Refining *in vitro* QPatch cardiac ion channel and MEA iPSC-derived cardiomyocyte assays for CiPA

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Introduction

The Comprehensive *in vitro* Proarrhythmia Assay (CiPA) initiative is a new cardiac safety testing proposal sponsored by the FDA to refine the current ICH S7B and E14 guidelines. Two components of CiPA utilise *in vitro* electrophysiological assays that require validation using a toolbox of compounds with defined clinical proarrhythmic risk[1]. Here we outline our progress to optimise these electrophysiological assays to meet the CiPA goal of predicting human cardiac liability.

1. Optimisation of compound testing format to quantify the potency of CiPA toolbox test compounds using automated patch clamp (Sophion QPatch) assays (HTS sub-team).
2. Demonstration that proarrhythmic liability can be accurately predicted using induced pluripotent stem cell-derived cardiomyocytes (iPSC-CM) on a multi-electrode array (MEA) system (Axion Maestro).
   - CiPA toolbox compounds from each risk classification (low, medium, and high) were tested against two commercial iPSC-CM cell lines (Ncardia vCor.4U and Cellular Dynamics International iCell Cardiomyocytes^2).
   - Recommended FDA methodologies were followed for cell culture and compound application.
   - Metrion validation results were compared to published datasets.
3. Validation of MEA data by comparison to gold-standard manual patch clamp (MPC) electrophysiological recordings of cardiac action potentials (AP) made from the same iPSC-CM cell lines.

Materials and Methods

Automated Patch Clamp (APC): Potency testing of compounds was performed using the gigaseal QPatch48 platform (Sophion, Denmark) using the conventional whole-cell configuration and single hole chips. Standard recording solutions and voltage protocols in line with CiPA guidelines were used. In this study variations to the compound application paradigm were investigated.

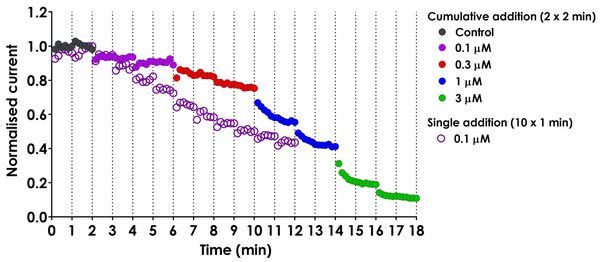
MEA Assay: Human iPSC-CM were obtained from Ncardia (vCor.4U) and Cellular Dynamics International (iCell cardiomyocytes^2) and seeded according to manufacturer's instructions onto Axion Maestro 96 well MEA plates. Plates were incubated at 37 °C (5 % CO_2) for 7-10 days and 100 % medium exchanges were performed every 2-3 days. Compounds were serially diluted in DMSO followed by a 1000-fold dilution into media. The final DMSO concentration did not exceed 0.1 %. Metrion's MEA experiments utilise serum-containing media for compound addition, this has been shown to be important for correctly identifying high risk compounds[2]. Compounds were tested using a cumulative acute application paradigm (30 min/conc) in accordance with FDA studies[3]. Parameters reported from Metrion's MEA assay were ≥ 10 % change in the Fridericia corrected field potential duration (ΔFPDc) and incidence of arrhythmic events (≥ 1 well).

Manual Patch Clamp (MPC): AP were recorded from iCell cardiomyocytes^2 7-10 days after cell seeding. Recordings were made at room temperature in current clamp mode using perforated patch (100 µg/ml gramicidin). Data were acquired with EPC10 amplifiers and PatchMaster software (HEKA Elektronik, Germany). Analog signals were low-pass filtered at 10 kHz before digitisation at 20 kHz. Spontaneous AP were analysed with CAPA software (SSCE UG, Germany).

