Using new *in vitro* cardiac ion channel assays and *in silico* models to predict proarrhythmic risk with automated patch clamp data

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Introduction

The FDA's Comprehensive *in vitro* Proarrhythmia Assay (CiPA) initiative is designed to remove the over-reliance on hERG data to predict human clinical cardiac risk[1], with recent results suggesting that inclusion of additional cardiac ion channels and assays (e.g. peak and late $Na_v1.5$, $Ca_v1.2$, dynamic hERG[2]) improve risk predictions of *in silico* action potential models[1]. The CiPA working groups currently use a mixture of manual and automated patch clamp (APC) platform data, but future CiPA drug screening will likely rely on APC data.

We show that high quality APC data from CiPA cardiac assays can accurately predict proarrhythmic risk of some, but not all, drugs in FDA *in silico* models, however, two areas require improvement:

1. The potency of CiPA compounds, such as verapamil, against $Ca_v1.2$ is influenced by the voltage protocol used and the state- and frequency-dependence of $Ca_v1.2$ channel inhibition.
2. Compounds exhibiting slow binding on-rates underperform in certain assays, including our current dynamic hERG assay.

Materials and Methods

***In vitro* cardiac ion channel assays:** CHO or HEK cells stably expressing cardiac ion channels were cultured and harvested using optimised protocols. All data were generated on the gigaseal QPatch48 system (Sophion).

***In silico* modelling:** Potencies for compounds from each CiPA risk category either generated using Metrion's APC assays or using FDA's manual patch clamp were applied to the official FDA optimised dynamic O'Hara-Rudy (ORd) model[3] which was downloaded from the FDA's Github site and used on Rstudio and qNet scores and threshold values generated[1].

1. *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 $Ca_v1.2$[5], therefore, we assessed a number of voltage protocols to determine whether low micromolar potency could be determined.

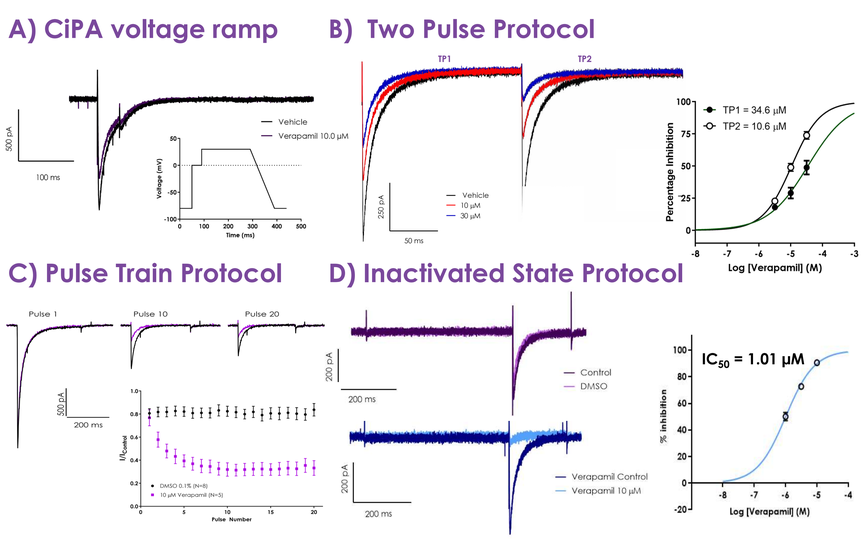

Figure 1: Assessment of *in vitro* $Ca_v1.2$ inhibition by verapamil using multiple voltage protocols with APC.
(A) The CiPA step ramp protocol showed that 10 µM verapamil had minimal block. **(B)** A two pulse protocol was used to confirm use/inactivated state preference. **(C)** Use of a 20 pulse 1 Hz train showed a use dependent effect. **(D)** A combined inactivated and use dependent block protocol revealed the strongest inhibition by verapamil.

2. Dynamic hERG assay

Metrion has previously optimised and validated the Milnes protocol on the QPatch 48 to yield acceptable stability in current amplitude and kinetics[6]. 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 2).

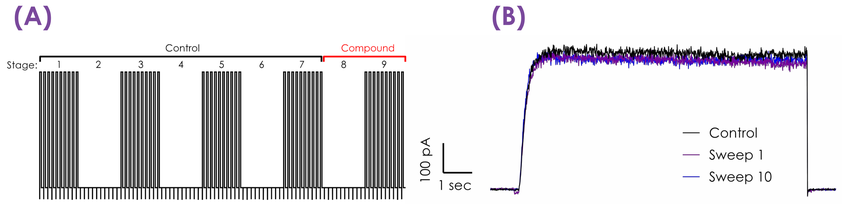

Figure 2: Modified Milnes protocol.
(A) The Milnes protocol consists of 9 stages, each 250 seconds long. Depolarising stages (1,3,5,7,9) consist of 10 depolarisations for 10 seconds each with a sweep-to-sweep interval of 25 seconds. A single concentration of compound is applied during stages 8 and 9. **(B)** Fundamental to reliable kinetic fitting is a stable baseline, as shown in Metrion's optimised assay conditions suitable for compound testing.

