Is There a Need to Add another Dimension (Time) to the Evaluation of the Arrhythmogenic Potential of New Drug Candidates in vitro?

Running title: Townsend; Arrhythmias: moving beyond acute $I_{K_r}$ block

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Most drug therapy regimens expose the human body to a foreign chemical for several hours to days and even years. Hence, before a new drug is approved by regulatory agencies, extensive safety studies are conducted to ensure that exposure to the drug will not cause undesirable effects in patients. A major cause for adverse events and drug attrition is cardiovascular toxicity\textsuperscript{1,2}. Drug developers have attempted to identify these issues earlier in medicine development to reduce risks to human volunteers in clinical trials and costs of pursuing the development of unsafe drugs. In 2005, the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) issued a guideline for the examination of new drug candidates in a series of \textit{in vitro} and \textit{in vivo} tests to assess their arrhythmogenic potential\textsuperscript{3}. Inhibition of the delayed rectifier potassium current (\(I_{Kr}\)) in the heart has been linked to the majority of drug-induced arrhythmias. As a result, \textit{in vitro} safety testing has been focused on acute drug effects on \(I_{Kr}\) or hERG, the potassium channel that underlies \(I_{Kr}\)\textsuperscript{4}. The development of high-throughput automated methods to measure hERG currents in heterologous expression systems has fueled the emphasis of \textit{in vitro} testing on \(I_{Kr}\) early in drug discovery and perhaps prevented the development of new medicines by discarding compounds prematurely. This testing paradigm has been challenged over the past several years with the realization that verapamil and a number of other drugs which inhibit \(I_{Kr}\) at therapeutic concentrations do not cause arrhythmias in patients. Verapamil has compensatory effects on other cardiac ion channels such that action potential duration (APD) is not affected by this drug and it is safe to administer to patients\textsuperscript{5,6}. In addition, arsenic trioxide, pentamidine and other drugs associated with a prolonged QT interval and cardiac arrhythmias do not block \(I_{Kr}\) acutely but instead inhibit its trafficking to the cell surface when applied for prolonged (overnight or longer) periods of time\textsuperscript{7}. More recently, Lu and colleagues\textsuperscript{8} showed that prolonged application of nilotinib and related anticancer drugs that
inhibit the phospho inositol 3 kinase (PI3K) pathway increased APD and caused early after depolarizations in canine ventricular myocytes. Intracellular infusion of phosphatidyl inositol 3, 4, 5-trisphosphate (PIP3) reversed these effects. Lu and colleagues were also able to demonstrate a link between prolonged APD and increases in late sodium currents, $I_{\text{Na-L}}$, in those cells. Most surprising perhaps was their finding that terfenadine, a well-known anti-histamine associated with $I_{\text{Kr}}$ block and cardiac arrhythmias, exerted the same effects on $I_{\text{Na-L}}$ in a PIP3-sensitive fashion. The report in this issue of Circulation by Yang and colleagues\(^9\) that several proarrhythmic drugs thought to be “selective” $I_{\text{Kr}}$ inhibitors could also increase late sodium currents after prolonged (≥5-hr) exposure further highlights the limitations of examining acute $I_{\text{Kr}}$ effects as a sole in vitro predictor of arrhythmias.

In their publication, Yang and colleagues describe the effects of acute versus “chronic” (≥ 5 hours) exposure to dofetilide on action potentials in mouse cardiomyocytes and human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). In both mouse and human cells, prolonged exposure to dofetilide increased APD and caused both early and delayed after depolarizations. While these effects could be attributed to $I_{\text{Kr}}$ block in human cells, adult mouse cells do not express $I_{\text{Kr}}$. In the latter, dofetilide must therefore be affecting other important determinant(s) of cardiac excitability. The authors then showed that prolonged dofetilide exposure increased late sodium currents in mouse cardiomyocytes, hiPSC-CMs and Chinese hamster ovary (CHO) cells transfected with the cardiac sodium channel Nav1.5. Peak sodium currents were also increased but sodium channel protein levels were unchanged. These findings prompted Yang and colleagues to examine the gating properties of sodium channels after a 5-hr exposure to dofetilide. Channel inactivation was shifted towards more positive potentials by about 20 mV, yielding window currents between -60 and -40 mV. Channel recovery from
inactivation was faster and the rate constants for fast and slow inactivation of macroscopic currents were increased. Taken together, these changes in channel gating are consistent with increased peak and late sodium currents. The mechanism by which the PI3K pathway regulates sodium channel gating remains to be determined. But the results from Yang and colleagues show it is conserved in mouse cardiomyocytes, human stem cell-derived cardiomyocytes and hamster ovary cells. The protein kinase Akt, a downstream effector of PI3K, may be involved, as the authors observed reduced Akt phosphorylation following prolonged exposure to dofetilide. In addition, the PI3K inhibitor LY294002 decreased Akt phosphorylation and increased late sodium currents in cells expressing Nav1.5.

