# In silico assessment of drug safety in human heart applied to late sodium current blockers

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**Abbreviations:** AP, action potential; APD, action potential duration; EAD, early afterdepolarization; IC_{50}, half inhibition concentration; I_{Kr}, rapidly activating rectifying K⁺ current (hERG); I_{Ks}, slowly activating rectifying K⁺ current; I_{NaL}, late sodium current; I_{In}, inward rectifying K⁺ current; I_{net}, net membrane current; LQT3, long QT 3; pIC_{50}, −log_{10}IC_{50}; QT_{int}, QT interval; RRD, reverse rate-dependence; SF, safety factor for conduction; TDR, transmural dispersion of repolarization; GS967, (6-(4-(trifluoromethoxy) phenyl)-3-(trifluoromethyl)-[1,2,4]triazolo[4,3-a]pyridine)

Drug-induced action potential (AP) prolongation leading to Torsade de Pointes is a major concern for the development of anti-arrhythmic drugs. Nevertheless the development of improved anti-arrhythmic agents, some of which may block different channels, remains an important opportunity. Partial block of the late sodium current (I_{NaL}) has emerged as a novel anti-arrhythmic mechanism. It can be effective in the settings of free radical challenge or hypoxia. In addition, this approach can attenuate pro-arrhythmic effects of blocking the rapid delayed rectifying K⁺ current (I_{Kr}). The main goal of our computational work was to develop an in-silico tool for preclinical anti-arrhythmic drug safety assessment, by illustrating the impact of I_{NaL}/I_{Kr} ratio of steady-state block of drug candidates on “torsadogenic” biomarkers. The O’Hara et al. AP model for human ventricular myocytes was used. Biomarkers for arrhythmic risk, i.e., AP duration, triangulation, reverse rate-dependence, transmemural dispersion of repolarization and electrocardiogram QT intervals, were calculated using single myocyte and one-dimensional strand simulations. Predetermined amounts of block of I_{NaL} and I_{Kr} were evaluated. “Safety plots” were developed to illustrate the value of the specific biomarker for selected combinations of IC_{50} for I_{Kr} and I_{NaL} of potential drugs. The reference biomarkers at baseline changed depending on the “drug” specificity for these two ion channel targets. Ranolazine and GS967 (a novel potent inhibitor of I_{NaL}) yielded a biomarker data set that is considered safe by standard regulatory criteria. This novel in-silico approach is useful for evaluating pro-arrhythmic potential of drugs and drug candidates in the human ventricle.

## Introduction

The emerging importance of the role of an enhanced late sodium current (I_{NaL}) in mammalian ventricle as a contributor to the pathogenesis of acquired and hereditary disease has resulted in this current being a target for anti-arrhythmic drug development. Under relatively common pathological conditions, I_{NaL} density is enhanced significantly (2- to 5-fold) in ventricle. These conditions include heart failure, oxidative stress, hypoxia, ventricular hypertrophy and LQT-related mutations. When I_{NaL} is increased, the action potential duration (APD) of human ventricular myocytes lengthens. This may lead to initiation and/or maintenance of arrhythmias such as Torsade de Pointes (TdP). In all such cases the repolarization reserve is reduced.

Several experimental and clinical studies have demonstrated significant anti-arrhythmic effects of I_{NaL} blockers, such as ranolazine. However, ranolazine and other compounds in development, which are relatively selective for I_{NaL}, may also block other ion channels such as delayed rectifier potassium channels (I_{Kr}). This effect can result in action potential (AP) prolongation. Recently, a potent and selective inhibitor of cardiac I_{NaL}, GS967, has been reported to suppress experimental arrhythmias in female rabbits. It is a requirement of the process of drug development to evaluate the ratio for I_{NaL}/I_{Kr} blockade for this drug candidate.

For this purpose, detailed understanding of the role of the ionic currents involved in the different phases of AP repolarization (early, intermediate and late phases) is essential. The delicate balance of the small ionic currents, which underlie the AP plateau, determines the impact of these drugs on AP prolongation and other biomarkers for arrhythmic risk (e.g., AP triangulation). Although several experimental and theoretical studies of this
have yielded substantial information. Further investigation based on data and principles from human ventricle is required to fully understand ionic mechanisms underlying drug-induced changes in APD.

It is noteworthy that APD prolongation alone appears to be insufficient to define “torsadogenic” risk. Additional biomarkers for arrhythmic risk must be identified and evaluated when defining anti-arrhythmic drug safety. Indeed, changes in QT interval (QTc), reverse rate-dependence (RRD) of APD prolongation and transmural dispersion of repolarization (TDR) have been also proposed as “torsadogenic” indicators.

Within the past 3 y, computer simulations have been employed in drug development programs with the goal of assessing in silico risk for drug-induced cardiac arrhythmia. However, only Mirams et al. and Sarkar et al. utilized human AP models, in addition to models of the rabbit and dog ventricular APs.

