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COMMENTARY

Ions, equations and electrons: the evolving role of computer simulations in cardiac electrophysiology safety evaluations

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Reliable preclinical cardiac safety evaluations of drug candidates are essential for selecting the best therapeutic agents. Advanced automated patch clamp technologies now allow for characterizing drug effects on multiple cardiac currents, enabling subsequent simulations of integrated electrophysiological responses on cellular, tissue and organ levels. In this issue, Mirams *et al*. summarize the strengths and limitations of models and simulations predicting drug-induced electrophysiological responses, emphasizing delayed repolarization and Torsades de Pointes pro-arrhythmia. The utility of computational approaches is contingent upon realistic models of ventricular electrophysiology, robust characterization of drug-channel interactions and an understanding of channel-myocyte interactions and pro-arrhythmic mechanisms. Simulations evaluating effects on repolarization (hazard identification) should aid in selecting safer drug candidates early in drug discovery, while simulations evaluating risk of Torsades de Pointes (incorporating known risk factors) should quantify pro-arrhythmic risk and reduce the need for costly clinical QT studies later in development. The wider adoption of realistic models and simulation studies will depend on simulation performance compared with 'gold standard' clinical findings.

LINKED ARTICLE

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Abbreviations

I_{Ca}, L-type calcium current; I_{Kr}, hERG current; I_{Ks}, slowly activating delayed rectifier current; I_{Na}, fast sodium current

It is well appreciated that delayed ventricular repolarization caused by drugs (or congenital cardiac ion channel mutations) is associated with a rare but potentially lethal arrhythmia termed Torsades de Pointes (named for the 'twisting of points' pattern on the electrocardiogram). I_{Kr} (the current that flows through the hERG channel) is a delayed rectifier repolarizing potassium current (encoded by the *K_v11.1* gene) that is activated during the action potential plateau to initiate ventricular repolarization. Drug-induced block of I_{Kr} is recognized as a primary contributor to Torsades de Pointes, typically in the setting of excessive drug concentration or multiple additional risk factors. Indeed, the link between I_{Kr} block and pro-arrhythmia is the basis for using the *in vitro* functional I_{Kr} assay for screening of small molecule drug candidates in drug discovery, and its inclusion as one cornerstone of the ICH S7B guidance (ICH S7B, 2005). However, it is also well recognized that I_{Kr} represents only one of many currents defining cardiac repolarization in man. Consistent with this concept, a recent study of 39 drugs demonstrated that QTc prolonging drugs are five to seven times more likely to demonstrate low safety margins (≤30, calculated as the ratio of the IC_{50} value for I_{Kr} block vs. therapeutic free plasma concentration) than drugs that do not prolong QTc (Gintant, 2011). These results can be attributed (at least in part) to simultaneous block of depolarizing current(s) (including calcium and late sodium currents) mitigating block of the repolarizing I_{Kr} (Bril *et al*., 1996; Fermini and Fossa, 2003). While qualitative descriptions of multi-channel block can be found, few detailed characterizations are available to predict the extent to which I_{Kr} block is mitigated by drug effects on other currents. Further, most studies performed to evaluate delayed repolarization are typically done under steady-state

conditions and one stimulation rate, rather than the varying rhythm associated with the initiation of Torsades de Pointes (true pro-arrhythmic risk).

With the adoption of automated high-throughput patch clamp techniques it is now possible to evaluate potency of current block of select ion channels expressed in heterologous expression systems for larger numbers of drug candidates. Ion channel profiles derived from such screening campaigns [including e.g. I_{Kr}, I_{Ks} (slowly activating delayed rectifier current), I_{Na} (fast sodium current) and I_{Ca} (L-type calcium current)] theoretically provide the means for efficiently detecting effects on repolarization when coupled with action potential simulations based on ventricular models. Such efforts to identify potential hazard based on delayed repolarization (a surrogate marker of potential pro-arrhythmia) are thus possible without resorting to laborious, expensive and time-consuming myocyte, tissue or animal repolarizing studies. Such computational approaches could reduce or prevent the practice (often in early drug discovery) of prematurely de-prioritizing compounds that reduce I_{Kr} (in so-called early 'frontloading safety studies') without consideration of a more integrated electrophysiological response. Indeed, it can be argued that beneficial drugs such as verapamil and fluoxetine would probably not have been developed based upon their I_{Kr} blocking potency. Not unexpectedly, a recent simulation study suggested that a drug's 'pro-arrhythmic risk' (defined based on prior historical risk assessments) was better predicted based on the integrated effects of three currents (I_{Kr}, I_{Na}, I_{Ca}) compared with I_{Kr} block alone (Mirams *et al.*, 2011). This study also concluded that increased action potential duration correlated best with Torsades risk, despite others suggesting steep restitution curves or triangulation as predictive markers of pro-arrhythmia. More extensive ion channel screening on less studied cardiac currents may also provide early detection of drug effects, thus avoiding unexpected effects on cardiac repolarization (e.g. by reduction of I_{K1} setting the ventricular resting potential). Analogous to experience interpreting I_{Kr} data, questions regarding 'threshold' values and 'safety margins' when translating *in vitro* to human studies for less studied currents will require further refinement.

