A Simulation Tool to Assess the Pro-arrhythmic Potential of Ion Channel Blockers

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Abstract

Under pathological conditions, such as LQT3, drugs that selectively block late Na⁺ current (INaL) exert antiarrhythmic effects by reducing action potential duration (APD). Some of these compounds also block the delayed rectifier K⁺ current (IKr) exerting an opposite effect. This study was designed to determine the preclinical safety assessment of ranolazine and an experimental compound (A) with multi-ion channel blocking properties.

Using the O’Hara et al. action potential (AP) model for human ventricular myocytes, APDs and QT intervals were calculated in cellular and 1-D tissue simulations, respectively, for different degrees of block of INaL and IKr under LQT3 (with enhanced INaL) conditions.

“Safety plots” were represented in a color scale with respective APDs and QT intervals that correspond to different combinations of IC50s for IKr and INaL of potential drugs. The reference APDs and QT intervals corresponding to LQT3 conditions (enhanced INaL), were shortened or prolonged depending on the IC50s of the drugs. Drugs with increasing selectivity for INaL block: compound A > ranolazine yielded 20 and 0% APD or QT interval shortening, respectively, that would be considered safe. This in-silico model appears to be useful in predicting proarrhythmic potential of drugs, and may be suitable for preliminary screening and drug design.

1. Introduction

Under pathological conditions in which late Na⁺ current (INaL) is abnormally enhanced, such as inherited channelopathies (LQT3), heart failure, acute hypoxia, and exposure to reactive oxygen species, drugs that block INaL exert antiarrhythmic effects by reducing action potential duration (APD), and decreasing the duration of QT interval [1-3]. One of the most INaL-specific blocker currently available is ranolazine, which preferentially blocks INaL over fast INa [2,4] and is approved by the FDA. Ranolazine has been used in experimental studies to eliminate early after depolarizations (EADs) under situations of heart failure [2] or in the case of exposure to reactive oxygen species [3].

However, some of the available antiarrhythmic drugs inhibiting INaL, present risks in their therapeutic profile depending on their effects on other currents (e.g., IKr). Indeed, the concomitant inhibition of INaL and IKr may lead to a complex modulation of repolarization, and even QT-prolongation [5]. For instance, Ranolazine cannot be considered a pure INaL blocker because it also blocks IKr at concentrations only 1.5- to 2-fold higher than those at which it blocks INaL [6,7]. Wu et al. observed action potential duration (APD) prolongation in rabbit hearts exerted by ranolazine but no EADs formation or ventricular arrhythmias [8].

It is thus complex to define safety profiles of drugs with mixed actions. The preclinical assessment of drug-induced ventricular arrhythmia represents a major concern for regulators, and is typically based on experimental studies. Recently, in silico techniques enrich the cardiotoxicity evaluation of drugs under design, using computational models [9,10].

Using a computational model, this study was designed to determine the preclinical safety assessment of ranolazine and an experimental compound with multi-ion channel blocking properties. Safety of the drug was assessed by the identification of the ratio of IKr / INaL block required for the drug to be safe in terms of decreasing APD and QT interval.

2. Methods

Simulations were carried out at cellular level considering an endocardial human ventricular cell using the latest human ventricular AP model by O’Hara et al. [11] (ORd). Steady-state action potential duration was computed at 90% of repolarization (APD90).

At the tissue level, simulations were conducted considering a fiber of 165 cells composed by endocardial, M, and epicardial cells as described in [11] and shown in...
Figure 1. Pseudo-ECGs were computed and the corresponding QT intervals were calculated after achieving steady-state. The stimuli applied were 1.5 the stimulation threshold in amplitude and 2 ms in duration, as well as for the unicellular simulations.

Figure 1. 1D transmural tissue composed by 60 endocardial cells, 45 M-cells and 60 epicardial cells. Stimulation was applied to the endocardial edge. Pseudo-ECG was computed at an electrode 2 cm apart from the epicardial zone.

In both cellular and tissue simulations pathological control conditions were considered as normal physiological conditions defined in ORd model with a 10-fold increased $I_{\text{Nat}}$, as a surrogate for LQT3. APD$_{90}$ and QT intervals were computed for control pathological conditions and for different ratios of $I_{\text{Kr}}/I_{\text{Nat}}$ blockade.

The results for APD$_{90}$ and QT interval were summarized graphically in “safety plots” (see Figures 3 and 4). The safety plot represents a matrix with APD$_{90}$ or QT interval values in a color scale, corresponding to different ratios of $I_{\text{Kr}}/I_{\text{Nat}}$ blockade. The blockade of $I_{\text{Nat}}$ is indicated in the vertical axis and the $I_{\text{Kr}}$ blockade in the horizontal axis by the half inhibition concentration (IC$_{50}$) of the potential drug for each current. The blockade of the currents applied in the simulations and the IC$_{50}$s are related as follows:

$$b = \frac{1}{1 + \frac{D}{IC_{50}}} \quad (1)$$

where (1-b) is the multiplicative factor of the current applied in ORd, [D] stands for the concentration of a potential drug (5 µM in our simulations), and IC$_{50}$ is the half inhibition concentration of the potential drug for the corresponding current.

The safety plot provides information about APD$_{90}$ or QT interval values corresponding to different potential drugs with different specificities for $I_{\text{Nat}}$ and $I_{\text{Kr}}$ applied under LQT3 conditions, giving thus an estimation of their safety.

3. Results and discussion

This section describes the results of the simulations conducted for a pathological situation in which $I_{\text{Nat}}$ current was increased 10-fold.

