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

Beatriz Trenor[1], Julio Gomis-Tena[1], Jose M Ferrero[1], Sridharan Rajamani[2], Luiz Belardinelli[2], Javier Saiz[1]

[1]Universitat Politècnica de València, Valencia, Spain
[2]Gilead Science, Fremont, CA, USA

Abstract

Under pathological conditions, such as LQT3, drugs that selectively block late Na^+ current (I_{NaL}) exert antiarrhythmic effects by reducing action potential duration (APD). Some of these compounds also block the delayed rectifier K^+ current (I_{Kr}) 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 I_{NaL} and I_{Kr} under LQT3 (with enhanced I_{NaL}) conditions.

"Safety plots" were represented in a color scale with respective APDs and QT intervals that correspond to different combinations of IC_{50}s for I_{Kr} and I_{NaL} of potential drugs. The reference APDs and QT intervals corresponding to LQT3 conditions (enhanced I_{NaL}), were shortened or prolonged depending on the IC_{50}s of the drugs. Drugs with increasing selectivity for I_{NaL} 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 (I_{NaL}) is abnormally enhanced, such as inherited channelopathies (LQT3), heart failure, acute hypoxia, and exposure to reactive oxygen species, drugs that block I_{NaL} exert antiarrhythmic effects by reducing action potential duration (APD), and decreasing the duration of QT interval [1-3]. One of the most I_{NaL}-specific blocker currently available is ranolazine, which preferentially blocks I_{NaL} over fast I_{Na} [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 I_{NaL}, present risks in their therapeutic profile depending on their effects on other currents (e.g., I_{Kr}). Indeed, the concomitant inhibition of I_{NaL} and I_{Kr} may lead to a complex modulation of repolarization, and even QT-prolongation [5]. For instance, Ranolazine cannot be considered a pure I_{NaL} blocker because it also blocks I_{Kr} at concentrations only 1.5- to 2-fold higher than those at which it blocks I_{NaL} [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 cardiotoxocity 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 I_{Kr} / I_{NaL} 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 (APD_{90}).

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.

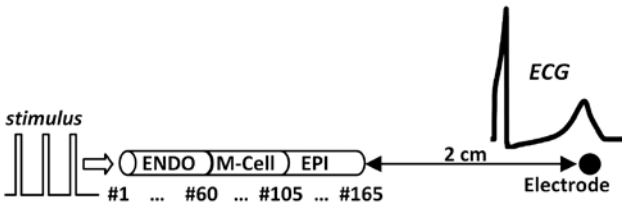

Figure 1. 1D transmural tissue composed by 60 endocardial cells, 45 M-cells and 60 epicardial cells. Stimulation was applied to the endocardial edge. Pseudo-ECG was computed at an electrode 2 cm apart from the epicardial zone.

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_{NaL}, as a surrogate for LQT3. APD_{90} and QT intervals were computed for control pathological conditions and for different ratios of I_{Kr}/I_{NaL} 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_{Kr}/I_{NaL} blockade. The blockade of I_{NaL} is indicated in the vertical axis and the I_{Kr} bockade 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:

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

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_{NaL} and I_{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_{NaL} 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_{Kr}/I_{NaL} 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_{NaL} enhancers [12]. The application of 5 µM of a potential drug very specific for I_{NaL} blockade, i.e. IC_{50} for I_{NaL} of 0.1 µM and IC_{50} for I_{Kr} of 1000 µM (which corresponds to an insignificant block of I_{Kr} and a 98% block of I_{NaL}) 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_{NaL}. Shortening of APD has also been observed experimentally with selective (although non purely selective) blockers of I_{NaL} [2,3]. Conversely, a very specific drug for I_{Kr} blockade (IC_{50} of 1 µM) and not for I_{NaL} (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_{Kr} block leads to significant increase of APD, EADs generation or non-repolarization [13,14]. Finally, when the IC_{50} ratio for I_{Kr} and I_{NaL} 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_{Kr} and I_{NaL} can prolong the APD [8].

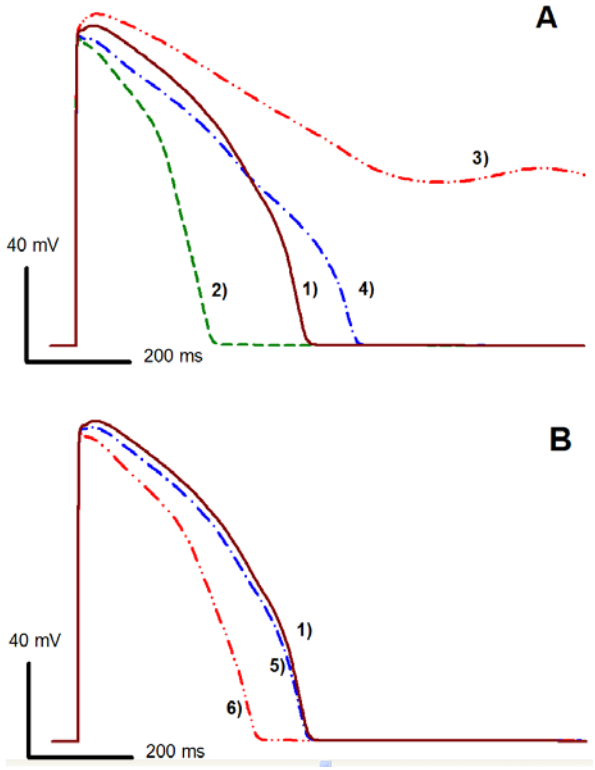

Figure 2. APs obtained in the cellular simulations for LQT3 pathological control conditions (trace 1) in panels A and B, and for the application of potential drugs with different specificity ratios for I_{Kr} and I_{NaL} blockade (other traces) under LQT3 pathological conditions.

