Investigating the importance of temperature dependent hERG channel block in proarrhythmic risk assessment

M.J. Windley,[1] S.A. Mann,[1,2] J.I. Vandenberg[1,2] and A.P. Hill,[1,2] [1]Victor Chang Cardiac Reseach Institute, 405 Liverpool St, Darlinghurst, NSW 2052, Australia and [2]St Vincent's Clinical School, University of New South Wales, NSW 2052, Australia.

Drugs that block the hERG potassium channel in the heart are the major cause of acquired long QT syndrome and are subsequently associated with the risk of developing fatal cardiac arrhythmias. Current regulatory guidelines are based around measurement of the IC_{50} of hERG channel block to predict proarrhythmic propensity. However, there is a growing appreciation that the kinetics of drug block may also be an important metric in assessing risk. Furthermore, while measurement of hERG block is routinely measured at room temperature in high throughput assays, it is documented that the potency of drug block can vary significantly between room and physiological temperatures. It remains to be seen whether this is also the case for the kinetics of drug block, and whether affinity and kinetic parameters measured at room temperature can be extrapolated to physiologically relevant temperatures.

In this study we examined the influence of temperature on the kinetics of cisapride block of the hERG channel. The kinetics of drug block were assessed using a microfluidic device with a solution exchange time of <30 ms. Whole-cell channel currents were evoked from CHO cells stably expressing hERG channels from which we were able to directly measure the rates of association and dissociation *via* fitting of a single exponential to the current record during drug washon and washoff at 22, 27, 32 and 37°C. The IC_{50} of cisapride block (22.9 ± 1.1 nM at 22°C, n = 4-6) was not significantly temperature dependent (ANOVA, $P<0.05$). By comparison, both the on and off rates of cisapride block were dependent on temperature. By raising the temperature from 22 to 37°C the on and off rates were increased by 5.5- and 4.2-fold, respectively. These changes in the kinetics of cisapride block could not be explained by diffusion alone, which would only predict an increase in rates of around 2-fold.

These results show that while temperature does not affect the affinity of cisapride binding to the hERG channel, it does alter the kinetics of the interaction. Existing protocols used to assess proarrhythmic risk do not incorporate measurement of binding kinetics and in most cases are not performed at physiological temperatures. As a result, the current approach is missing a lot of mechanistic information that may help more accurately assign risk to compounds in development. Our study therefore highlights the importance of measuring the kinetics of drug binding at physiological temperatures or at the very least having a better understanding of the temperature dependence of binding affinity and kinetics, both of which are likely to vary considerably between drugs. We suggest that this improved understanding of the mechanistic basic of hERG block will improve our ability to predict which drugs are more likely to induce potentially fatal cardiac arrhythmias.