The main goals of this project were to identify the relative role of ionic currents at defined phases of repolarization in human ventricle and to use this information to reveal and illustrate the impact of \( I_{NaL} \) and \( I_{Kr} \) block on selected biomarkers that define arrhythmic risk. Our analysis reveals the biophysical basis for reverse rate-dependence in human ventricle. In addition, a new simulation tool denoted the “safety plot” is developed and utilized to assess drug safety for \( I_{NaL} \) blockers.

Results

Effects of \( I_{NaL} \) and \( I_{Kr} \) block on repolarization of diseased human ventricular AP. Single isolated myocyte simulations were conducted to reveal the effects of ranolazine and GS967 on AP waveform and to study the changes of several “plateau” ionic currents at defined stages during repolarization. Figure 1A shows the APs for baseline (left) together with the effects of ranolazine (center) and GS967 (right). Control AP is also shown (see dashed lines) in the three cases for reference. Ranolazine had no significant effects, apparently, changing APD_{90} to 100.9%, APD_{50} to 103% and APD_{50} to 105% of baseline values. In contrast, GS967 decreased APD_{90} to 86.9%, APD_{50} to 87.2% and APD_{50} to 91.1% of baseline. The higher selectivity of GS967 for \( I_{NaL} \) (IC_{50} of 0.13 μM and > 10 μM for \( I_{NaL} \) and \( I_{Kr} \), respectively) compared with ranolazine, can account for its effect on APD compared with ranolazine. The differences in the changes of AP at the selected phases of repolarization can be explained by the different sizes and functional roles of the repolarization currents. For example, Figure 1B shows the small role of \( I_{NaL} \) at 90% of repolarization but also illustrates the more important role of the current at 30% and 60%. A similar pattern also holds for \( I_{Kr} \) and \( I_{Kr} \) (Fig. 1C) at 90% of repolarization. However, the waveform of repolarization also depends on the delicate balance of many other ionic currents (e.g., \( I_{Kr} \), \( I_{NaL} \)). Thus, the \( I_{NaL} \) (Fig. 1E) is a key variable to “track.” In general, in human ventricle a relatively specific drug for \( I_{NaL} \) has greater effects on the early phase of repolarization. This effect on early as opposed to late repolarization has been termed an increase in triangulation.

Rate-dependent effects of \( I_{NaL} \) and \( I_{Kr} \) block on repolarization. To begin to explore the effects of \( I_{NaL} \) and \( I_{Kr} \) blockers on rate-dependent APD changes, APs at two different steady-state simulation frequencies were simulated. Test compounds with different degrees of specificity for the selected ion channels were “applied” by varying the IC_{50} for \( I_{NaL} \) and \( I_{Kr} \). In all the cases the amount of block was calculated for a drug at a concentration of 5 μM. Figure 2 shows APs and several underlying ionic currents for a basic cycle length (BCL) 500 ms (continuous trace) and 2000 ms (discontinuous trace). The superimposed data depict (1) baseline (column 1), (2) in the presence of a drug specific for \( I_{Kr} \) (IC_{50} of 10^{-5} and 10^{-3} M for \( I_{Kr} \) and \( I_{NaL} \), respectively) in column 2, (3) a drug specific for \( I_{NaL} \) (IC_{50} of 10^{-5} and 10^{-7} M for \( I_{Kr} \) and \( I_{NaL} \), respectively) in column 3 and (4) a drug with the same specificity for these two ion channels (IC_{50} of 10^{-5} M for \( I_{Kr} \) and \( I_{NaL} \)) in column 4. As expected, these results demonstrate that a drug more specific for \( I_{Kr} \) (column 2) prolongs APD_{90}, and this effect is larger at low frequencies (discontinuous trace) than at high frequencies (continuous trace), 124% and 118% of baseline at 0.5 Hz and 2 Hz, respectively. The so-called reverse rate-dependence effect exerted by \( I_{Kr} \) blockers, i.e., a greater APD prolongation at low frequencies (see Fig. 2C) can be in part explained by \( I_{Kr} \) accumulation at the higher frequency (residual activation), as experimentally observed by others. We note how in the O’Hara et al. (ORd) model the contribution of \( I_{Kr} \) does not change significantly with frequency. \( I_{Kr} \) is larger at the higher frequency due to residual activation.

Note that if the drug is more selective for \( I_{NaL} \) (column 3), APD_{90} is further shortened at low frequencies (69% and 78% of baseline value at 0.5 Hz and 2 Hz, respectively). This is because the contribution of \( I_{NaL} \) to net current is relatively large at low frequencies.

When the drug has the same specificity for \( I_{NaL} \) and \( I_{Kr} \) (column 4), the changes in APD_{90} are similar for both cycle lengths (113% and 112% of baseline value at 0.5 Hz and 2 Hz, respectively). Note that the reverse rate-dependence effect due to \( I_{Kr} \) block is neutralized by \( I_{NaL} \) block, as has been observed in rabbit ventricular myocytes. This pattern of changes holds for APD_{50} and APD_{50}. Our results show that the \( I_{NaL}/I_{Kr} \) ratio of blockade of potential drugs has an important effect on reverse rate-dependence, which is an indicator to evaluate drug safety.

