Predicting Cardiac Proarrhythmic Risk Exclusively Using Automated Patch Clamp Data

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Introduction

Recent work by FDA and HESI CiPA working groups indicate that *in vitro* hERG, $Na_v1.5$ and $Ca_v1.2$ potency data in addition to dynamic hERG kinetic data is required to accurately predict proarrhythmic risk[1]. Below we explore two key challenges in exclusively using automated patch clamp for risk prediction:

1. Metrion has previously shown the ability to implement the difficult Milnes[2] protocol on QPatch[3], but the challenges of producing full concentration response formats required for *in silico* models are unknown.
2. $Ca_v1.2$ inhibition values for compounds such as verapamil have been variable, with literature IC_{50} values >10 µM compared to the 202 nM IC_{50} obtained by manual patch clamp in the original CiPA study[4,5]. The similar potency of verapamil against $Ca_v1.2$ and hERG make it a Multiple Ion Channel Effect (MICE) compound and "rescues" it from being classed as a high risk proarrhythmia compound as shown by Li *et al.*[1]
3. QPatch dynamic hERG and $Ca_v1.2$ data from multiple voltage protocols were used to assess proarrhythmic risk using the FDA's *in silico* model.

Materials and methods

Dynamic hERG assay: CHO cells expressing the human ether-a-go-go related gene (hERG) potassium channel ($K_v11.1$) 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.

***In vitro* cardiac ion channel assays:** CHO or HEK cells stably expressing cardiac ion channels (hERG,$Ca_v1.2$,$Na_v1.5$) were cultured and harvested using optimised protocols. Standard QPatch cell suspension, sealing and whole-cell protocols were utilized, with minor adjustments for each channel to improve assay efficiency.

***In silico* modelling:** Optimised dynamic O'Hara Rudy model was downloaded from the FDA's Github site and used on Rstudio.

1. Dynamic hERG assay

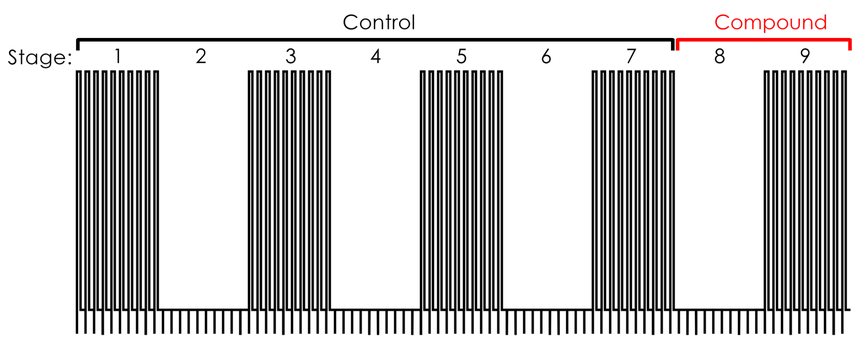
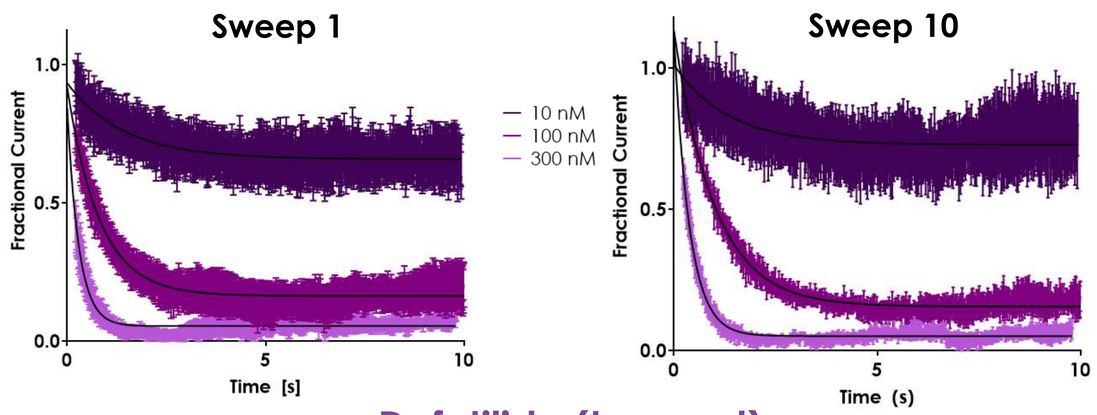


Figure 1: Modified Milnes protocol. The Milnes protocol consists of 9 stages, each 250 seconds long. Depolarising stages (1,3,5,7,9) consist of 10 depolarisations for 10 seconds each with a sweep-to-sweep interval of 25 seconds. A single concentration of compound is applied during stages 8 and 9.