In first instance, simulations were carried out at cellular level and APD$_{90}$ values were computed for different ratios of $I_{\text{Kr}}/I_{\text{Nat}}$ blockade. The reference APD yielded 439 ms corresponding to the pathological situation with no current blockade, and the AP is depicted in Figure 2 trace 1). This APD is indeed longer than control APD in ORd model (270 ms), as has been demonstrated in experimental studies using $I_{\text{Nat}}$ enhancers [12]. The application of 5 µM of a potential drug very specific for $I_{\text{Nat}}$ blockade, i.e. IC$_{50}$ for $I_{\text{Nat}}$ of 0.1 µM and IC$_{50}$ for $I_{\text{Kr}}$ of 1000 µM (which corresponds to an insignificant block of $I_{\text{Kr}}$ and a 98% block of $I_{\text{Nat}}$) yielded an APD$_{90}$ of 252 ms (trace 2) in Figure 2). The shortening of APD$_{90}$ indicates the safety of a very specific blocker for $I_{\text{Nat}}$. Shortening of APD has also been observed experimentally with selective (although non purely selective) blockers of $I_{\text{Nat}}$ [2,3]. Conversely, a very specific drug for $I_{\text{Kr}}$ blockade (IC$_{50}$ of 1 µM) and not for $I_{\text{Nat}}$ (IC$_{50}$ of 1000 µM) would provoke no repolarization (trace 3) in Figure 2). These results are in agreement with experimental studies in which $I_{\text{Kr}}$ block leads to significant increase of APD, EADs generation or non-repolarization [13,14]. Finally, when the IC$_{50}$ ratio for $I_{\text{Kr}}$ and $I_{\text{Nat}}$ is 1/0.1 APD$_{90}$ was slightly increased (533 ms). These results can be compared to the experimental observations from Wu et al., in which blockers of $I_{\text{Kr}}$ and $I_{\text{Nat}}$ can prolong the APD [8].

Figure 2. APs obtained in the cellular simulations for LQT3 pathological control conditions (trace 1) in panels A and B, and for the application of potential drugs with different specificity ratios for $I_{\text{Kr}}$ and $I_{\text{Nat}}$ blockade (other traces) under LQT3 pathological conditions.
Figure 2 panel B shows the APs corresponding to the particular cases of the blockades exerted by ranolazine (trace 5) and a drug under design: compound A (trace 6). IC50s for INaL and IKr are 6 and 12 µM for ranolazine [15], and 0.2 and 8 µM for compound A, respectively. The decrease in APD90 with respect to the control pathological conditions are 0.9% and 23.7%, respectively.

The safety plot represented in Figure 3 summarizes all the cases tested in the cellular simulations and gives an orientation of safety in drugs design.

Figure 3 represents in a color scale the APD90 for the different IC50 selected for IKr and INaL, always considering 5 µM of the potential drug. Longer APD90s are represented in red and shorter APD90s in blue. The pathological reference APD90 is 439 ms and is represented in the bottom right corner (indicated by the black square) where IKr is normal (high IC50 implies a very low block for the concentration of the drug) and INaL is 10-fold increased. As we go up in the right edge INaL is progressively blocked (IC50 for INaL decreases) and APD90 shortens, however if we move to the left in the bottom edge, IKr is blocked (IC50 for IKr decreases) and APD90 is increased.

But what happens for other combinations of block? Where is the safety barrier? White lines join the IC50 combinations for which APD90 is 120%, 110%, 100%, 90%, and 80% of the pathological reference APD90. Up from the 90% barrier, would imply beneficial effects of the drug, as APD90 is reduced. However, the left side of the 110% barrier implies dangerous effects of the drug prolonging APD90. Ranolazine, represented by the pink circle, is indeed located in the safe part of the matrix. So is compound A, represented by a pink triangle.

In second instance, simulations were carried out at tissue level considering a fiber of 165 cells composed by endocardial, M, and epicardial cells as described in [11]. Pseudo ECGs were computed and the corresponding QT intervals are shown in the safety plot of Figure 4. The reference QT interval corresponds to the pathological situation with no blockade (shown in the bottom right corner of the safety plot with a black square). The results obtained in our simulations indicate that compound A is safer than ranolazine, as it reduces the QT interval to around 80% of its control value.

Figure 4. QT safety plot. 2D QT map as a function of IC50 (in µM) for IKr (horizontal axis) and INaL (vertical axis), for a drug concentration of 5 µM. The color legend is expressed in ms. Ranolazine is represented by the pink circle and compound A by the pink triangle. White lines join IC50 combinations for which QT interval is 10% or 20% increased or decreased with respect to the reference pathological QT interval, represented in the right bottom edge of the matrix, where only INaL is 10-fold enhanced.

Similar maps of APD and QT interval were presented by other groups [9,10] to assess cardiotoxicity considering the joint block of IKr and IKs. Indeed, cardiac safety assessment has traditionally been only based on hERG, and this has the risk of producing either false positive or negative results. The consideration of multichannel effects improves substantially the
cardiotoxicity assessment. In the present simulation study, the main goal was different, as we aimed at the estimation of an IC\textsubscript{50} ratio for I\textsubscript{Kr}/I\textsubscript{NaL} to assure cardiac safety.

4. Conclusion

The results obtained in the present simulations provide a helpful tool for drug safety assessment. These results can be considered a proof of concept, suggesting that systems of prediction based on computer modeling can be suitable and give an orientation of the electrophysiological effects of the drug under design, and can be used for preliminary screening in drug discovery.

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