To further investigate the ionic mechanisms of reverse rate-dependence observed in the presence of \( I_{NaL} \) and \( I_{Kr} \) blockers, we calculated the net current. Indeed, as postulated by Banyasz et al., RRD is an intrinsic property of these human ventricular cells; stimulus frequency modulates APD, so that at low frequencies APD is longer. In all cases, when APD is long, the net current is very small. As a consequence, any change in the very small net current (e.g., due to drug effect) causes prominent changes in APD. The opposite effects take place at high frequencies when APD is shorter and the net outward current is larger. We computed the net current at the instant of time corresponding to APD_{90} for the four cases considered in Figure 2 (baseline, drug 1, 2 and 3), always assuming 5 μM of the drugs with variable specificities for \( I_{NaL} \) and \( I_{Kr} \). These changes were evaluated at different steady-state cycle lengths (from BCL 500 ms to 2000 ms). The relationship between the net current and the APD_{90} is illustrated in Figure 3. These results are in accordance with experimental
has an important effect on rate-dependent changes in APD. To illustrate this, multiple sets of ventricular myocyte simulations were performed at different but constant stimulation frequencies for selected combinations of $I_{\text{NaL}}$ and $I_{\text{Kr}}$ blockade. Potential drugs observations of Banyasz et al.: That is, longer APDs tend to correspond to lower net currents regardless of the drug used.

**APD and rate dependence safety plots.** As described above, our results show the $I_{\text{Net}}/I_{\text{K}}$, ratio of blockade of potential drugs has an important effect on rate-dependent changes in APD. To illustrate this, multiple sets of ventricular myocyte simulations were performed at different but constant stimulation frequencies for selected combinations of $I_{\text{NaL}}$ and $I_{\text{Kr}}$ blockade. Potential drugs
having IC\textsubscript{50} for I\textsubscript{NaL} in the range 10\textsuperscript{-6} to 10\textsuperscript{-3} M, and IC\textsubscript{50} for I\textsubscript{Kr} in the range 10\textsuperscript{-7} to 10\textsuperscript{-5} M were tested at a fixed 5 μM concentration. The effects of different concentrations (3, 5 and 8 μM) at a stimulation frequency of 1 Hz can be observed in Figure S2.

Figure 4 illustrates these findings in the form of a safety plot, using a color scale for APD\textsubscript{90} values. Relatively large values for the biomarker (APD\textsubscript{90}) are represented in red, and relatively small APD values are shown in blue. The circle represented in bottom right corner corresponds to the baseline condition (I\textsubscript{NaL} is enhanced 2-fold). Here, essentially no current block takes place (a pIC\textsubscript{50} results in 0.995 of I\textsubscript{NaL} and I\textsubscript{Kr}). APD\textsubscript{90} is 353.3 ms in this case. Consideration of data in the right edge of the safety plot, shows that when I\textsubscript{NaL} is progressively blocked (IC\textsubscript{50} for I\textsubscript{NaL} decreases, and thus pIC\textsubscript{50} increases) the biomarker decreases (APD\textsubscript{90} is 252.1 ms in the top right corner). Data to the left in the bottom edge, corresponding to a progressive block of I\textsubscript{Kr} (IC\textsubscript{50} for I\textsubscript{Kr} decreases, and pIC\textsubscript{50} increases), lead to an increase of the biomarker [APD\textsubscript{90} is 741.2 ms with the induction of an early-after depolarization (EAD) in the left bottom corner].

But what happens for other combinations of block? Where is the safety barrier? Black lines join the IC\textsubscript{50} combinations for which the biomarker is 120%, 110%, 100% and 90% of baseline value, represented in the bottom right corner. The 90% barrier would depict beneficial effects of the drug, as the biomarker is reduced. In contrast, biomarker values, which fall to the left side of the 110% barrier, imply dangerous effects of the drug increasing the biomarker.

Figure 5 represents safety plots using APD\textsubscript{90}, APD\textsubscript{60}, APD\textsubscript{30} and triangulation as biomarkers, and the safety plots in Figure 6 illustrate the rate-dependence, i.e., the effect of a BCL change on APD\textsubscript{90}. Ranolazine, represented by the black circle, can be positioned in the matrix, based on its approximately IC\textsubscript{50} of 6 and...
12 μM for $I_{NaL}$ and $I_{Kr}$, respectively. Note that this drug is located in the “safe” part of the matrix. Also the test compound GS967 (IC$_{50}$ of 0.13 and >10 μM for $I_{NaL}$ and $I_{Kr}$, respectively), represented by a black triangle, is apparently safer than ranolazine. At high frequencies (first column) shorter APDs and triangulation (blue and green colors) are observed. In contrast, the results at low frequencies (second column) show longer APDs (red and yellow colors). As expected, the decrease in APD exerted by GS967 is more pronounced at low frequencies and especially APD$_{90}$ whereas the slight increase of APD exerted by ranolazine does not result in any significant changes (approximately 110% of the baseline value).