Metrion has previously optimised and validated the Milnes protocol on the QPatch to yield acceptable stability in current amplitude and kinetics[3]. This has allowed the evaluation of a small number of challenging compounds (e.g. slow on-rate) using a composite concentration response assay format (Figure 1). Trapping of drugs in the hERG channel was determined for verapamil, terfenadine, cisapride and dofetilide by composite 3-pt mini-IC_{50}. The trapping parameter (vhalf-trap) was compared with published literature values (Table 1).

Cisapride (non-trapped)



Dofetilide (trapped)

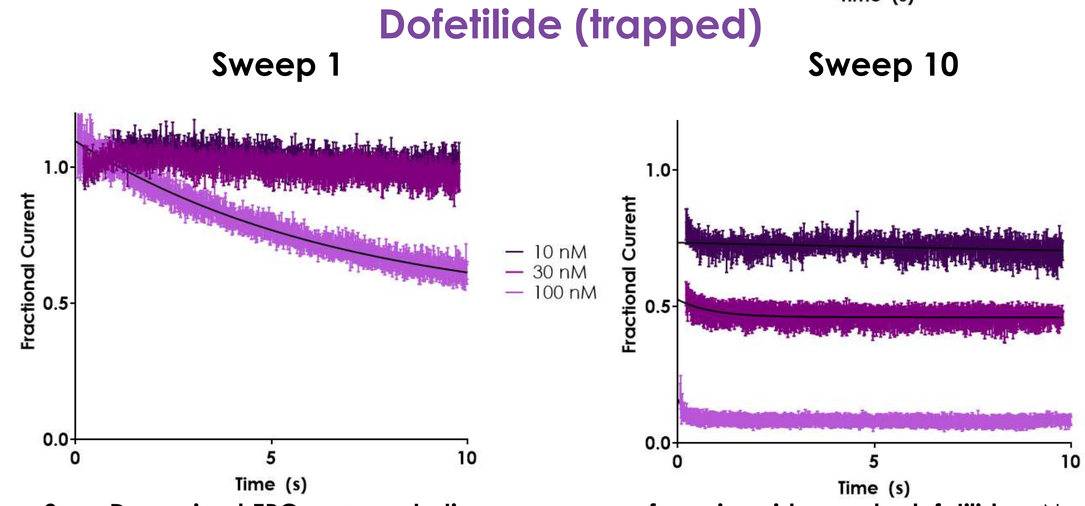


Figure 2: Dynamic hERG concentration responses for cisapride and dofetilide. Normalised concentration response data for cisapride and dofetilide show the ability of the QPatch Milnes protocol assay to discriminate between the minimal trapping of cisapride and the high degree of trapping of dofetilide.

Parameter	Cisapride		Dofetilide		Terfenadine		Verapamil	
	APC	MP	APC	MP	APC	MP	APC	MP
Trapping (-200 to -1 mV)	-179.5	-167.4	-1.02	-1.15	-23.38	-81.66	-70.58	-96.94

Table 1: Comparison of the level of dynamic hERG trapping. The vhalf-trap parameter was calculated using the FDA's optimised dynamic O'Hara-Rudy model using Metrion's automated patch clamp (APC) QPatch data and compared to FDA published manual patch clamp data (MP).

2. *In vitro* $hCa_v1.2$ assessment

One of the largest differences in compound potencies between automated and manual patch clamp data has been for $Ca_v1.2$[4], with these values being key to "rescuing" the predictive risk of compounds such as verapamil. Previous publications and in-house experiments showed use-dependent and inactivated state preference for verapamil inhibition of $Ca_v1.2$[6], therefore we assessed a number of voltage protocols to determine whether a low micromolar potency could be determined.

