Action Potential Waveform Analysis in Human iPSC-Cardiomyocytes Enables Mechanistic Assessment of Multichannel Cardiac Effects

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Background and Purpose

Recent regulatory changes, including the FDA Modernisation Act 2.0 and the UK government's "Replacing animals in science" strategy, have accelerated the adoption of human-relevant New Approach Methodologies (NAMs) or Non-Animal Alternatives (NAAs) for nonclinical safety assessment. In cardiac safety evaluation, reliance on single ion channel assays, such as the rapid delayed rectifier potassium channel hERG (K_V11.1), may fail to capture the integrated electrophysiological effects that underlie proarrhythmic risk.

Parameters derived from action potential waveform analysis, including triangulation and repolarisation reserve, are recognised as contributors to arrhythmogenic liability. Human induced-pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) provide a translationally relevant system in which integrated multi-ion channel cardiac effects can be assessed.

Aim

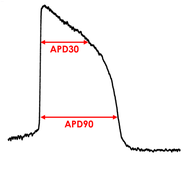
The aim of this study was to evaluate whether optical voltage imaging of hiPSC-CM action potential waveforms can detect compound-induced electrophysiological changes beyond hERG inhibition alone and enable mechanistic interpretation through correlation with ion channel electrophysiology data.

Materials and Methods

hiPSC-CMs (iCell²; Fujifilm CDI) were seeded into 96-well plates. After 8 days, cells were loaded with voltage-sensitive dye (BeRST[1]) and placed in the Lumencor VOLTA scanner, set to 28°C. Action potentials were measured optically for 40 s using fluorescence (10 kHz; excitation 660 nm/emission 680 nm). Compounds were applied (10 concs; 0.02 nM – 30 µM), incubated for 30 min, followed by a 40 s recording. Action potential duration at 30% (APD_{30}) and 90% repolarisation (APD_{90}), and beat rate were quantified using VOLTA analysis software. Clinical QTc risk analysis of $ycAPD_{90}{}^3$ was performed using the thresholds and equations according to Kilfoil *et al.*, 2021[2]. Triangulation was calculated per well as APD_{30}/APD_{90} and normalised to vehicle (0.1% DMSO), where a decrease in ratio indicates an increase in triangulation (**Figure 1 a**).

Whole-cell voltage clamp experiments were performed at 23°C on CHO cells stably expressing hERG using the Sophion QPatch48 platform. Recording solutions: extracellular (in mM) NaCl 140, KCl 2, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 10, glucose 5; intracellular (in mM) KF 120, KCl 20, HEPES 10, EGTA 10. Currents were elicited using the voltage protocol shown in **Figure 1 b**.

1 a



1 b

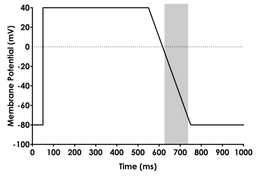


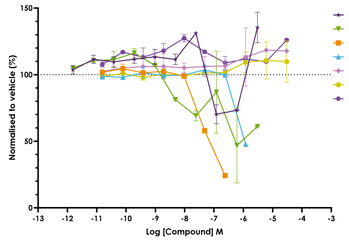
Figure 1.
a) Example hiPSC-CM action potential showing APD_{30} and APD_{90}.

b) QPatch hERG voltage protocol used in this study

Results

Figure 2. Effect of compounds on APD_{30}

2 a



2 b

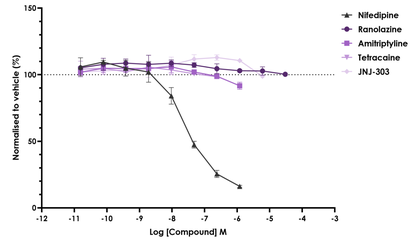
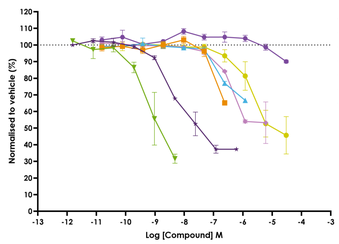


Figure 3. Effect of compounds on triangulation ratio (APD_{30}/APD_{90})