APD triangulation data (Fig. 5D) reveal that both ranolazine and GS967 slightly increase this parameter with respect to the baseline value. Specifically, ranolazine further increases APD$_{90}$ more than APD$_{50}$ whereas GS967 decreases APD$_{50}$ more than APD$_{90}$ at each stimulation rate.

Finally, Figure 6 highlights how drugs very specific for $I_{NaL}$ (such as GS967) decrease APD$_{50}$, APD$_{90}$ and APD$_{30}$ rate-dependence, calculated as the difference between APD at minimum frequency and APD at maximum frequency. In the case of ranolazine, the rate dependence (RD) is unchanged (100% of baseline), due to the fact that the block of $I_{Kr}$ would provoke large reverse rate-dependence, which is neutralized by the concomitant block of $I_{NaL}$ by the drug.

Effects of $I_{NaL}$ and $I_{Kr}$ blockers on QT interval and transmural dispersion of repolarization. Simulations were performed at tissue level based on an in silico fiber of 165 cells composed of a fixed number of endocardial, M and epicardial cells as described in O’Hara et al. Pseudo-ECGs were computed and the corresponding QT intervals were measured. In addition, repolarization times of selected myocytes within the fiber were calculated, and transmural dispersion of repolarization was defined as the difference between the maximum and the minimum repolarization times in the fiber. Figure 7A shows APs measured in the central cells of each part of the tissue (endo-, midmyo- and epicardial tissues) under baseline conditions (left), in the presence of 5 μM ranolazine (center) or GS967 (right). Figure 7B shows the pseudo-ECG for these conditions. Note that QT interval was increased slightly by ranolazine (107% of the baseline value) but was decreased by GS967 (91.4% of the baseline value). Finally, repolarization times at selected myocytes within the fiber are depicted in Figure 7C, and TDR is indicated in the curves. Note that ranolazine and GS967 decreased TDR to 81.5% and 54.2% of the baseline value, respectively.

Safety plots based on QT interval and transmural dispersion of repolarization data. Figure 8 summarizes the values of QT$_{90}^*$ and TDR for different combinations of $I_{NaL}$ and $I_{Kr}$ blockade in different safety plots for 3 μM (left), 5 μM (center) and 8 μM (right) of potential drugs. The reference QT$_{90}^*$ and TDR correspond to the baseline conditions (right bottom corner). The results obtained in our simulations indicate that GS967 is safer than ranolazine, as it reduces the QT$_{90}^*$ down to 90% of its baseline value for the lower concentration.

With regard to the TDR simulations shown in Figure 8B, the two drugs that were assessed reduced TDR quite significantly. This is of interest as TDR is being seriously considered an important biomarker for arrhythmic risk, and very few studies have tested the effects of drugs on this biomarker. The reduction of the TDR exerted by these drugs is notable, highlighting their beneficial effects.

Discussion

Major findings. Our computational work, based on a current and very comprehensive mathematical model of the human ventricular AP, provides novel insights into the roles of $I_{NaL}$ and $I_{Kr}$ block in the modulation of well accepted biomarkers for proarrhythmic risk. Our approach further illustrates and documents the utility of computational methods as one potential assessment tool in Safety Pharmacology. The principal findings and insights from our work are (1) demonstration that it is essential to study the role of selected drug targeted currents ($I_{NaL}$, $I_{Kr}$, $I_{Kr}$) at defined time points of AP repolarization; (2) novel insight into the ionic mechanisms responsible for reverse rate-dependence of antiarrhythmic agents: delayed rectifier K$^+$ currents exhibit a relatively large effect on the net current which governs the initiation of repolarization and modulates the repolarization waveform; (3)

**Figure 3.** Instantaneous net current measured at APD$_{90}$ as a function of APD$_{90}$ for different combinations of $I_{NaL}$/IC$_{NaL}$ ratios (different symbols). For each curve, corresponding to a specific combination of $I_{NaL}$/IC$_{NaL}$ ratio, simulations were performed at increasing BCLs from 500 ms to 2000 ms in each curve. Baseline corresponds to conditions where only $I_{NaL}$ is enhanced 2-fold and no drug is applied.
mechanism-based tool, which can be used to advantage during the initial phases of drug development.