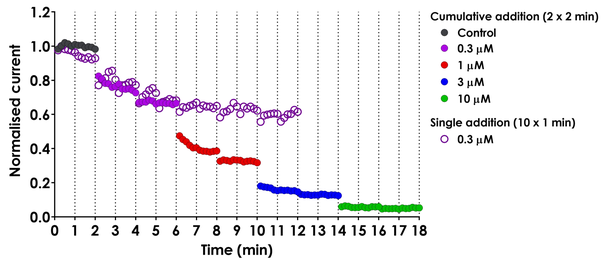
1. Optimising CiPA QPatch assays for reliable potency determination

Potency determination using QPatch requires attention to the on-rate of CiPA toolbox compounds

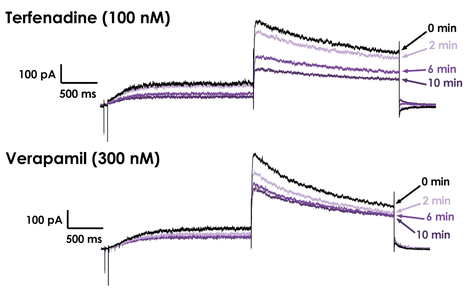
A) Time-dependent hERG block by Terfenadine



B) Time-dependent hERG block by Verapamil



C) Effects of single compound additions on hERG currents



D) hERG potency values in each testing format

Compound testing format	Potency IC_{50} (µM)	
	Terfenadine	Verapamil
Cumulative 2 x 2 min	0.56 ± 0.12	0.53 ± 0.16
Single addition 10 x 1 min	0.10	0.25

Figure 1: Comparison of two compound testing formats to determine the potency of slow and fast acting hERG channel blockers
Compounds with slower on-rates required longer incubation periods to reach steady state inhibition and their potency is underestimated with a standard cumulative concentration testing paradigm. **A-B:** Example normalised current vs. time plots for block of the hERG currents by Terfenadine and Verapamil obtained using either a cumulative (2 x 2 min additions of four escalating concentrations per cell) or single (10 x 1 min additions of one concentration per cell) compound testing format. **C:** Concordant current traces from single addition protocol showing the differences in time to reach steady state block at the IC_{50} concentration for Terfenadine and Verapamil. **D:** Table showing the potency values calculated using the testing formats; a single addition protocol leads to a composite IC_{50} calculated by aggregating inhibition values from single cells whereas the potency in a cumulative testing format is the mean (±SD) IC_{50} fit derived from N>3 individual cells

2. Prediction of proarrhythmic risk using iPSC-CM assays on a MEA system

MEA assays developed at Metrion align with CiPA risk classification and regulatory body publications

Compound	CiPA TdP Risk	ΔFPDc						Arrhythmia					
		Metrion vCor.4U	Metrion iCell^2	JiCSA iCell (4,5,6)	FDA iCell (3)	FDA Cor.4U (3)	FDA iCell (2)	Metrion vCor.4U	Metrion iCell^2	JiCSA iCell (4,5,6)	FDA iCell (3)	FDA Cor.4U (3)	FDA iCell (2)
Diltiazem	Low	↓	↓	↓	↓	↓	↓	-	-	-	-	-	-
Mexiletine	Low	↑	↑	↑	=	=	=	-	-	-	-	-	-
Ranolazine	Low	↑	↑	↑	↑	↑	↑	-	-	+	+	-	+
Verapamil	Low	↓	↓	↓	↓	↓	↓	-	-	-	-	-	-
Chlorpromazine	Medium	↑	↑	=	=	=	↑	+	-	+	-	-	+
Cisapride	Medium	↑	↑	↑	↑	↑	↑	+	+	+	+	-	+
Ondansetron	Medium	↑	↑	↑				+	+	+			
Terfenadine	Medium	↑	↑	↑	=	↑	↑	+	-	-	-	-	+
Bepridil	High	↑	↑	↑	=	↑	↑	-	+	-	-	-	-
D,L-Sotalol	High	↑	↑	↑				+	+	+			
Dofetilide	High	↑	↑	↑	↑	↑	↑	+	+	+	+	+	+
Quinidine	High	↑	↑	↑	↑	↑	↑	+	+	+	-	+	+

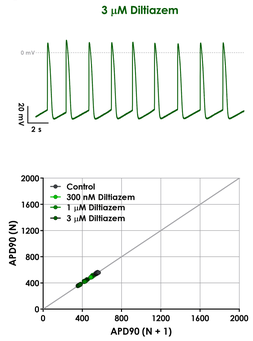
Key
↑ : Increased FPDc
↓ : Decreased FPDc
= : No change
+ : Arrhythmia/EAD events
- : No arrhythmic events

