Kinetic evaluation of slow onset hERG blockers through development of an enhanced automated electrophysiology assay panel

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Overview

In this study we demonstrate the importance of the rapid adaptation of the frontline cardiac safety assay to improve the predictivity of the AstraZeneca human ether-e-go-go (hERG) screening process. The object of the study was to firstly to better understand binding kinetics of our new candidate molecules and secondly to introduce a new assay format enable to better quantify the potency of compounds with slower binding kinetics. As a result of the study, we identified classes of compounds that have a slow onset binding. When tested under extended incubation conditions we were able to generate a more accurate, 10-fold shift in potency. This new extended assay format allows us to accurately characterize early drug candidates from a safety standpoint to ultimately improve the accuracy of this decision-making assays earlier in the drug discovery pipeline.

Introduction

Evaluation of early-stage drug candidates for cardiotoxicity liabilities against human ether-a-go-go (hERG) cardiac potassium channel using patch-clamp assay remains one of the gold standards for elimination of chemical substances that potentially trigger different life-threatening cardiac arrhythmias. hERG is a voltage-dependent potassium channel, which plays one of the most crucial roles in repolarization of cardiac action potentials. The blockage and modification of the normal functioning of hERG channel leads to prolonged QT interval, which results in increased risk of polymorphic ventricular tachycardia (Torsades de Pointes, TdP) and cardiac arrest. Apart from the effective detection of ion channel blockage it is important to label the compounds that affect protein trafficking and folding. Whilst effective for most compound classes standard hERG screening workflows will underestimate the potency of compounds with slow binding (slow on-set) kinetics. Here we describe a novel workflow that enabled us to identify these slower binders earlier in our safety screening cascade.

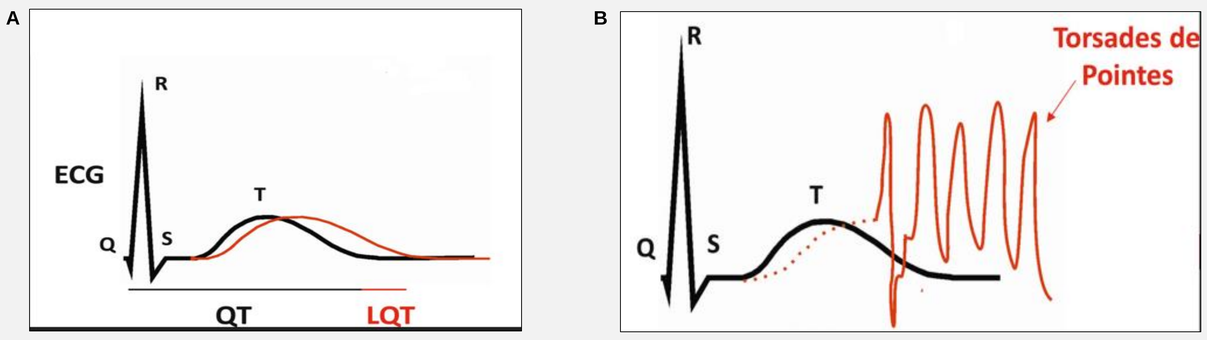


Fig.1 A. Demonstration of a normal ECG (black) and distorted one (red) as a result of prolonged QT interval due to delayed repolarization of blocked $I_{Kr.}$ B. Normal ECG (black) and acquired Torsades de Pointes (red) due to drug-induced hERG blockage. Figure source: El Harchi et al. (2022).

