

In Silico Assessment for Drug Design of Multi-ion Channel Blockers

B. Trenor¹, Julio Gomis-Tena¹, Jose M Ferrero¹, Javier Saiz¹

¹Universitat Politècnica de València, Valencia, Spain, btrenor@eln.upv.es

Abstract

This study was designed to determine the preclinical safety assessment of multi-ion channel blockers. Drugs blocking late Na⁺ current (I_{NaL}) exert antiarrhythmic effects by reducing action potential duration (APD). These compounds usually block also the delayed rectifier K⁺ current (I_{Kr}) exerting an opposite effect. We used the O'Hara et al. action potential (AP) model for human ventricular myocytes, and calculated APDs and QT intervals in cellular and 1-D tissue simulations, respectively, for different degrees of block of I_{NaL} and I_{Kr} under enhanced I_{NaL} conditions. We represented in a color scale APDs and QT intervals that correspond to different combinations of IC₅₀s for I_{Kr} and I_{NaL} of potential drugs. When the reference APDs and QT intervals were shortened the potential drug could 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

In situations such as inherited channelopathies (LQT3), heart failure, acute hypoxia, and exposure to reactive oxygen species, the late Na⁺ current (I_{NaL}) is abnormally enhanced. Under these circumstances, 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

cardiotoxicity evaluation of drugs under design, using computational models [9,10].

This study was designed to determine the preclinical safety assessment of ranolazine and an experimental compound with multi-ion channel blocking properties, using a computational model. 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 assesses about the drug safety in the design process.

2. Methods

We used the latest human ventricular AP model by O'Hara et al. [11] (ORd) to carry out simulations considering an endocardial human ventricular cell. 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 60 endocardial, 45 M, and 60 epicardial cells as described in [11]. Pseudo-ECGs were computed in an electrode placed at 2 cm from the epicardial edge of the tissue, 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.

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. APD₉₀ and QT intervals were computed for control pathological conditions and for different ratios of I_{Kr}/I_{NaL} blockade.

The results for APD₉₀ and QT interval were summarized graphically in "safety plots" (see Figure 2). The safety plot represents a matrix with APD₉₀ 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} blockade in the horizontal axis by the half inhibition concentration (IC₅₀) of the potential drug for each current. The blockade of the currents applied in the simulations and the IC₅₀s are related as follows:

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

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 IC₅₀ is the

half inhibition concentration of the potential drug for the corresponding current.

The safety plot provides information about APD₉₀ 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

We considered a pathological situation in which I_{NaL} current was increased 10-fold.

Firstly, simulations were carried out at cellular level and APD₉₀ 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 1 trace a). This APD is 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 μ M of a potential drug very specific for I_{NaL} blockade, i.e. IC₅₀ for I_{NaL} of 0.1 μ M and IC₅₀ for I_{Kr} of 1000 μ M (which corresponds to an insignificant block of I_{Kr} and a 98% block of I_{NaL}) yielded an APD₉₀ of 252 ms (trace b) in Figure 1). The shortening of APD₉₀ 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 (IC₅₀ of 1 μ M) and not for I_{NaL} (IC₅₀ of 1000 μ M) would provoke no repolarization (trace c) in Figure 1). 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 IC₅₀ ratio for I_{Kr} and I_{NaL} is 1/0.1 APD₉₀ 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].

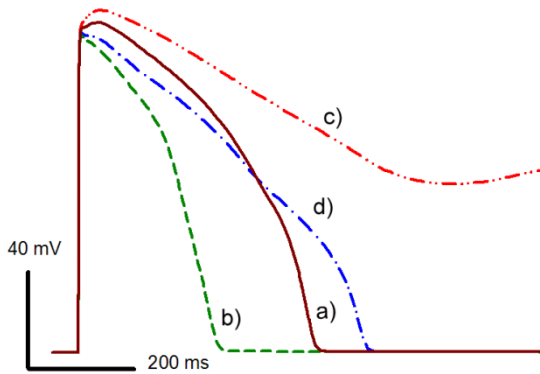


Figure 1. APs obtained in the cellular simulations for LQT3 pathological control conditions (trace 1) in panels A and B, and for the application of potential drugs with different specificity ratios for I_{Kr} and I_{NaL} blockade (other traces) under LQT3 pathological conditions.