Trapping of compounds in the hERG channel was determined for cisapride, dofetilide, terfenadine and verapamil. An example of a non-trapped (cisapride) and a trapped (dofetilide) compounds are shown in Figure 3 and the trapping parameter (vhalf-trap) determined was compared with published literature values (Table 1).

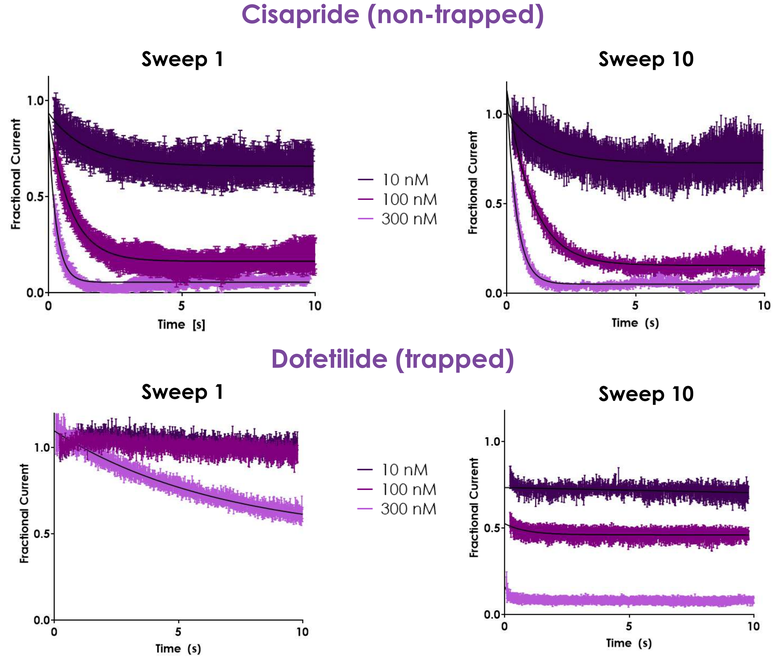

Figure 3: Dynamic hERG concentration responses for cisapride and dofetilide.
Normalised concentration response data for cisapride and dofetilide show the ability of the QPatch Milnes protocol assay to discriminate between the minimal trapping of cisapride and the high degree of trapping of dofetilide.

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 (the membrane voltage at which half of the drug-bound channels are closed) 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).

3. *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 (Figure 4).

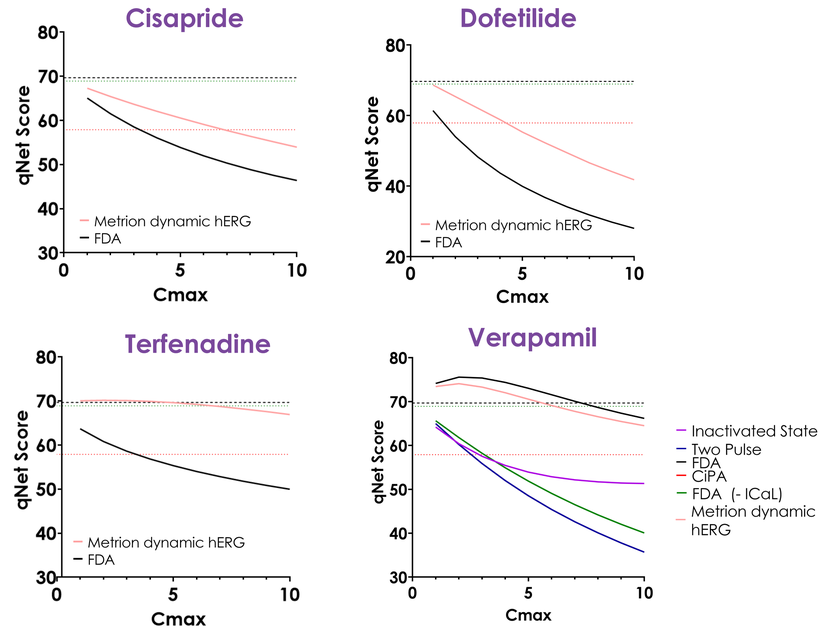

Figure 4: *In silico* cardiac action potential modelling using an optimised O'Hara Rudy model.
Comparisons of the qNet score as a function of C_{max} were determined for cisapride, dofetilide, terfenadine and verapamil using the FDA test set data (manual patch) alone (blank line) or when Metrion's dynamic hERG APC data was substituted (pink line). The choice of voltage protocol used to determine verapamil potency affected the qNet score.

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

- $Ca_v1.2$ 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.
- Metrion exploited its dynamic hERG assay to assess the utility of APC data to predict proarrhythmic 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 the 10 sweep protocol (Figure 2).

References

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