Incorporating the CiPA paradigm into an integrated cardiac risk assessment

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INTRODUCTION

- Safety-related attrition remains a major issue in drug discovery and development with cardiovascular toxicity accounting for around 20% of both preclinical and clinical failures.
- Currently, compounds are tested for inhibition of the hERG potassium channel since this is associated with QT interval prolongation and life-threatening arrhythmia, including Torsades de Pointes (TdP).
- The Comprehensive In Vitro Proarrhythmia Assay (CiPA) initiative proposes more thorough investigation of cardiac electrophysiology preclinically, by (1) testing at additional ion channel targets, (2) *in silico* modelling of ion channel data to predict effects on the cardiac action potential (AP) and (3) verification of predicted effects in human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs).
- The aim of this work was to deploy the 3 pre-clinical elements of CiPA, define a compound's proarrhythmic risk, and apply this data to an integrated cardiac risk assessment.

MATERIALS AND METHODS

- The activity of a panel of compounds (Dofetilide, Nifedipine, Bepridil, Verapamil and Quinidine) was tested against 7 cardiac ion channels (the "CiPA ion channel panel"; hERG, hNaV1.5 peak and late current, hCaV1.2, hKir2.1, hKvLQT1 and Kv4.3). These ion channels were stably expressed in recombinant cell lines and ionic currents measured by automated patch-clamp at ambient temperature (Figure 1, PatchLiner, Nanion Technologies). Data from two exemplar compounds is shown.
- The resulting IC_{50}, % inhibition and Hill Coefficients were used as inputs for the in-silico Action Potential (isAP) model to simulate the impact on AP duration (e.g. APD_{90}), amplitude and Vmax (maximum rate of depolarisation) in virtual left ventricular cardiomyocytes. The model was originally described by Davies *et al*. (2012) Am J Physiol 302: H1466-H1480. The R-Shiny platform used in this work is an accessible, PC-based version of the O'Hara Rudy model as adopted by the FDA (Li et al (2019) Clin Pharmacol Ther 105: 466-475). Inputs were also used from a hERG dynamic/kinetic study (Milnes et al. (2010) JPTM 61:178-191).
- Impedance and field potential measurements were made using human induced pluripotent stem cell (hiPSC) cardiomyocytes (Cor.4U® from Ncardia) on the xCELLigence RTCA CardioECR platform (ACEA Biosciences).

RESULTS

Ion channel panel - Automated patch-clamp

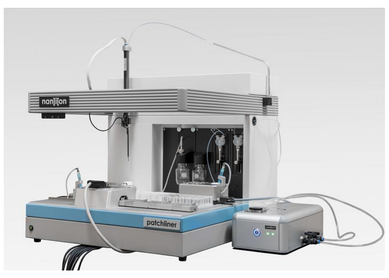


Fig 1. Patchliner (Nanion Technologies)

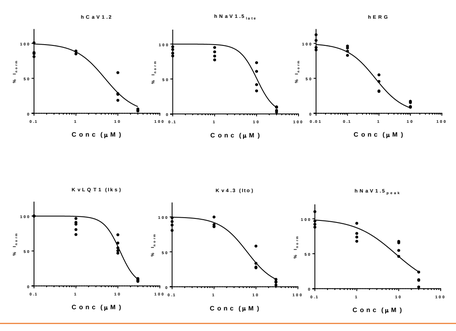


Fig 2. Exemplar ion channel data for Verapamil

	Verapamil		Quinidine	
	IC_{50} (μM)	nH	IC_{50} (μM)	nH
hERG	1.3	0.8	1.2	0.9
hNaV1.5peak	14	1.4	12	1.5
hNaV1.5late	13	1.0	17	1.3
hCaV1.2	7.4	1.1	12	0.6
hKvLQT1 (Iks)	40	1.0	6.4	1.4
hKv4.3 (Ito)	29	1.1	12	1.0
hKir2.1	NE		NE	

NE No effect

AP simulation – *in silico* modelling

Fig 3. Verapamil simulation at 81nM*

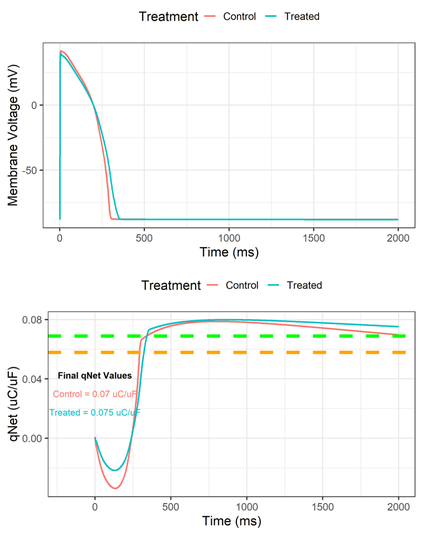
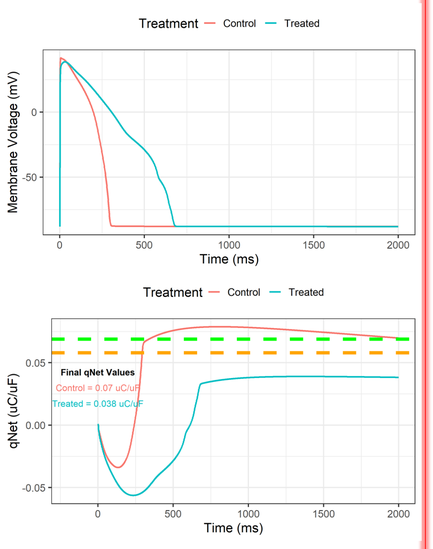