We also considered in our simulations the particular cases of the blockades exerted by ranolazine and a drug under design: compound A. IC₅₀s for I_{NaL} and I_{Kr} are 6 and 12 μ M for ranolazine [15], and 0.2 and 8 μ M for compound A, respectively. The decrease in APD₉₀ with respect to the

control pathological conditions are 0.9% and 23.7%, respectively.

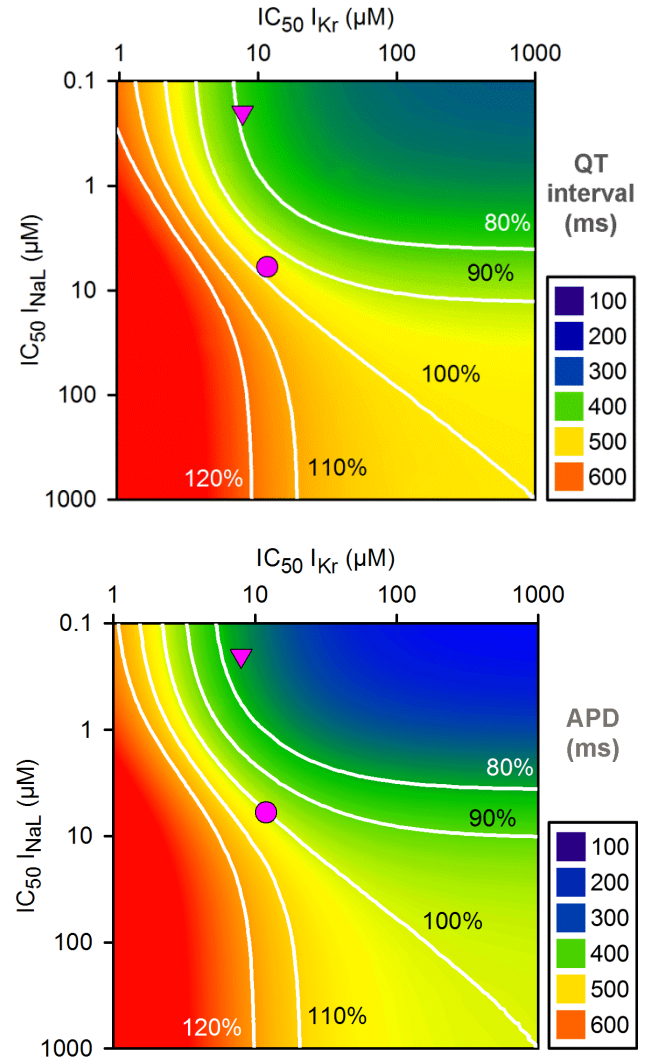


Figure 2. APD₉₀ (top panel) and QT interval (low panel) safety plots. 2D APD₉₀ and QT interval maps as a function of IC₅₀ (in μ M) for I_{Kr} (horizontal axis) and I_{NaL} (vertical axis), for a drug concentration of 5 μ M. The color legend is expressed in ms. Ranolazine is represented by the pink circle, and compound A by the pink triangle. White lines join IC₅₀ combinations for which APD₉₀ or QT interval are 10% or 20% increased or decreased with respect to the reference pathological values represented in the right bottom edge of the matrix, where only I_{NaL} is 10-fold enhanced.

The safety plot represented Figure 2 (top panel) summarizes all the cases tested in the cellular simulations and gives an orientation of safety in drugs design. APD₉₀ for the different IC₅₀ selected for I_{Kr} and I_{NaL} are represented in a color scale for the different IC₅₀ selected for I_{Kr} and I_{NaL}, always considering 5 μ M 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 I_{Kr} is normal (high IC₅₀ 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 (IC₅₀ for I_{NaL} decreases) and APD₉₀ 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. But what happens for other combinations of block? