New CiPA cardiac ion channel cell lines and assays for *in vitro* proarrhythmia risk assessment

Edward SA Humphries, Robert W. Kirby, Louise Webdale and Marc Rogers

Metrion Biosciences Ltd, Riverside 3, Granta Park, Cambridge, CB21 6AD, U.K.

Introduction

New cardiac safety testing guidelines are being developed as part of the FDA's Comprehensive *in vitro* Proarrhythmia Assay (CiPA) initiative, which aims to remove the reliance on screening against the hERG channel by expanding the panel to include other human ventricular ion channels such as $Na_v1.5$, $Ca_v1.2$, $K_v4.3$/KChiP2.2, $K_{ir}2.1$ and $K_v7.1$/KCNE1. In addition, the CiPA working groups have recently identified two additional ion channel assay readouts required for *in silico* models to reliably predict proarrhythmia. The first is a 'late' $Na_v1.5$ assay, as inhibition of persistent inward current can affect repolarisation and mitigate proarrhythmia (e.g. Ranolazine). The second is a kinetic hERG assay that measures drug trapping using the Milnes voltage protocol[1] and improves the prediction of proarrhythmia risk[2]. Here we describe validation of these additional CiPA assays on the gigaseal QPatch48 automated patch clamp platform.

Materials and Methods

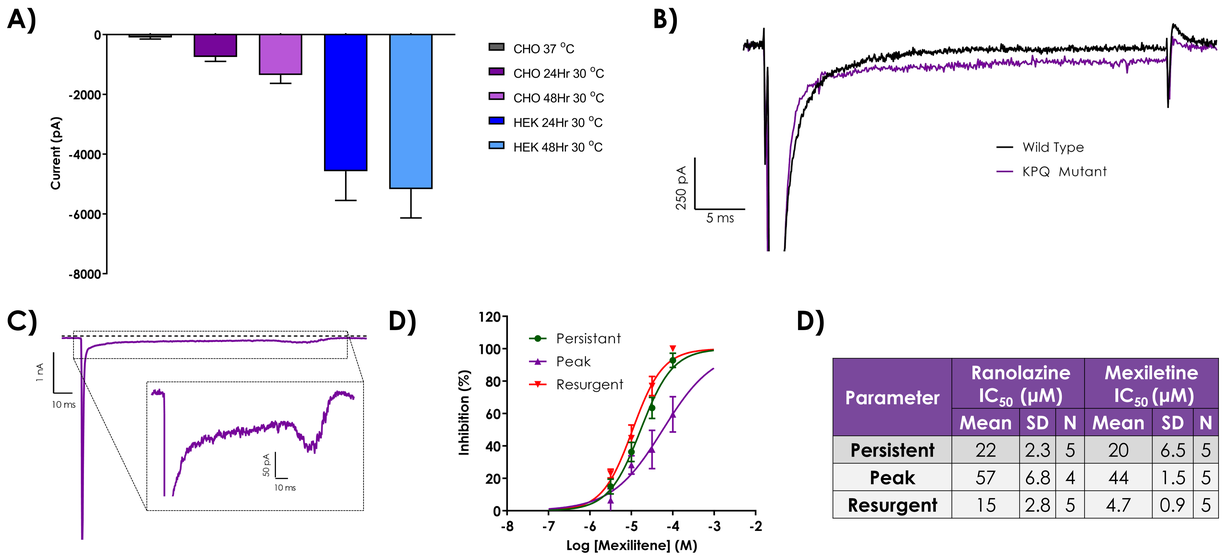
$Na_v1.5$ (KPQ) late sodium assay: HEK293 and CHO cells were transfected using standard liposomal transfection reagents with $Na_v1.5$ (ΔKPQ) mutant cDNA obtained commercially and verified by sequencing. All data are from single hole chips. Standard QPatch cell suspension, sealing and whole-cell protocols were utilized, with minor adjustments to obtain a high proportion of gigaohm seals.