Fig 4. Quinidine simulation at 3237nM*

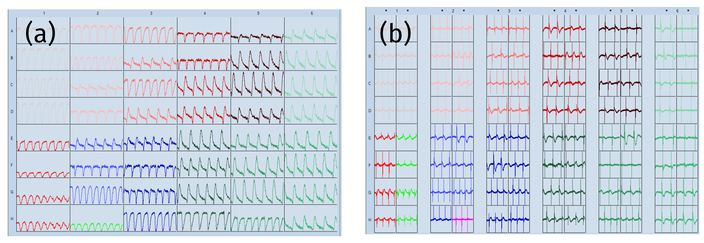


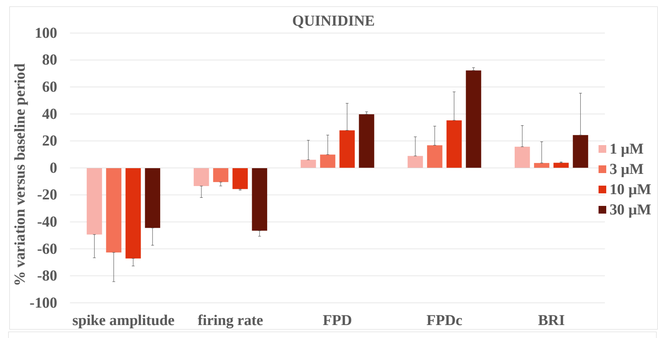
In silico modelling predicted a prolongation of action potential duration with Quinidine.

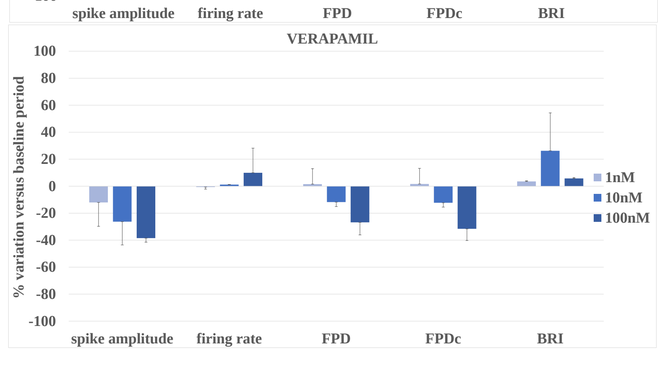
* Simulation concentrations reflect free Cmax

hiPSC-CMs measurement

Fig 5. Impedance (a) and Field Potential (b) were measured simultaneously in 48 wells







- High-quality, functional automated patch-clamp data was generated on a number of reference compounds (Dofetilide, Nifedipine, Verapamil, Quinidine and Bepridil). Verapamil and Quinidine data are shown here to exemplify a low and high proarrhythmic risk (Figure 2 and Table).
- Using the R-Shiny platform, *in silico* AP simulation was performed with both hERG static block and hERG dynamic block inputs.
- *In silico* AP simulations of verapamil data predict no change in AP duration or qNet (Figure 3). In hiPSCs, no prolongation in Field Potential Duration (FPD) parameters was recorded (Figure 5). Accordingly, the CiPA paradigm would class verapamil as "low proarrhythmic risk".
- Quinidine was predicted to prolong action potential duration *in silico (*Figure 4). In hiPSCs Quinidine demonstrated a dose-dependent increase in FPD parameters demonstrating the concordance between the two methods (Figure 5). According to the CiPA paradigm, quinidine would be classed as "high proarrhythmic risk"
- Beat rate index (BRI) is a measure of cardiac contractility. At the top concentration tested (1μM), verapamil stopped the hiPSCs from beating; this is a negative inotropic effect.

CONCLUSIONS

- By deploying ion channel profiling, *in silico* modelling and field potential measurements, compounds can be classified with different degrees of proarrhythmic risk (low, medium or high). Moreover, hiPSC-CMs permit the study of chronic exposure of compounds (up to several days) as well as short-term exposure recommended by CiPA.
- Verapamil had a clear effect on cardiac contractility, despite being classified as "low proarrhythmic risk". The ability to measure not only action potential duration but also cardiac contractility is powerful. This direct effect on cardiac contractility is biologically significant and should be considered as part of an integrated cardiac risk assessment.
- Collectively, these data sets provide critical information to the project on risks associated with progressing into *in vivo* studies and ultimately into the clinic.