3 a



3 b

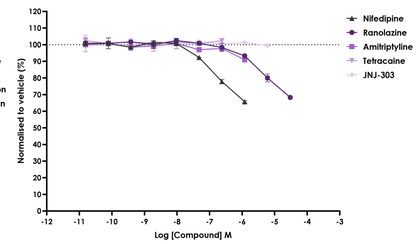
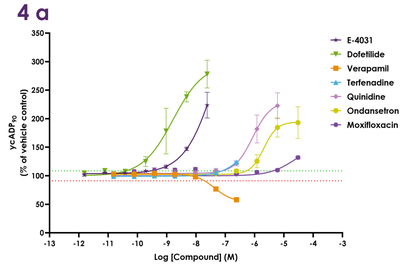


Figure 4. Effect of compounds on $ycAPD_{90}{}^3$

4 a



4 b

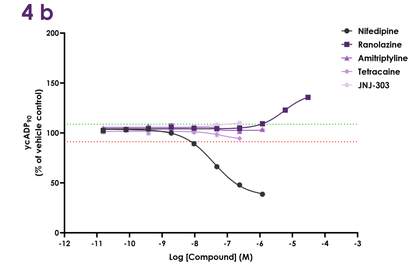


Table 1. Summary of estimated clinical QTc risk

Compound	$ycAPD_{90}{}^3$ – Exposure estimated to produce 10 ms increase in clinical QTc (nM)	Expected Free Therapeutic Plasma Conc. (EFTPC; nM)[4]	Ratio of estimated exposure to EFTPC
E-4031	0.11	-	-
Dofetilide	0.05	0.3	0.17
Verapamil	-*	-	-
Terfenadine	37.80	-	-
Quinidine	46.94	-	-
Ondansetron	272.97	229.5	1.19
Moxifloxacin	3565.64	2792	1.28
Nifedipine	-*	-	-
Ranolazine	792.78	-	-
Amitriptyline	-	-	-
Tetracaine	-	-	-
JNJ-303	30.24	-	-

An estimation of clinical endpoints using methods derived from a previous comprehensive translational analysis[2]
*Shortened $ycAPD_{90}$

Table 2. Summary hERG ion channel data

Compound	Mean IC_{50} (µM)	Inhibition at max. conc. (%)	Max. conc. (µM)	N
Dofetilide	0.014	95.4	0.1	12
Verapamil	0.674	94.0	10	16
Terfenadine	0.116	97.2	0.3	11
Quinidine	1.15	91.4	10	16
Ondansetron	5.22	68.2	10	12
Moxifloxacin	82.80	55.7	100	16

Results Summary

- Compound-mediated effects on APD_{30} were more consistent and more pronounced for some compounds, such as Verapamil and Nifedipine, indicating reduced inward calcium current (**Figure 2 b**).
- The selective hERG inhibitors, Dofetilide and E-4031, produced an early and pronounced increase in triangulation (**Figure 3 a**).
- Multi-ion channel effect compounds, such as Verapamil, Terfenadine, Quinidine and Ondansetron, all showed concentration-dependent increases in triangulation at higher exposures (**Figure 3 a**), consistent with modulation of inward (e.g. I_{CaL}) and outward (e.g. I_{Kr}) currents.
- Dofetilide and E-4031 increased APD_{90} and triangulation at low nM concentrations (**Figures 3 a and 4 a**) but also affected APD_{30} at µM concentrations (**Figure 2 a**), which is where Early Afterdepolarisations (EADs) were observed in raw traces (data not shown), indicating instability of the action potential.
- Compounds with multi-ion channel effects, such as Quinidine and Ondansetron showed less pronounced effects on APD_{30} (**Figure 2 a**), despite the presence of EADs.

Conclusions

Action potential waveform analysis in hiPSC-CMs enables mechanistic differentiation of multi-ion channel cardiac effects beyond hERG inhibition and action potential duration, supporting its application in human-relevant cardiac safety assessment.

These findings demonstrate that optical voltage imaging of hiPSC-CM action potentials provides mechanistic insight into multichannel cardiac effects that cannot be captured by hERG alone.

References

[1] Huang, Y. L., Walker, A. S., & Miller, E. W. (2015). A Photostable Silicon Rhodamine Platform for Optical Voltage Sensing. J Am Chem Soc, 137(33), 10767-10776.
[2] Kilfoil, P. *et al.*, (2021). Characterization of a high throughput human stem cell cardiomyocyte assay to predict drug-induced changes in clinical electrocardiogram parameters. Eur J Pharmacol, 912, 174584.
[3] Yamamoto, W. et al., (2016). Electrophysiological characteristics of human iPSC-derived cardiomyocytes for the assessment of drug-induced proarrhythmic potential. PloS One, 11(12).
[4] ICH E14/S7B Q&As Training Material Examples Supplemental File (2022).