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Application Report

# CiPA hERG Milnes kinetic assay on QPatch

To meet the FDA's CiPA requirements for improved *in silico* action potential modelling and arrhythmia prediction, we have successfully created and implemented the challenging Milnes hERG cardiac safety assay


## Summary

- High fidelity QPatch hERG kinetic data closely mimics FDA's manual patch clamp Milnes protocol data
- Stable hERG current profile during repetitive long depolarizing test pulses
- Assay can detect changes in hERG amplitude and decay kinetics due to drug binding and trapping
- Pharmacologically validated with clinical drugs showing a wide range of drug trapping activity


## Introduction

Cardiac safety side-effects remain the leading cause of new compound attrition during the drug discovery process (Valentin and Redfern, 2017), suggesting that more robust preclinical *in vitro*, *ex vivo* and *in vivo* assays and models are required to predict human clinical risk. Currently the industry is moving away from an over-reliance on the human Ether-a-go-go related potassium channel (hERG, $K_V$11.1) assay and QT prolongation readouts by developing new initiatives that provide a more balanced assessment of patient risk, focusing in particular on cardiac arrhythmia liability. The FDA's Comprehensive *in vitro* Proarrhythmia Assay (CiPA) initiative and Japanese regulatory authorities (JiCSA, CSAHi) aim to more accurately model and predict arrhythmia risk by including additional human cardiac ion channels in patch clamp electrophysiology screening panels. This broader dataset is then used in sophisticated *in silico* models of the human ventricular action potential to predict arrhythmias. After 5 years of extensive collaborative effort by working groups in the UK, EU, US and Japan, it has become apparent that incorporating data obtained from the 'big 6' panel of cardiac ion channel assays (hERG, $Na_V$1.5, $Ca_V$1.2, $K_V$LQT1, $K_V$4.3, and $K_{ir}$2.1) into standard *in silico* action potential models allows the accurate prediction of proarrhythmic liability of most, but not all, clinical drugs with well characterised risk profiles.

Recent work by FDA and CiPA working groups indicate that addition of hERG-drug kinetic data obtained with the so-called 'Milnes' voltage protocol (Milnes *et al.*, 2010; Figure 1) to a modified 'dynamic' O'Hara-Rudy *in silico* model correctly predicts the cardiac liability of compounds such as quinidine and cisapride (Li *et al.*, 2017). 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 (Pearlstein *et al.*, 2016). 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.

We report here the successful implementation of the 'Milnes' voltage protocol on a high quality automated patch clamp (APC) platform that enables accurate determination of dynamic drug-hERG kinetics.

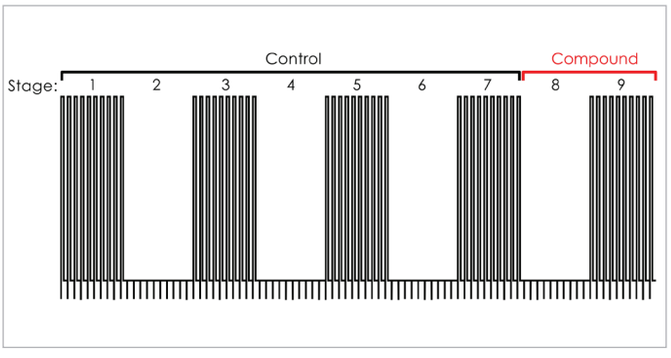



Fig. 1: Milnes dynamic hERG voltage protocol. Cells clamped at -80mV were depolarized for 10 seconds every 25 seconds during each test pulse, which was repeated 10 times during each pulse train (Stage 1, 3, 5, 7, 9). Cells were then held at -80 mV for 250 seconds (Stage 2, 4, 6, 8) before the 10-pulse train stage was repeated. During Stage 8 cells were exposed to compound before a final 10-pulse train was applied to assess drug trapping.

## Results and discussion

### DMSO Stability

Cells were voltage clamped at -80 mV and hERG currents were elicited by 10 test pulses comprising a 10 second depolarization applied every 25 seconds (Stage 1), after which cells were held at -80 mV for 250 seconds (Stage 2). This cycle was then repeated (Figure 1) to allow a stable baseline to be achieved. Importantly, after assay optimization, all test pulse responses in DMSO (Stage 9) were similar in profile to that of the last control sweep (Stage 7; Figure 2). A stable baseline is pivotal to the dynamic hERG assay, as changes in hERG current kinetics would appear as drug trapping.