Yang and colleagues also found that effects on late sodium currents were not limited to dofetilide or terfenadine. They reported that other I_{Kr} blockers also increased I_{Na-L} in CHO cells transfected with Nav1.5. D-sotalol and E-4031, both methanesulphonanilides like dofetilide, increased I_{Na-L} after prolonged exposure. They also observed increased late sodium currents, although to a lower extent, with three unrelated drugs, the antipsychotics haloperidol and thioridazine and the antibiotic erythromycin. Finally, moxifloxacin and verapamil did not alter the amplitude of I_{Na-L}. This range of effects on late sodium currents could significantly contribute to the variety of pro-arrhythmic activities of these I_{Kr} blockers. Increased late sodium currents are a known cause of cardiac arrhythmias. Several mutations in SCN5A, the gene encoding the cardiac sodium channel Nav1.5, underlie the long QT syndrome LQT3. These mutations lead to various levels of late sodium currents, all very small relative to peak current amplitudes (≤3%) but large enough to disrupt action potentials and cause arrhythmias.

This paper may show us the tip of the iceberg with regard to the effects of prolonged drug exposure on cardiac action potentials and their underlying ionic currents. Additional studies are
warranted to determine the prevalence of drug effects on late sodium currents. Most early safety pharmacology studies on cardiac ion channels are performed on automated electrophysiology instruments. The latter however generally do not have the sensitivity to measure late sodium current amplitudes reliably ($I_{\text{Na}-L} < 0.5\%$ of peak sodium currents in the transfected CHO cells described in this study). Therefore manual patch clamp studies would be necessary to accurately detect changes in late sodium channel currents after prolonged drug exposure. Testing the effects of prolonged drug exposure on the PI3K signaling pathway (e.g. Akt phosphorylation) could be a first step to identify potential modulators of late sodium currents. However, drugs could increase $I_{\text{Na}-L}$ and cause heart rhythm abnormalities through direct interactions with the channel or through other pathways. These effects would be missed if drugs were only tested in PI3K pathway assays. The alpha-1 adrenergic receptor antagonist and proarrhythmic drug alfuzosin was shown to increase $I_{\text{Na}-L}$ in human embryonic kidney 293 cells. It would be interesting to determine whether these effects are sensitive to PIP3. Finally, Yang and colleagues showed that ATX II, a peptide toxin that binds to sodium channels, increased $I_{\text{Na}-L}$ in a PIP3-independent manner.

The need to examine the effects of drug candidates on multiple cardiac ion channels and review the current testing paradigm is the focus of a recently initiated public-private project called Comprehensive in vitro Proarrhythmia Assay (CiPA). This project proposes, in part, the examination of drug effects on various cardiac channels and their integration in silico by computer models of human cardiac electrophysiology, with the aim to propose a new paradigm for the assessment of the pro-arrhythmic potential of new drugs. However most cardiac ion channel assays and modeling efforts examine acute exposure to drugs and do not consider prolonged or chronic effects. For example they do not incorporate effects on channel...
trafficking. The latter have been well-documented for several drugs and the hERG channel. But recent studies do point out the need to include binding kinetics to refine in silico models. The findings of Yang and colleagues are therefore timely and should stimulate discussions in the field of cardiac safety on the necessity to look at prolonged effects of drug candidates on the various determinants of cardiac excitability.

**Conflict of Interest Disclosures:** None.

**References:**


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