$Ca_v1.2$ Two Pulse Protocol

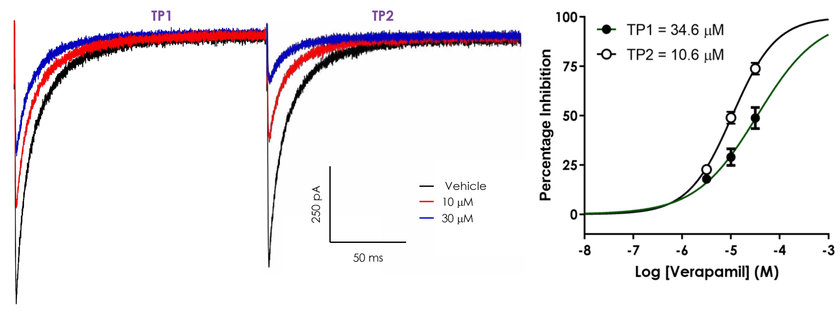


Figure 3: Two pulse protocol: initial experiments aimed to confirm use / inactivated state preference for verapamil

$Ca_v1.2$ Pulse Train Protocol

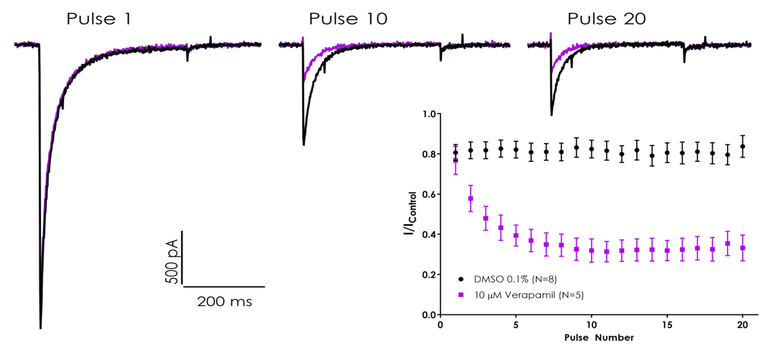


Figure 4: Pulse Train: Increased use dependence was assessed using a 20 pulse 1Hz train