Methods

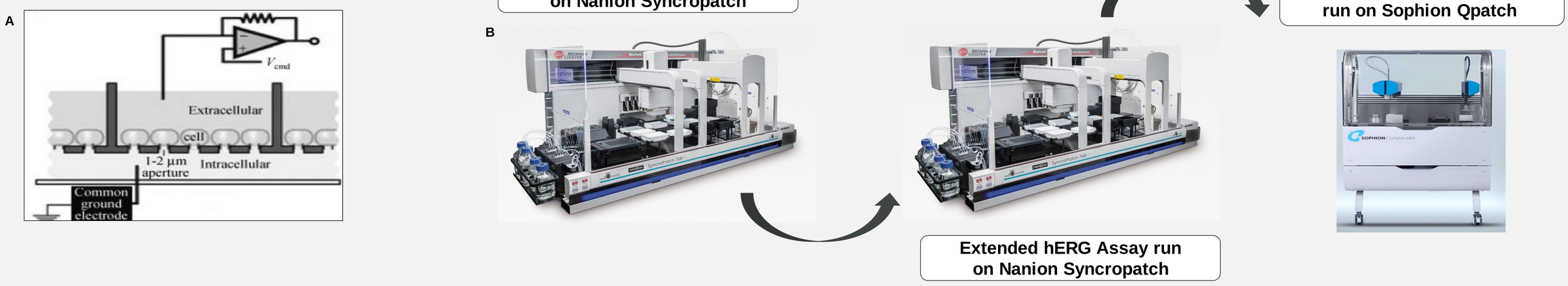


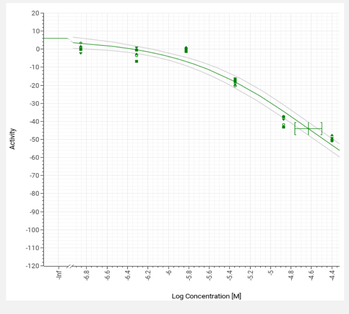
Fig. 2. A. Automated plate-based patch clamp setup. Figure source: Chen, Peihua, et al. (2009). B. Schema of an established patch-clamp assay workflow.

An automated patch clamp (APC) hERG assay is now one of indispensable technologies used cardiotoxicity safety assessment of new chemical entities in drug discovery industry. However, despite following widely-used approaches, it is important to adapt them to compounds with various binding kinetics.

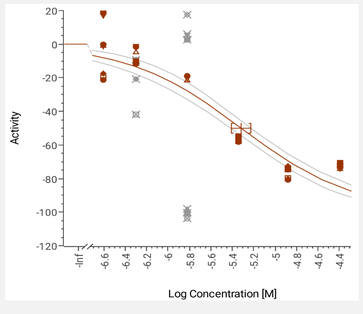
An established workflow of Frontline, Extended and Augmented hERG assays allows to gradually increase compound exposure time from 3 min to 15 min and 27 min, respectively. This, consequently, guaranties a balance of a throughput in cardiac safety assays and accuracy of the potency measurement.

Results

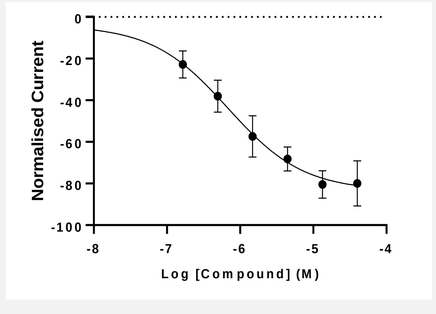
Slow onset compound in Fronline hERG Assay



Slow onset compound in Extended hERG Assay



Slow onset compound in Augmented hERG Assay



IC50 > 20 - 40µM → IC50 = 4.8µM → IC50 = 0.68µM

Building new capabilities in cardiotoxicity safety space, we observed that 30% slow onset compounds detected in a Frontline assay demonstrated 10x shift of potency in Extended assay, which enabled safety team to accurately determine the potency of drug candidates at early stages of drug discovery process.

Summary

- 21% of compounds tested in Fronline hERG assay in AstraZeneca demonstrated slow onset binding, meaning that the potency values are not accurately determined in the assay. In order to address the issue, an efficient workflow constituting of Frontline, Augmented and Extended hERG assays was established at AstraZeneca using various automated patch clamp platforms.
- The introduction of an extended hERG assay into the safety portfolio allowed safety team to accurately determine the potency of early drug candidates and lead decision-making process earlier in the development of new drug candidates.