Dynamic hERG assay: CHO cells expressing the human ether-a-go-go related potassium channel (hERG, $K_v11.1$) were obtained from B'SYS. Cells were cultured and harvested using our optimised QPatch protocols. Standard QPatch cell suspension, sealing and whole-cell protocols were utilized, with minor adjustments to obtain a high proportion of gigaohm seals and acceptable whole-cell hERG current amplitude and stable current kinetics.

1. $Na_v1.5$ (ΔKPQ) late sodium cell line and assay validation

An additional CiPA channel component required for accurately predicting proarrhythmia is the 'late' or persistent sodium current. This small current persists throughout the cardiac action potential after initial inactivation of over 99% of sodium channels[3]. The small amplitude of the wildtype late current is not amenable to automated patch clamp recordings so activators such as ATXII and veratridine have been used induce late openings. However, large shifts in IC_{50} values for such drugs as Ranolazine occur between each activator, which can also open endogenous sodium currents. Metrion aimed to remove the need to activate the 'late' Nav1.5 current using non-specific pharmacological tools by creating a cell line expressing a long QT syndrome mutation (ΔKPQ), which exhibits an enhanced persistent current[4].

Robust expression of $Na_v1.5$ ΔKPQ currents suitable for QPatch screening



D)

Parameter	Ranolazine IC_{50} (μM)			Mexiletine IC_{50} (μM)		
	Mean	SD	N	Mean	SD	N
Persistent	22	2.3	5	20	6.5	5
Peak	57	6.8	4	44	1.5	5
Resurgent	15	2.8	5	4.7	0.9	5

Figure 1: $Na_v1.5$ KPQ current assessment A) Polyclonal cell lines (CHO and HEK) were assessed for peak current size with varying days in culture at 30°C. **B)** Side by side comparison of $Na_v1.5$ WT and KPQ cells using the QPatch cell clone facility **C)** Representative trace of optimised voltage protocol showing an increasing in current size (c.f panel B). Pharmacological validation was performed, **D)** IC_{50} curves for Mexiletine and **E)** IC_{50} parameters for Ranolazine and Mexiletine against $Na_v1.5$ ΔKPQ.

2. Dynamic hERG assay

Recent work by FDA and CiPA working groups indicate that addition of hERG kinetic data obtained with the so-called 'Milnes' voltage protocol[1] to a modified 'dynamic' O'Hara-Rudy *in silico* model improves cardiac liability prediction[2]. The kinetics of drug binding and unbinding to the hERG channel underlies compound potency, but there is evidence that compounds which become trapped in the pore of the channel carry a greater clinical risk[1]. Up to now only high fidelity manual patch clamp recordings have been used to reliably measure hERG channel binding kinetics and drug trapping, both important aspects of drug action and potency as well as cardiac liability.

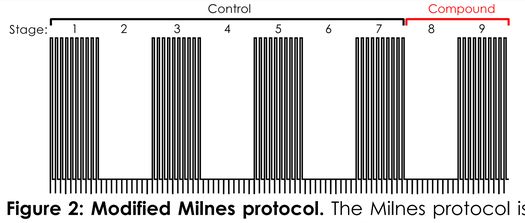


Figure 2: Modified Milnes protocol. The Milnes protocol is comprised of 9 stages each 250 seconds long. Depolarising stages (1,3,5,7,9) consist of 10 depolarisations of the channel for 10 seconds with a sweep to sweep internal of 25 seconds.