$Ca_v1.2$ Inactivated State Protocol

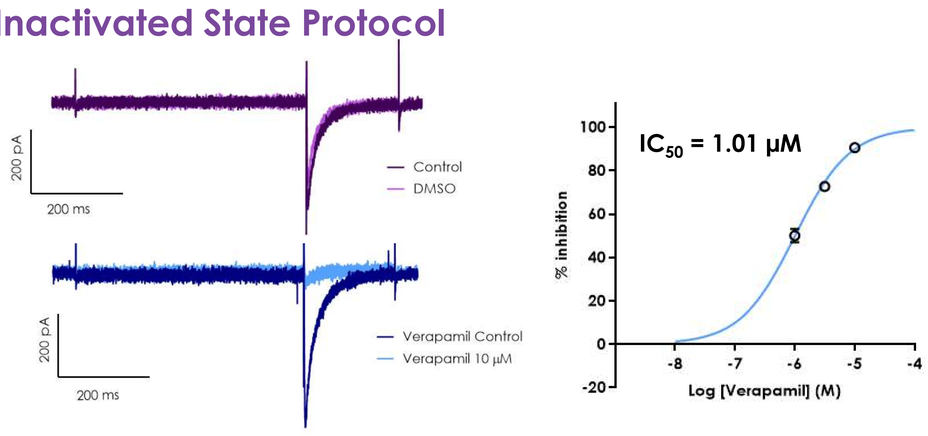
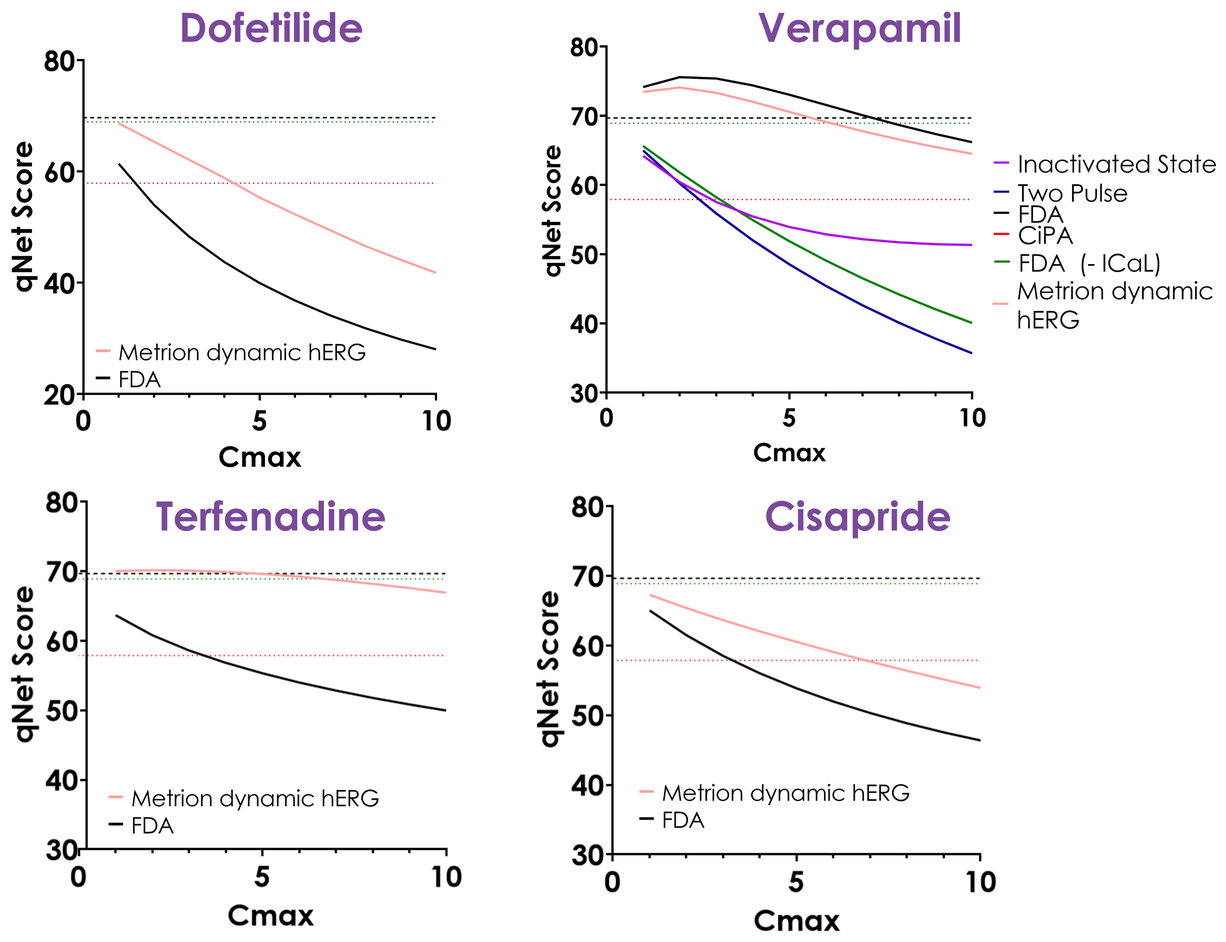


Figure 5: Inactivated state: We assessed an inactivated and use dependence voltage protocol against verapamil

in silico modelling

Combining Metrion's dynamic hERG data and more physiological $Ca_v1.2$ potency values, we assessed the utility of fully automated patch clamp data for cardiac safety assessment using the most recent FDA published *in silico* cardiac action potential model (Optimised dynamic O'Hara Rudy model).



Compound	Risk		Possible Reason
	MP	APC	
Cisapride	Medium	Medium	N/A
Dofetilide	High	Medium	hERG potency (not in steady state)
Terfenadine	Medium	Low	hERG potency (not in steady state)
Verapamil	Low	High	$Ca_v1.2$ potency differences

Table 2: Comparison of automated vs manual patch clamp proarrhythmia predictions. *In silico* prediction of qNet proarrhythmic scores from automated patch clamp (APC) or manual patch clamp data (MP).

Conclusions

- Metrion exploited its dynamic hERG assay to assess the utility of automated patch clamp data to predict proarrhythmia risk. hERG kinetic data align with manual patch recordings, but some compounds still exhibit small potency shifts, potentially due to slow or incomplete block during 10 sweep protocol (Fig. 1).
- $Ca_v1.2$ potency for verapamil was increased 30 fold using use-dependent and inactivated state voltage protocols, to better align with manual patch clamp data. Further work is required to improve this protocol for CiPA.

References

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