**Mechanisms for reverse-rate dependence of drug-induced APD prolongation.** The repolarization of AP is determined by the very delicate balance of ionic currents.13 A very small change in this balance (net current) caused by a drug may have important consequences on AP morphology and thus on myocyte electrophysiological properties. This concept was first recognized by classical cardiac electrophysiologists35 and originally was termed all-or-none repolarization. Many subsequent studies have provided basis for understanding the ionic mechanisms for repolarization, and the concept of repolarization reserve, through mathematical modeling.13,36 The main goal of the present study (oriented to \(I_{NaL}\) and \(I_{Kr}\) block) was to reveal the effects of established or in development anti-arrhythmic drugs on repolarization in human ventricle using computational methods. Our results show that a new and very selective blocker for \(I_{NaL}\) (GS967) has a relatively large effect on the early phase of repolarization (significant decrease of APD\(_{90}\)) in comparison with its effects on the late phase of repolarization (APD\(_{90}\)) whereas other currents, e.g., \(I_{K1}\), strongly modulate APD\(_{90}\). Similar pattern of results has been reported,10 where GS967 reduced APD\(_{50}\) more than APD\(_{90}\) in isolated rabbit myocytes. Previously, somewhat similar results were obtained by Goineau et al.37 in rabbit Purkinje fibers. Lidocaine increased AP triangulation, by reducing APD\(_{30}\) more than APD\(_{90}\). These findings show that the net impact on AP morphology must be evaluated as a net balance of multiple ion channel conductances.

In our simulations, as demonstrated in the safety plots of Figure 5, the changes in triangulation due to \(I_{NaL}\) block also depend on the amount of \(I_{Kr}\) block, i.e., on the drug specificity. If we consider a pure \(I_{NaL}\) blocker (moving upwards in the right edge of the safety plots of Figure 5D) AP triangulation tends to diminish as specificity for \(I_{NaL}\) increases. Figure 2 illustrates a plausible ionic mechanism for this. The observed decrease in AP triangulation in response to selective blockers of \(I_{NaL}\) is in accordance with the experimental observation that agents that enhance \(I_{NaL}\) have the opposite effect: an increase in triangulation.38,39 Our results also provide insight into a previous paper that reported an increase in AP triangulation following selective \(I_{Kr}\) block.40

Another new mechanistic insight from our simulations is that the ratio \(I_{NaL}/I_{Kr}\) of block by drug candidates can strongly influence the drug-induced RRD of the APD even under steady-state conditions. Most contemporary drug discovery or Safety
Pharmacology initiatives consider RRD as an important biomarker for pro-arrhythmic actions. It is well known that Class III antiarrhythmic agents, such as dofetilide and other selective blockers of I_{Kr}, include RRD effects. RRD in human ventricle was reproduced by our simulations (see Fig. 6). Specifically, our results showed that selective block of I_{NaL} led to APD shortening in a RRD manner, in accordance with experimental studies. These countering actions lead to a neutralization of the reverse rate-dependence of APD prolongation when a drug blocks both I_{NaL} and I_{Kr}. Similar effects have been reported in the setting of simultaneous block of I_{Kr} and I_{NaC}. In summary, the delicate and dynamic balance between I_{Kr} and I_{NaL} as a consequence of any relative affinity (I_{NaC}) differences for ion channel targets can explain RRD of APD in human ventricle.

Several hypotheses have been developed to explain the underlying ionic mechanisms for reverse RRD modulation of APD. It was first postulated that I_{Kr} accumulation (that is, residual activation) observed at relative high frequencies in guinea-pig myocytes was responsible due to the slow deactivation kinetics of this current. A somewhat similar phenomenon and species-dependent (see O’Hara et al.) can be observed in our results (Fig. 2), showing a steeper I_{Kr} increase at fast rates. Here, the kinetics of I_{Kr} could be a significant factor for the RRD of APD prolongation exerted by I_{Kr} blockers. However, I_{Kr} cannot be the only cause of RRD. Thus, even in the setting of I_{Kr} block by HMR1556, RRD APD prolongation was also observed in canine ventricular myocytes.

Quite recently, Banyasz et al. have suggested that RRD was an intrinsic property of human ventricular cells. Indeed, at low frequencies, when APD is relatively long, the net repolarizing current is very small. Under these conditions any change in the plateau currents can lead to significant changes in APD. Our results provide insight into this. Note that the calculated curvilinear relationship of the net current correlates strongly with APD_{90} (Fig. 3). We conclude that in human ventricle intrinsic biophysical properties of I_{Kr} and I_{NaL} and their combined contribution to I_{net} result in the basis for reverse rate-dependence of APD.

Safety of I_{NaL} blockers. Drug-induced APD prolongation, the associated dispersion in transmural repolarization in the human ventricle and TdP inducibility have emerged as significant concerns in drug safety evaluations. Increases in these parameters can be a major obstacle for drug approval. In this context, I_{NaL} is emerging as a promising pharmacological target. Inhibition of this component of Na+ current markedly reduces the TdP inducing capability of agents that prolong the QT interval. Furthermore, I_{NaL} block is likely to have an additional anti-arrhythmic effect, especially in conditions which are characterized by enhanced I_{NaL} due to genetic or acquired causes. These include LQT3, heart failure, hypoxia and free radical challenge.