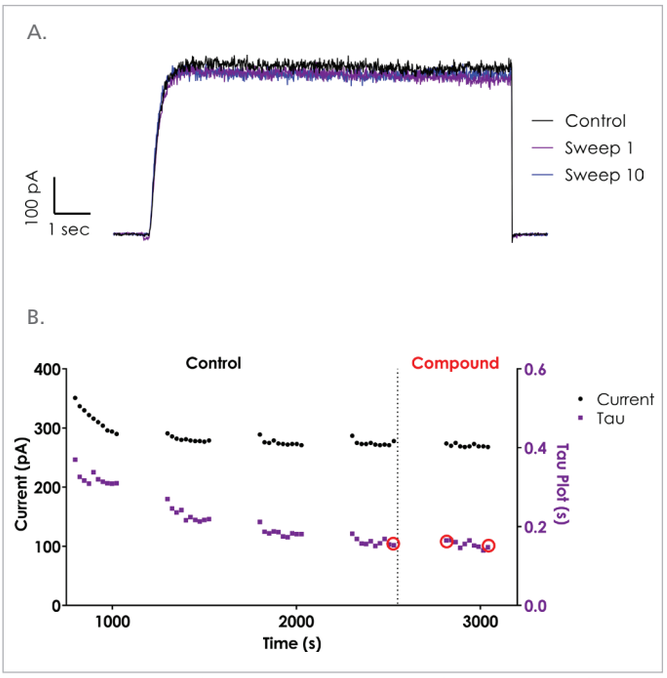
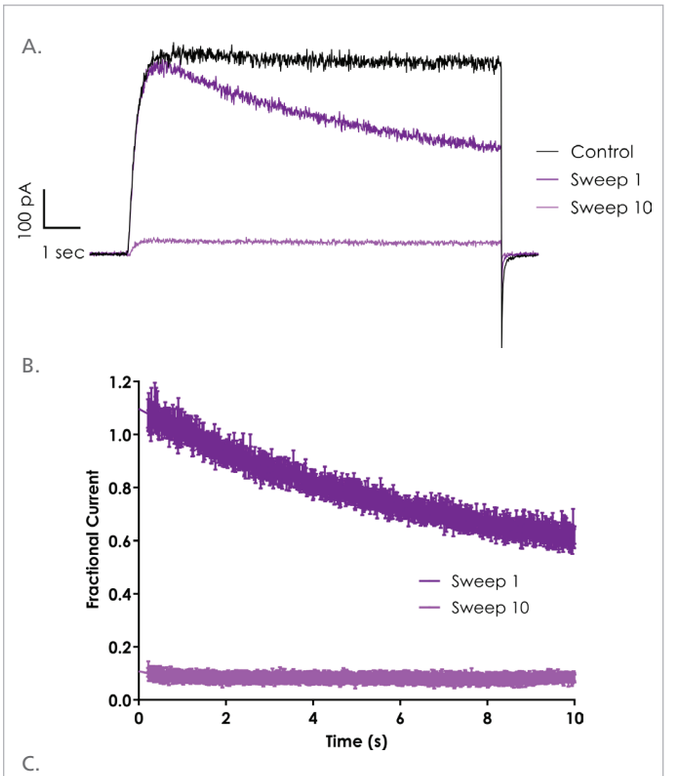



Fig. 2: DMSO stability of hERG current and kinetics after application of Milnes protocol. A) hERG current kinetics in Stage 9 were similar to that of the final control depolarization at the end of Stage 7 showing a stable baseline (red circles in B indicate traces shown). B) Current amplitude and Tau activation fits for the experiment shown in A.

### Measuring hERG drug trapping

Two drugs with known trapping properties, dofetilide and terfenadine, were tested using Metrion's optimised dynamic hERG assay using the Milnes voltage protocol. Cells were exposed to a single concentration of dofetilide (10, 30, 100 nM) or terfenadine (1, 30, 100 nM) in each experiment. The first test pulse in the presence of dofetilide, a clinically known proarrhythmic drug (Figure 3), showed clear drug binding and inhibition during the depolarizing step, reaching near plateau at 10 seconds. After 10 consecutive depolarizations no further time-dependent drug binding was observed, as dofetilide remains trapped in the pore and block is instantaneous. Similarly, terfenadine, an antihistamine removed from the market due to its propensity to elicit ventricular arrhythmia, also showed clear trapping in the hERG pore (Figure 3).