Stable hERG currents in dynamic hERG QPatch assay

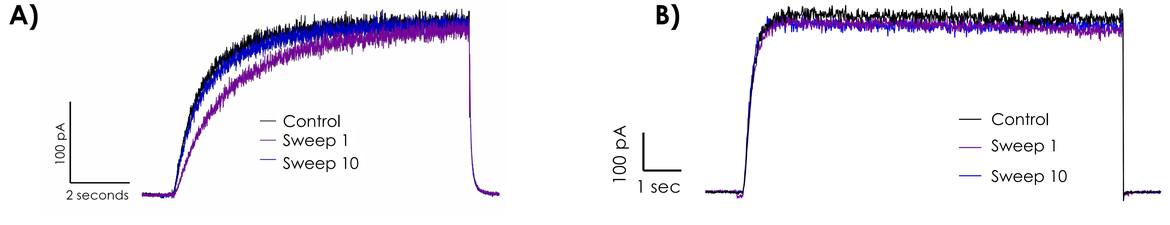
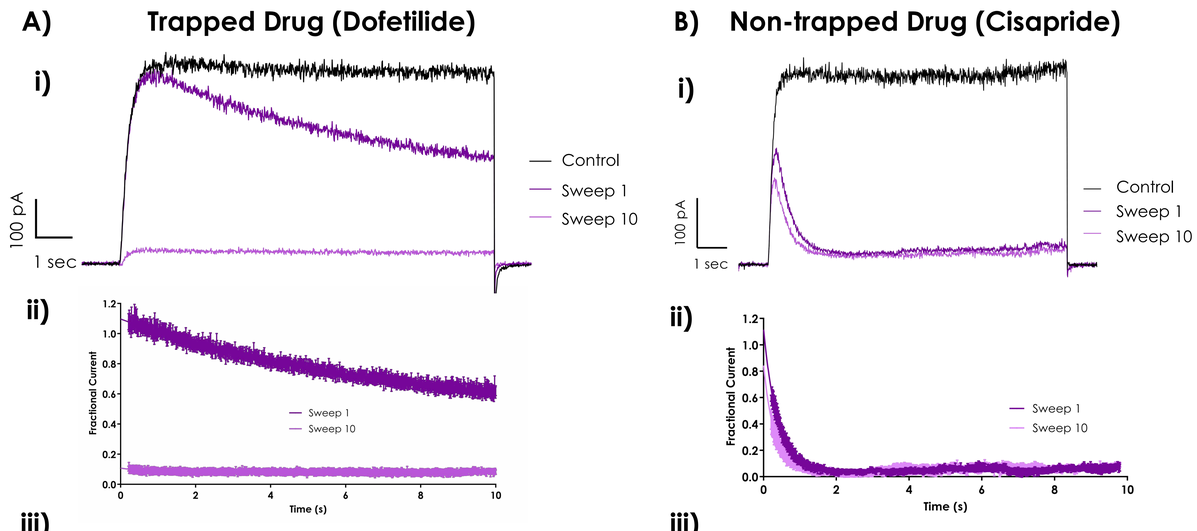


Figure 3: Development of a stable baseline A) Initial experiments showed altered first pulse kinetics in stages (1,3,5,7,9) and significantly slower activation of hERG at room temperature compared to that at more physiological temperatures used in both Milnes *et al.*[1] and Li *et al.*[2] **B)** Metrion's optimised dynamic hERG assay with a stable current profile suitable for compound testing.

Dynamic hERG assay detects drug trapping



Parameter	% trapped	
Drug	Dofetilide	Terfenadine
Peak Reduction	97	83

Parameter	% trapped	
Drug	Cisapride	Verapamil
Peak Reduction	26	34

Figure 4: Drug binding profile of a trapping (Dofetilide) and non-trapping (Cisapride) in our dynamic hERG assay Ai) Representative trace of Dofetilide (100 nM), **ii)** Average fractional hERG current for sweep 1 and 10 in the presence of Dofetilide (100 nM), **iii)** Summary data of trapping drugs. **Bi)** Representative trace of Cisapride (300 nM), **ii)** Average fractional hERG current for sweep 1 and 10 in the presence of Cisapride (300 nM). **iii)** Summary data table of non-trapping drugs.

References

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2. Li *et al.* 2017. Circ Arrhythm Electrophysiol. PMID: 28202629
3. Catterall *et al.* 2007. Toxicon. PMID: 17239913
4. Chandra, Starmer and Grant 1998. Am J Physiol. PMID: 9612375
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Conclusions

Metrion have produced two additional QPatch assays to improve it's CiPA cardiac safety assay panel:

- $Na_v1.5$ ΔKPQ 'late' current assay to reliably measure low amplitude persistent inward currents
- Dynamic hERG assay to allow assessment of drug binding and trapping

www.metrionbiosciences.com

edward.humphries@metrionbiosciences.com