Our simulations demonstrate that selective block of I_{NaL} (GS967) can decrease well-accepted biomarkers for arrhythmic risk. These include APD, reverse rate-dependence, triangulation, QT_{90} and transmural dispersion of repolarization. This insight is in accordance with experimental findings. Indeed, Belardinelli et al. have reported that in rabbit ventricular myocytes, GS967 almost completely restored the normal APD after it had been markedly increased with ATXII. In control conditions, GS967 had a slight tendency to decrease APD, with the effect being larger for APD_{90} than for APD_{95}. There is also ample experimental and theoretical evidence that I_{NaL} enhancement can have opposite pro-arrhythmic effects, including an increase of triangulation, reverse rate-dependence of APD prolongation measured in transgenic mice with LQT3 or the peak to end interval of the T-wave, which closely approximates TDR, in rabbit ventricular wedges. Perhaps more importantly, many experimental studies have shown that inhibition of I_{NaL} can markedly reduce the risk of drug-induced TdP, e.g., by I_{Kr} blockers. Thus, the combined application of I_{NaL} blockers with I_{Kr} blockers can improve the safety profile. This concept was first illustrated by the simulation work of Noble et al. and is confirmed by our computational results. Note that ranolazine suppressed early afterdepolarizations (EADs) and reduced the increase in TDR induced by the selective I_{Kr} blocker d-sotalol in canine cardiac wedges. However, the net effect and clinical consequence of multiple channel blockade (mainly I_{Na} and I_{Kr}) by ranolazine is a modest increase in the mean QT interval by 2–6 ms. This important experimental observation was also reproduced by our results (see Fig. 8), whereas more selective blockers of I_{NaL} (such as GS967) reduced QT interval.

Safety plots as a tool for anti-arrhythmic drug development. At present, the preclinical assessment of drug-induced ventricular arrhythmia, a major concern for the international cardiac safety pharmacology community, is based mainly on experimental studies. Recently, however, advanced computational technology for in-silico assessment of the efficacy and safety of specific drugs has emerged as a complementary and potentially valuable tool.

Notable research efforts have been made to link molecular dynamics to biophysical models. Other detailed models of drug-ion-channel interaction take into account the rate of binding and unbinding and can be reproduced in either Hodgkin-Huxley or Markov models formulations. For example a recent study on the atrial-selectivity of ranolazine is based on a markovian model of its inhibiting effects on the sodium channels.

In the present study we have used a classical measure of the drug action, by employing IC_{50} data, that is the fraction of block of the targeted channel conductance. A recent computational study by Mirams et al. provided interesting insights into TdP prediction following simultaneous applications of many different ion channel blockers. Other computational studies have assessed the effects of I_{Kr} and/or I_{NaL} blockers on several biomarkers for arrhythmic risk as a proof of concept in the preclinical phase of development of drugs. Our work complements and extends these approaches. We have evaluated for the first time the safety of drugs with different ratios of I_{Kr}/I_{NaL} block, using a recent and very detailed human AP model. Safety was estimated by accepted torsadogenic indicators: APD prolongation, triangulation, reverse rate-dependence, QT_{in} and TDR. The sizes and shapes of the safety zones vary from one biomarker to the other, but a general pattern of behavior can be observed: As the affinity for I_{NaL} block increases, safety (blue and green colors) increases. We note that the safety plot corresponding to the biomarker AP triangulation.
Figure 5. For figure legend, see page 257.
has the most extensive unsafe zone, whereas TDR safety plots have the smallest unsafe zones. In our simulated safety plots a 2-fold enhancement of $I_{\text{NaL}}$ was considered. Based on our analyses we predict that a pathological situation in which $I_{\text{NaL}}$ is further enhanced would increase the size of the safety zone. Indeed, if the enhanced $I_{\text{NaL}}$ has a major role in generating the biomarker parameter, then a specific blocker of this current would tend to restore normal conditions.

**Limitations of this study.** We acknowledge several limitations of our approach at this stage of its development. Caution should be exercised when placing a data set in the safety plots if the simulations have been conducted at different stimulation frequencies. The efficacy of a drug can change significantly with heart rate. In the case of ranolazine, the observed $I_{\text{Kr}}$ block is independent of stimulus frequency, whereas its IC$_{50}$ for $I_{\text{NaL}}$ decreases with increased frequency. This property was not evaluated in our approach because the required data for GS967 block at different frequencies are not available. After the IC$_{50}$ changes as a function of stimulation frequency of a specific drug have been specified, this drug can be correctly positioned in the safety plot and the effects on the different biomarkers can be evaluated.

We also acknowledge that, as pointed out by consensus from the Cardiac Physiome Initiative, development of complex models can include propagation of errors or uncertainty in (1) data selection, (2) interpolation or (3) interpretation. It was principally for these reasons that we selected the ORd model as the fundamental computation platform. The experimental data used to build the model are from the human heart and are very extensive. Nonetheless, the ORd model was developed to model normal physiological AP waveforms, and considers the controversial presence of a large number of M cells in a ventricular strand. Our application extends this data set to a substrate that is a target for clinical anti-arrhythmic agents or drug candidates new in development.