Table 1: Comparison of MEA iPSC-CM data obtained at Metrion to published data
Compounds from each CiPA risk classification were screened against vCor.4U and iCell cardiomyocytes^2 on the Maestro MEA using a cumulative acute application protocol[3]. Overall, good correlation was observed between our data and published datasets[2-6]. Our MEA assay correctly identified all low risk compounds as non-arrhythmic and all high risk compounds showed a significant FPDc prolongation. All high risk compounds resulted in arrhythmic events in both cell lines, with the exception of Bepridil which failed to generate EAD/arrhythmic events in vCor.4U cells.

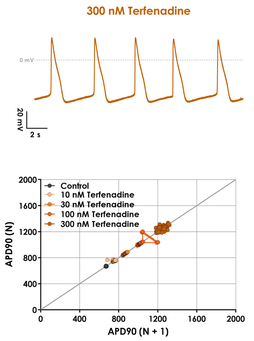
3. Manual patch clamp validation of MEA iPSC-CM data

Metrion's MEA results were verified by manual patch clamp AP data

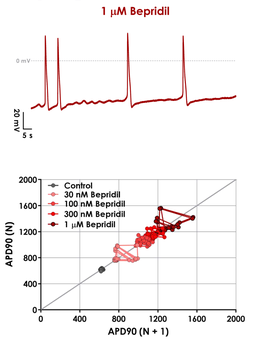
A) Low Risk



B) Intermediate Risk



C) High Risk



D) Comparison of MEA and MPC datasets

Compound	CiPA TdP Risk	iCell^2 ΔFPDc / ΔAPDc		iCell^2 Arrhythmia	
		MEA	MPC	MEA	MPC
Diltiazem	Low	↓	↓	-	-
Ranolazine	Low	↑	↑	-	-
Chlorpromazine	Medium	↑	↑	-	+
Terfenadine	Medium	↑	↑	-	+
Bepridil	High	↑	↑	+	+
D,L-Sotalol	High	↑	↑	+	-
Dofetilide	High	↑	↑	+	+
Quinidine	High	↑	↑	+	-

Key
↑ : Increased FPDc/APDc
↓ : Decreased FPDc/APDc
= : No change
+ : Arrhythmia/EAD events
- : No arrhythmic events

Figure 2: Comparison of MEA and manual patch clamp data recorded from CDI iCell cardiomyocytes^2
Compounds from each CiPA risk classification were screened against spontaneous AP recorded from iCell^2 using MPC. **A-C**: *Top panels* show representative AP in the presence of Diltiazem (green), Terfenadine (orange), and Bepridil (red). *Bottom panels* show Poincaré plots which indicate APD90 stability over the final 2 min of compound addition, with expected increase in variability with higher proarrhythmic risk. **D:** Table comparing the change in FPDc/APDc (MEA/MPC) and arrhythmic event incidence. Good correlation was observed across the two assays, with FPDc/APDc prolongation aligning for all compounds. Differences were observed for arrhythmic event detection, with MPC able to detect arrhythmias for medium risk compounds, supporting calls for the inclusion of MPC to confirm CiPA risk profiles.

Conclusions

Metrion show that the application of APC and cardiac electrophysiology expertise is required to optimise and validate *in vitro* CiPA cardiac safety assays capable of reliably predicting proarrhythmic risk:

1. **QPatch ion channel assays** - Close attention to compound testing format is required to assess the potency of CiPA toolbox compounds due to their slow on-rate on automated patch clamp systems.
2. **iPSC-CM MEA assays** - Utilisation of protocols matching those developed by the FDA resulted in data that aligned well to published findings[2] and highlights the importance of including serum during compound application.
3. **Manual patch clamp recordings** - Gold standard electrophysiology technique helped validate plate-based MEA screening data and identified arrhythmic events that went undetected for two compounds on the MEA platform.

References

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(3) Blinova *et al.* (2017) Toxicol Sci. **155**(1);234-247.
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(6) Nozaki *et al.* (2016) Regul Toxicol Pharmacol. **77**;75-86

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