Development of high-throughput electrophysiological assay for the screening of hERG ion channel modulators using Sophion Qube 384

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Introduction

hERG (human Ether-a go-go-Related Gene) is a voltage gated ion channel expressed in cardiac tissue which is of particular concern in off-target pharmacology. Blockade of hERG can lead to prolongation of cardiac repolarisation (long QT syndrome) and ventricular arrhythmia (torsades de pointes). Drug induced LQTS is the most common reason for drug withdrawal in the last ten years; techniques are therefore required to address this and help to reduce attrition as early as possible in the drug development process.

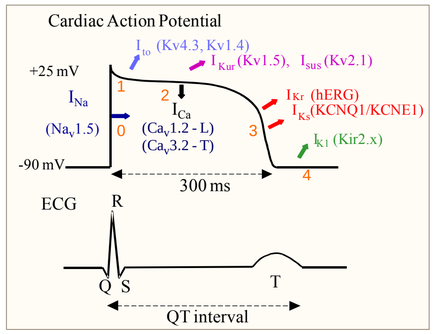

Figure 1: Selected ion channels (including hERG) associated with the Cardiac Action Potential

Second generation automated electrophysiology systems such as IonWorks Barracuda use perforated patch technology to allow c.600 dose response curves to be generated per day, but produce low fidelity megaOhm recordings with poor success rates. We have developed a more robust, reproducible high throughput whole cell patch clamp assay methodology using the Sophion Qube 384, which provides higher fidelity recordings and improved reliability in prediction of activity compared to other automated electrophysiology methods

Objectives

- To demonstrate expected activity of tool compounds at hERG using Sophion Qube
- To develop an assay to screen compounds for blockade of the channel

Methods

All experiments were conducted with cells stably expressing the hERG ($K_V11.1$) protein. Recordings were made in whole cell mode on the Sophion Qube. All recordings were single cell recordings in 384-well Qchips.

Results

Current recordings

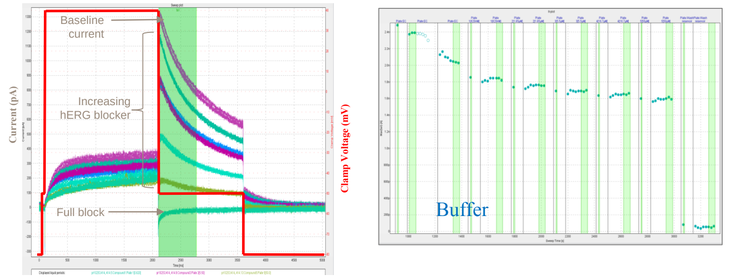

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Figure 2: hERG recordings produced on Sophion Qube: Stimulation protocol and resultant currents (left) and I-t plot in the absence of compound (right). Representative traces from the same cell are superimposed to visualize the effect of additions of buffer (baseline), 5 increasing concentrations of a hERG blocker and 150μM Quinidine (full block). Repeated baseline recordings produce a consistent current with little rundown

Current & Seal Distributions

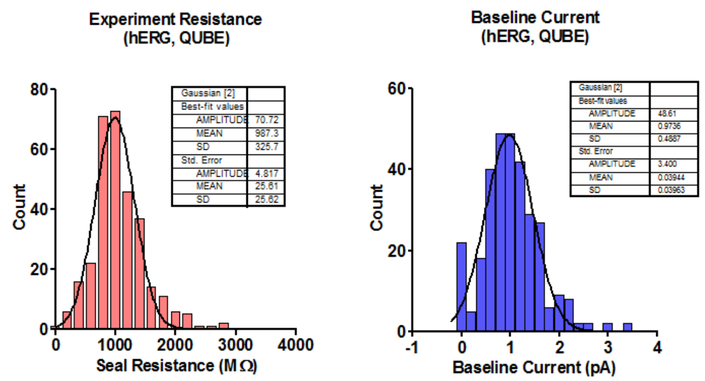

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Figure 3: Frequency distributions obtained from hERG responses on Qube. Favourable distributions of resistance and current are observed with this reagent with averages of ~ 1GΩ and ~1nA, respectively

Comparison of assay platforms

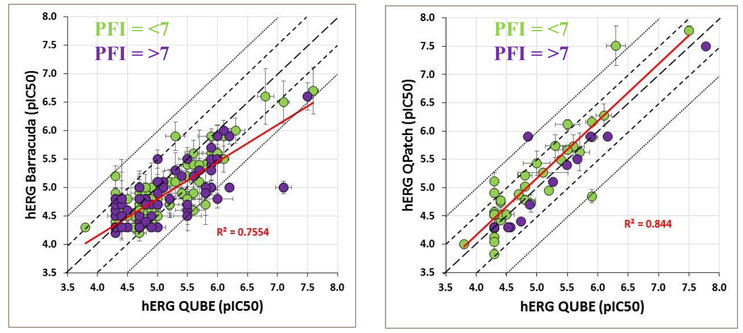

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Figure 4: Correlations of pIC_{50} values obtained for a compound set from IonWorks Barracuda (left) and Sophion QPatch (right) with equivalent data from Sophion Qube. The Qube data show better agreement with QPatch and lower variability (error bars are standard deviations)

Conclusions

- We have fully recorded hERG ion channel currents in whole cell configuration for the first time on a high throughput electrophysiology platform
- Data produced compares better with Q-Patch (gold standard) than previous method
- We have developed a 5-point screening assay which reliably flags hERG blockers for attrition reduction whilst providing increased data flow for *in silico* modelling

Acknowledgements

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References

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