We conclude that safety plots can provide a very valuable tool in the initial phases of drug development, specifically in the preclinical assessment of the arrhythmogenic risk of compounds that block a number of different ion channels. This tool not only overcomes many limitations of experimentation, but also its predictive capacity allows a better selection of experiments, reducing the cost of drug screening.

**Materials and Methods**

**Human ventricular myocyte model.** Simulations of the electrical activity of an endocardial human ventricular myocyte were performed using the human ventricular AP model developed by O’Hara et al. (ORd). This model is based on experimental data taken from 140 healthy human hearts; it encompasses the formulation of 18 ionic currents and carrier-mediated fluxes and a detailed formulation of steady-state and transient ion concentrations, including intracellular Ca$^{2+}$ transients. This model reproduces the electrophysiological behavior of all three types of human ventricular myocytes, with a high degree of fidelity, including alterations due to drug effects.

We have modified the formulation of $I_{\text{NaL}}$ in ORd model to closely match experimental data from Maltsev et al. In their...
we used C++ code run on an array of Dell cluster nodes with 64-bit AMD Opteron processors, running Linux and Sun Microsystems Grid Engine.

Human ventricular strand model. One-dimensional simulations of AP initiation and conduction were performed using a heterogeneous multicellular strand, which resembles some functional features of a ventricular transmural wedge preparation, as described in O’Hara et al. This strand was composed by 60 endocardial, 45 M and 65 epicardial cells.

Drugs. The two drugs that have been evaluated in this study are ranolazine and GS967 (6-(4-(trifluoromethoxy) phenyl)-3-(trifluoromethyl)-[1,2,4] triazolo[4,3-a]pyridine), a potent and selective inhibitor of $I_{NaL}$. Ranolazine has a potency of inhibition (IC$_{50}$) of 6 and 12 μM for the block of $I_{NaL}$ and $I_{Kr}$, respectively, and IC$_{50}$ values for GS967 are 0.13 and > 10 μM for the block of $I_{NaL}$ and $I_{Kr}$, respectively. These values were obtained in rabbit ventricular myocytes, as detailed in Belardinelli et al.

In this study a large number of inter-related sets of simulations were performed. In each, the hypothetical potential drugs were “applied” in selected combinations according to IC$_{50}$ for $I_{NaL}$ and $I_{Kr}$. The ranges of $10^{-7}$ to $10^{-3}$ M (pIC$_{50}$ from 7 to 3) and $10^{-6}$ to $10^{-3}$ M (pIC$_{50}$ from 6 to 3) were assessed respectively for $I_{NaL}$ and $I_{Kr}$. The pharmaceutical description pIC$_{50}$ (standing for $-\log IC_{50}$) was used. To simulate the steady-state effects of these drugs, $I_{NaL}$ and $I_{Kr}$ conductances were reduced with a multiplicative factor (1-b), related to the IC$_{50}$ as follows:

$$b = \frac{1}{1 + \frac{[D]}{IC_{50}}}$$

where [D] stands for the concentration of the potential drug. This value is 5 μM in our simulations, which is within the therapeutic concentration for ranolazine (1 to 10 μM).

Parameter definitions. All APs or other output parameters were measured after achieving steady-state conditions. Steady-state was then defined with an error of 1.9% in APD$_{90}$ after 100 stimulation pulses. Each applied stimulus was 1.5 the threshold and 2 ms in duration. In the strand simulations, the stimuli were applied at the endocardial end of the fiber.

All model equations and code were taken from O’Hara et al., which can be downloaded from rudylab.wustl.edu. Rapid integration methods are provided in the Supplemental Materials from O’Hara et al. For simulation of the basic human model, experiments on human ventricular myocytes, $I_{NaL}/I_{NaT}$ ($I_{NaT}$ denotes peak $I_{Na}$) ratio was approximately 0.1%. In our model, the maximum conductance ($g_{NaL}$) was fitted accordingly using voltage clamp simulations, yielding 0.018 mS/μF. The new APD$_{90}$ remains within experimental values. Details are given in the Supplemental material (Fig. S1).

This $I_{NaL}$ formulation was modified to simulate the effects of pathological conditions. Specifically, $g_{NaL}$ was enhanced 2-fold, as a surrogate for a genetic modification of the human $I_{NaL}$, which results in enhanced $I_{NaL}$, and has been denoted LQT3 syndrome, or to simulate part of the effects of free radical challenge, heart failure or hypoxia. We refer to this single modification of the ORd model as “baseline conditions” throughout the paper.

