PWD80cF. In MEA, 12 of 19 high and intermediate-risk compounds exhibited 10% or greater FPDcF prolongation, and EADs were observed in 9 of 19 compounds. All 9 low-risk compounds showed no effect or shortening of FPDcF (Table 2).

Table 2 Risk classification, hERG or Cav1.2 IC50 values, percent changes of repolarization phases, and EAD occurrence for 28 CiPA compounds in Ca transient and MEA measurements

Risk	Compounds	hERG IC50(µM)	CaV1.2 IC50(µM)	Ca Transient		MEA	
				PWD80cF (% change) around hERG IC50	EAD	FPDcF (% change) around hERG IC50	EAD
High (8)	Azimilide	0.166	19.2	21.0	✓	18.6	✓
	Bepridil	0.093	1.2	7.5	✓	2.1	
	Disopyramide	10.115	300	176.9	✓	39.3	
	D-l-sotalol	297.757	470.6	358.0	✓	49.9	✓
	Dofetilide	0.023	300	336.7	✓	59.0	✓
	Ibutilide	0.003	59.6	280.4	✓	31.7	✓
	Quinidine	0.428	17.4	59.8	✓	14.9	
	Vandetanib	0.213	7.9	12.3	✓	9.1	✓
Intermediate (11)	Astemizole	0.006	1.6	74.5	✓	17.5	✓
	Chlorpromazine	0.431	2.0	6.2		1.0	
	Cisapride	0.019	4.8	15.0	✓	11.9	✓
	Clarithromycin	86.612	300.0	144.4	✓	16.6	
	Clozapine	1.458	3.8	-10.8		-10.5	
	Domperidone	0.022	25.1	13.5	✓	5.5	
	Droperidol	0.014	12.1	6.6	✓	3.5	
	Ondansetron	1.024	300.0	105.5	✓	17.8	✓
	Pimozide	0.033	0.19	166.1	✓	32.1	
	Risperidone	0.186	87.9	116.4	✓	15.0	✓
	Terfenadine	0.063	0.57	21.1	✓	6.3	
Low (9)	Diltiazem	2.924	0.42	-36.6		-1.9	
	Loratadine	14.696	9.3	1.0		0.0	
	Metoprolol	170.907	300.0	88.1	✓	-4.7	
	Mexiletine	34.759	86.3	20.0	✓	7.8	
	Nifedipine	129.284	0.13	-39.1		-30.9	
	Nitrendipine	48.710	0.042	-43.0		-14.5	
	Ranolazine	5.179	300.0	10.6		8.3	
	Tamoxifen	1.702	3.4	3.4		2.2	
	Verapamil	0.479	0.17	-34.5		-41.5	

Table 3 Sensitivity and specificity for QT prolongation and EADs in Ca transients and MEA assays

QT prolongation	Sensitivity	Specificity	accuracy	EAD detection	Sensitivity	Specificity	accuracy
Ca-transient	80 %	67 %	76%	Ca-transient	91%	78 %	87%
MEA	65 %	100 %	77%	MEA	49 %	100 %	66%

The sensitivity values for QT prolongation and EAD detection were 80% and 91% in Ca transients, whereas the sensitivity values were 65% and 49% in MEA, respectively. The specificity values for QT prolongation and EAD detection were 67% and 78% in Ca transients, whereas the specificity values were 100% and 100% in MEA, respectively. The accuracy for QT prolongation was 76% in Ca transients and 77% in MEA. In contrast, the accuracy for EAD detection was 87% in Ca transients and 66% in MEA (Table 3). These results indicate higher sensitivity for Ca transients and higher specificity for MEA.