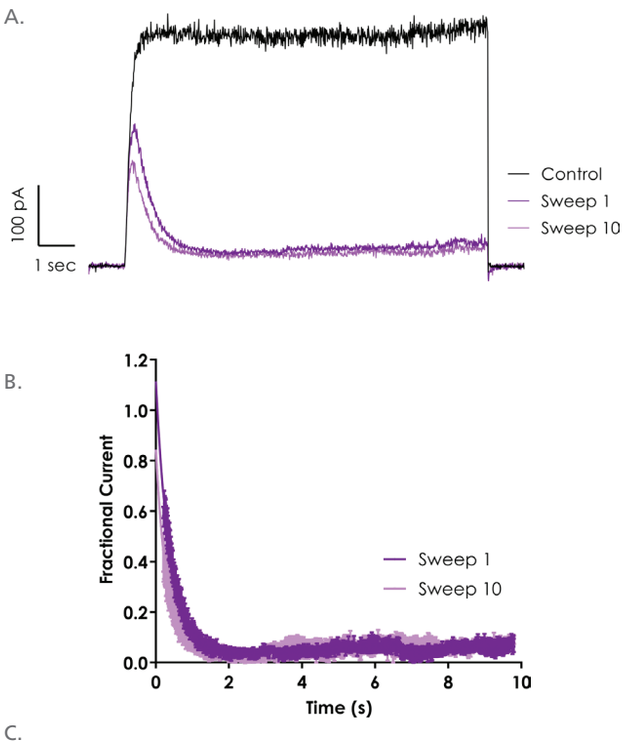

| Drug | Drug Trapping (%) |
|---|---|
| Dofetilide | 97 |
| Terfenadine | 83 |

Fig. 3: Example of a hERG drug trapping (dofetilide 100 nM). A) Representative hERG current traces before and after dofetilide application (Stage 9, sweeps 1 and 10). Time-dependent drug binding can be seen in sweep 1, but not in sweep 10, a characteristic of drug trapping. B) Average current during sweep 1 and 10, normalized to control sweep. C) % trapping values for known trapping drugs, dofetilide and terfenadine.

### Validation of Milnes' protocol with non-trapped drugs

To rule out the possibility that the QPatch recording configuration and our optimised experimental conditions were promoting hERG drug trapping or creating kinetic artefacts that could be mistaken for such a drug binding mechanism, we validated the Milnes protocol assay using cisapride and verapamil. Both drugs were previously shown by manual patch clamp to not exhibit drug trapping (Milnes et al., 2010; Li *et al*. 2017). Cisapride (300 nM) binds rapidly to the hERG channel, as seen in the hERG current traces during the first depolarizing sweep in drug (Figure 4). However, unlike trapped drugs, sweep 10 also shows clear time-dependent drug binding and inhibition of the current during the depolarizing step. This occurs as cisapride dissociates from the pore between voltage steps, and rebinds again once the channel opens during subsequent depolarizations. Verapamil also shows clear time-dependent binding during pulse 1 and 10

(raw data not shown), confirming its profile as a non-trapped drug in our QPatch dynamic hERG protocol assay (Table, Figure 4). Verapamil is a clinically safe drug that exhibits a hERG liability, and it has been suggested that the risk of arrhythmia with verapamil is mitigated due to its lack of hERG trapping, and inhibition of inward $Ca_V1.2$ L-type currents.




C.

| Drug | Drug Trapping (%) |
|---|---|
| Cisapride | 26 |
| Verapamil | 34 |

Fig. 4: Example of a compound lacking hERG drug trapping (Cisapride 300 nM) A) Representative hERG traces in the absence (Stage 7; sweep 10) and presence of cisapride (Stage 9; sweep 1 and 10). Time-dependent drug binding can be seen in the first and last sweeps, indicating a lack of trapping. B) Average fractional current during sweep 1 and 10 normalised to the control sweep. C) Trapping values for cisapride and verapamil, indicating low trapping profile.

## Conclusion

Reliable, predictive and cost-efficient cardiac safety screening is essential for developing safe and effective new drugs. Being able to operate all the necessary in vitro patch clamp cardiac ion channel assays on the same high-quality APC platform, including dynamic hERG kinetic protocols, will greatly facilitate these efforts.

## Methods

CHO cells expressing hERG were obtained from B'SYS. Cells were cultured and harvested using Metrion's 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 currents.

Assay conditions were optimized to ensure that hERG currents elicited by the 'Milnes' kinetic voltage protocol were stable in profile, including consistent activation kinetics and current amplitudes during the compound testing phase of the application protocol (typically after 3 - 6 application periods). All data are from single hole QPlates, to ensure accurate fitting of kinetic data to single cell recordings.

**References:**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