Introduction

New cardiac safety testing guidelines are being developed as part of the FDA’s Comprehensive in vitro Proarrhythmia Assay (CiPA) initiative, which aims to remove the reliance on screening against the hERG channel by expanding the panel to include other human ventricular ion channels such as Na\textsubscript{1.5}, Ca\textsubscript{1.2}, K\textsubscript{4.3/4.2/CNP2.2}, K\textsubscript{2.1} and K\textsubscript{7.1}/KCNE1. In addition, the CiPA working groups have recently identified two additional ion channel assay readouts required for in silico models to reliably predict proarrhythmia. The first is a ‘late’ Na\textsubscript{1.5} assay, as inhibition of persistent inward current can affect repolarisation and mitigate proarhythmia (e.g. Ranolazine). The second is a kinetic hERG assay that measures drug trapping using the Milnes voltage protocol and improves the prediction of proarrhythmia risk. Here we describe validation of these additional CiPA assays on the gigaseal QPatch48 automated patch clamp platform.

Materials and Methods

1. Na\textsubscript{1.5} (KPQ) late sodium cell line and assay validation

An additional CiPA channel component required for accurately predicting proarrhythmia is the ‘late’ or persistent sodium current. This small current persists throughout the cardiac action potential after initial inactivation of over 99% of sodium channels. The small amplitude of the wildtype late current is not amenable to automated patch clamp recordings so activators such as ATXII and veratridine have been used to induce late openings. However, large shifts in IC\textsubscript{50} values for such drugs as Ranolazine occur between each activator, which can also open endogenous sodium currents. Metrion aimed to remove the need to activate the ‘late’ Nav1.5 current using non-specific pharmacological tools by creating a cell line expressing a long QT syndrome mutation (KPQ), which exhibits an enhanced persistent current.

Robust expression of Na\textsubscript{1.5} KPQ currents suitable for QPatch screening

Dynamic hERG assay

Recent work by FDA and CiPA working groups indicate that addition of hERG kinetic data obtained with the so-called ‘Milnes’ voltage protocol to a modified dynamic O’Hara-Rudy in silico model improves cardiac liability prediction. 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. 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.

References

1. Milnes et al. 2010. JET. PMID: 21272394
5. Denac et al. 2000. Nau Sch Arch Pharm. PMID: 1113883
7. Li et al. 2017. Circ Arrhythm Electrophysiol. PMID: 28202629

Conclusions

Metrion have produced two additional QPatch assays to improve its CiPA cardiac safety assay panel:

- Na\textsubscript{1.5} KPQ ‘late’ current assay to reliably measure low amplitude persistent inward currents
- Dynamic hERG assay to allow assessment of drug binding and trapping