Despite significant progress, numerous limitations of modelling and simulations for cardiac safety screening are recognized, with many cited by Mirams *et al.* (2012). One concern is related to the veracity of automated *in vitro* determinations of block potency, which may be influenced by (i) functional differences between expressed channels in heterologous systems versus native myocytes (due to channel-associated subunits); (ii) differences in performance of automated patch platforms (including seal resistances); (iii) differences in voltage clamp protocols across platforms and laboratories (including rate, holding potentials, test potentials); (iv) differences in experimental conditions (e.g. room vs. physiological temperature); and (v) differences in anticipated versus actual *in vitro* drug concentrations (more likely with hydrophobic compounds adsorbing to tubing and test chamber). Such differences may substantially influence values for block (or sometimes augmentation) for compounds. Finally, discrepancies between *in vitro* simulation studies and *in vivo* QTc effects may arise due to plasma protein binding and membrane transporters affecting drug distribution and accumulation in the myocardium, as well as the presence of electrophysiologically active metabolites.

It is encouraging that some automated patch platforms now have the capacity to conduct experiments at physiological temperatures, probably a necessary requirement for better characterizing drug effects. It is likely that greater granularity of the kinetics of current block and unblock (beyond IC_{50} values) will be essential for robust stimulation studies, enhancing concordance with clinical observations to bolster present-day arguments against the need for thorough QT studies in the absence of preclinical signals. Data obtained from human cardiac stem cells (with ionic current as well as repolarization studies) may also provide additional (and more 'human-like') data for simulations; such validation efforts are presently ongoing.

Considering ionic current block as more than simply conductance block (the equivalent of reducing the number of conducting ion channels) may prove quite important in simulating proarrhythmic effects (i.e. beyond simple steady-state changes in repolarization). Such effects include allosteric block (when bound drug affects the gating transitions of channel states) and Markov models used to describe more complex drug–channel interactions in which drug binding (channel block) depends on the time a channel occupies different functional states. Such modelling requires more detailed experimental data (time- and voltage-dependent block, kinetics of block and unblock, 'envelopes of current' tests, etc.) and more demanding computational models. Finally, selection of the appropriate human ventricular computational model remains as complex as the often-debated role of ventricular phenotypic heterogeneity and remodelling that occurs in diseased ventricles. Ideally, it would be desirable to 'plug in' known drug-channel parameters and determine the risk of Torsades de Pointes in models with and without known risk factors (e.g. hypokalemia, gender, heart failure). At present, such investigations are largely limited to proarrhythmic animal models used to investigate proarrhythmic mechanisms or to provide confidence in pursuing drug candidates with recognized safety liabilities (Sugiyama, 2008; Oros *et al.*, 2010).

In mid- to late-stage clinical development, the effects of drug candidates on ventricular repolarization (QT interval prolongation, Darpo, 2010) are evaluated in well-controlled clinical thorough QT studies. These studies are used to (i) determine if further clinical data are warranted to assess pro-arrhythmic risk in subsequent clinical development, and (ii) inform subsequent use in the marketplace. Additional simulation studies with multiple compounds (rather than a few examples) are necessary to convincingly demonstrate the utility of computational approaches in defining delayed repolarization, with the ultimate goal of obviating the need for thorough QT studies. The wealth of data accumulated from numerous thorough QT studies (standardized following adoption of ICH E-14 guidelines) provide a clinical 'gold standard' to compare with computationally-derived simulations. These clinical studies provide pharmacodynamic and pharmacokinetic data (with plasma concentrations obtained corresponding to QT measurements) necessary for comparisons to simulation results. Given the numerous thorough QT studies benchmarking the QT prolonging effects of 400 mg moxifloxacin (the *de facto* positive standard for thorough QT

studies), it might be worthwhile to consider this drug as one standard for evaluating future simulation studies. Such studies could also probe T wave morphology changes, a well-characterized clinical effect of moxifloxacin. A recent simulation study noted substantial differences in the effects of block of two repolarizing currents (I_{Kr} and I_{Ks}) on guinea pig, dog and human ventricular repolarization models (O'Hara and Rudy, 2012). These results argue for human ventricular simulations as a valuable addition to future integrated cardiac safety evaluations. Additional robust, detailed characterization of drug effects and better models of ventricular repolarization (in three-dimensional space) will be necessary to delineate pro-arrhythmic risk.

As Mirams *et al*. (2012) recognize, simulations will not fully replace animal-based experimental models for cardiac safety testing in the near future, as additional indirect drug effects on currents (including up- or down-regulation of channel trafficking/expression, metabolic modulation) are not detected with typical screening approaches used today. However, simulations can provide insights into electrophysiological responses on cellular, tissue and organ levels given appropriate data, and thus increase confidence in the risk assessment (and probability of success) of novel drug therapies. Such studies are perhaps most useful for guiding preclinical as well as early phase 1 clinical studies, minimizing animal usage and informing internal decision making. Can virtual hearts and computer simulations predict delayed repolarization and/or human QT prolongation for drugs with multiple electrophysiological actions to save novel drug candidates? Or (more challengingly), can such approaches predict torsadogenic risk? Can simulations that provide insights regarding drug effects on PR and QRS intervals (an additional focus for evolving thorough QT studies) be far behind? Stay tuned for further developments.

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Conflicts of interest

None.

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