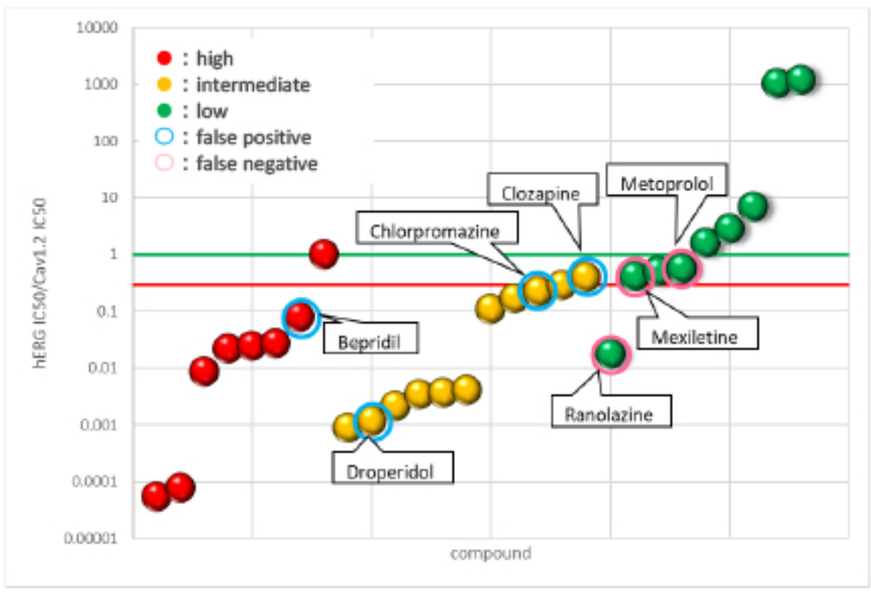

Fig. 1 Multi-ion channel-based risk classification of 28 CiPA Compounds.
The horizontal region between 0.3 and 1.0 indicates the threshold range for QT/EAD risk classification.

Clozapine and chlorpromazine were identified as false negatives in the Ca transient assay, whereas mexiletine and metoprolol were identified as false positives.
When analyzed using the hERG/Cav1.2 IC50s ratio in the multi-ion channel assay (MIC), these compounds were difficult to classify in terms of QT/EAD risk.
Ranolazine was separately determined to be a difficult-to-classify compound based on hERG/Nav late current analysis, whereas bepridil and droperidol were determined to be positive in the MIC analyses (Fig. 1).

Results 2

Table 4 Comparison of in vivo, Ca-transient, and MEA results for extra five compounds

Compounds	Pharmacological effects	*in vivo/in vitro* [1,2,3,4,5]		Ca Transient		MEA	
		QT interval	Arrhythmia	PWD80cF	EAD/ Arrest	FPDcF	EAD/ Arrest
E-4031	hERG/IKr block	Prolongation	✓	Prolongation	✓	Prolongation	✓
Chromanol 293B	IKs blockers	Prolongation	✓	Prolongation	–	Prolongation	–
Veratridine	blockade of Na^+ channel fast inactivation	Prolongation	✓	Prolongation	Arrest	Prolongation	✓
(±)-Bay K 8644	Ca enhancer	Prolongation	–	Prolongation	–	Prolongation	–
Mallotoxin	hERG enhancers	Shortening	✓ (VT)	Shortening trend	Arrest	Shortening trend	–

All five additional compounds showed the expected QT prolongation or QT shortening effects in both the Ca transient and MEA assays, and the results were consistent with findings from in vivo or in vitro animal studies. Arrhythmogenic activities, such as TdP and Ventricular Tachycardia (VT) that have been reported in animal studies, were not detected in the in vitro assays, except for E-4031 and veratridine (Table 4).

Conclusion

The Ca transient assay showed higher sensitivity and offers a shorter experimental period with smaller amounts of test compounds compared with the MEA assay, although it has lower specificity than the MEA assay.

Therefore, the Ca transient assay is applicable for minimizing proarrhythmic risk in the early stages of drug discovery.

In addition, the Ca transient assay and/or the MEA assay can predict effects consistent with those observed in currently used in vivo and in vitro animal assays for compounds with various pharmacological actions other than hERG blockade.

References

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