All model equations and code were taken from O’Hara et al., which can be downloaded from rudylab.wustl.edu. Rapid integration methods are provided in the Supplemental Materials from O’Hara et al. For simulation of the basic human model, from 7 to 3) and $10^{-6}$ to $10^{-3}$ M (pIC$_{50}$ from 6 to 3) were assessed respectively for $I_{NaL}$ and $I_{Kr}$. The pharmaceutical description pIC$_{50}$ (standing for $-\log IC_{50}$) was used. To simulate the steady-state effects of these drugs, $I_{NaL}$ and $I_{Kr}$ conductances were reduced with a multiplicative factor (1-b), related to the IC$_{50}$ as follows:

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Figure 7. (A): action potentials (APs) in endocardial (continuous line), Midmyocardial (dashed line) and epicardial (dotted-dashed line) cells at baseline and after ranolazine (5 μM) and GS967 (5 μM). (B): pseudo-ECGs computed and measured as described in Materials and Methods. (C): Repolarization time (RT) profile along the transmural fiber under baseline conditions, and during steady-state effects of ranolazine (5 μM) and GS967 (5 μM). Repolarization times are shown at 90% of repolarization in these three types of ventricular myocytes at baseline, and in the presence of 5 μM of Ranolazine and GS967. Transmural dispersion of repolarization (TDR) in ms is indicated for each case. Simulations were conducted at a BCL of 1000 ms.
Ionic currents $I_{NaL}$, $I_{Kr}$, the slow component of the delayed rectifier potassium current ($I_{Ks}$), and the inward rectifier $K^{+}$ current ($I_{K1}$) were also measured. Importantly, net current ($I_{net}$) was determined as the sum of all ionic currents in the ORd model. This current was continuously measured during the AP (Fig. 1E). $I_{net}$ was also calculated at a specific instant of time within the AP repolarization phase, i.e., 60% of repolarization (see Fig. 3).

Safety plot construction. We have developed an approach to summarize and illustrate the results of the required complete set of simulations. The effects of potential drugs, having different specificities for $I_{NaL}$ and $I_{Kr}$, on a specific biomarker (APD, triangulation, APD rate-dependence (RD), QT$_{int}$ and transmural dispersion of repolarization) can be illustrated on the plot. This has been achieved by constructing a color-coded map denoted “safety plot” (see Fig. 4; Fig. 5; Fig. 6; Fig. 8; Fig. S2). Each safety plot illustrates the values of the chosen biomarker (e.g., APD$_{90}$) in a color-coded scale as a function of the pIC$_{50}$ values for $I_{Kr}$ (horizontal axis) and $I_{NaL}$ (vertical axis). The simulations were performed for a fixed concentration of the potential drugs (5 μM). Thus, the block amount of both currents could be varied in some of the single myocyte simulations and was 1 Hz in 1D-fiber simulations.

Several accepted biomarkers for arrhythmic risk were calculated in our set of simulations: APD, triangulation, APD rate-dependence (RD), QT$_{int}$ and transmural dispersion of repolarization. APD values were determined at 90%, 60% and 30% of repolarization and are referred as APD$_{90}$, APD$_{60}$ and APD$_{30}$, respectively. By convention, triangulation was defined as the difference between APD$_{90}$ and APD$_{30}$. APD rate-dependence was calculated as the maximum APD$_{90}$ (corresponding to the minimum frequency of stimulation of 0.5 Hz) minus the minimum APD$_{90}$ (corresponding to the maximum frequency of stimulation of 2 Hz). In the multicellular simulations pseudo-ECGs were computed as described in O’Hara et al., and the corresponding QT intervals were measured. Finally, repolarization time (RT) in the selected myocytes of the fiber was computed as the sum of the activation time and the APD$_{90}$ of this cell. Based on this, transmural dispersion of repolarization was defined as the difference between the maximum and the minimum repolarization times along the heterogeneous fiber.

**Figure 8.** Safety Plot analysis based on computed QT interval (QT$_{int}$) (A) or transmural dispersion of repolarization (TDR) (B), as a function of pIC$_{50}$ for $I_{Kr}$ (horizontal axis) and $I_{NaL}$ (vertical axis). Drug concentrations of 3, 5 and 8 μM are considered. Ranolazine is represented by the circle and GS967 by the triangle. Black lines join IC$_{50}$ combinations for which QT$_{int}$ or TDR increase or decrease by 10% or 20% with respect to baseline values, represented in the right bottom edge of the matrix, i.e., where only $I_{NaL}$ is enhanced 2-fold. Simulations were conducted at a BCL of 1000 ms.
calculated from the corresponding pIC_{50}. The resulting sets of biomarker values relate molecular pharmacology actions at steady-state to accepted experimental and/or clinical measures of electrophysiological effect on APD_{90} or QT_{int}. This information is coupled with knowledge of regulatory agency standards for drug-induced changes (denoted by black lines). All simulations were performed under pathological conditions (with enhanced I_{NaL}).

Disclosure of Potential Conflicts of Interest

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Supplemental Material

Supplemental materials may be found here: http://www.landesbioscience.com/channels/article/24905

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