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). IC_{50}s for I_{NaL} and I_{Kr} are 6 and 12 µM for ranolazine [15], and 0.2 and 8 µM for compound A, respectively. The decrease in APD_{90} 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 APD_{90} for the different IC_{50} selected for I_{Kr} and I_{NaL}, always considering 5 µM of the potential drug. Longer APD_{90}s are represented in red and shorter APD_{90}s in blue. The pathological reference APD_{90} is 439 ms and is represented in the bottom right corner (indicated by the black square) where I_{Kr} is normal (high IC_{50} implies a very low block for the concentration of the drug) and I_{NaL} is 10-fold increased. As we go up in the right edge I_{NaL} is progressively blocked (IC_{50} for I_{NaL} decreases) and APD_{90} shortens, however if we move to the left in the bottom edge, I_{Kr} is blocked (IC_{50} for I_{Kr} decreases) and APD_{90} is increased.

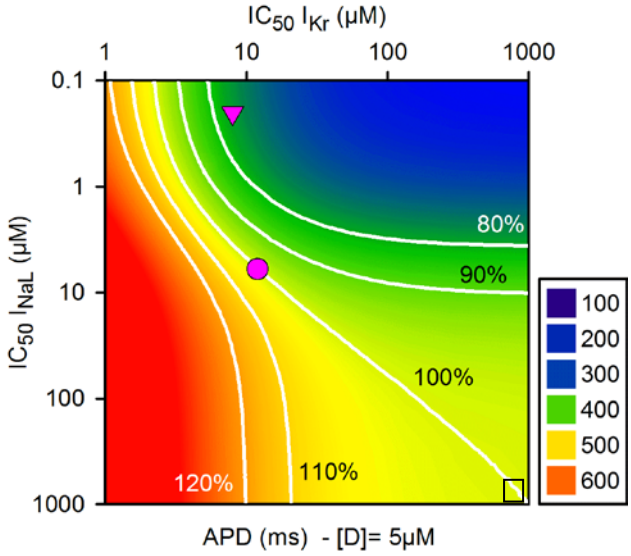

Figure 3. APD_{90} safety plot. 2D APD_{90} map as a function of IC_{50} (in µM) for I_{Kr} (horizontal axis) and I_{NaL} (vertical axis), for a drug concentration of 5 µM. The color legend is expressed in ms. Ranolazine is represented by the pink circle, and compound A by the pink triangle. White lines join IC_{50} combinations for which APD_{90} is 10% or 20% increased or decreased with respect to the reference pathological APD_{90}, represented in the right bottom edge of the matrix, where only I_{NaL} is 10-fold enhanced.

But what happens for other combinations of block? Where is the safety barrier? White lines join the IC_{50} positive or negative results. The consideration of combinations for which APD_{90} is 120%, 110%, 100%, 90%, and 80% of the pathological reference APD_{90}. Up from the 90% barrier, would imply beneficial effects of the drug, as APD_{90} is reduced. However, the left side of the 110% barrier implies dangerous effects of the drug prolonging APD_{90}. 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.

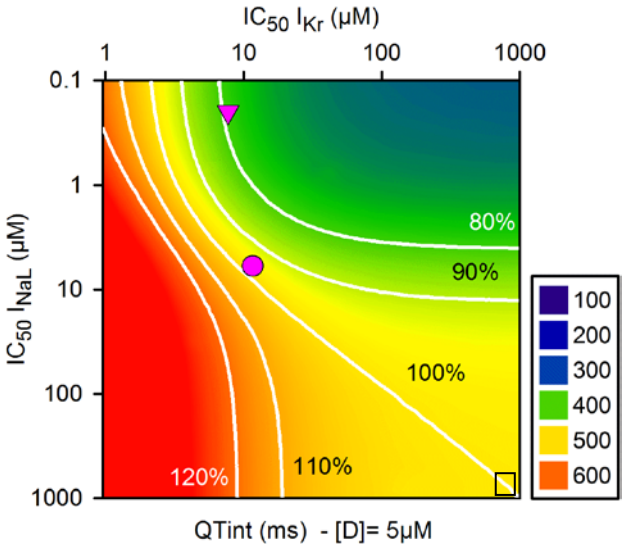

Figure 4. QT safety plot. 2D QT map as a function of IC_{50} (in µM) for I_{Kr} (horizontal axis) and I_{NaL} (vertical axis), for a drug concentration of 5 µM. The color legend is expressed in ms. Ranolazine is represented by the pink circle and compound A by the pink triangle. White lines join IC_{50} 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 I_{NaL} 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 I_{Kr} and I_{Ks}. 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_{50} ratio for I_{Kr}/I_{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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Address for correspondence.

Beatriz Trenor.
Departamento de Ingeniería Electrónica (7F)
Universidad Politecnica de Valencia
Camino de Vera s/n Valencia 46022. Spain.
btrenor@eln.upv.es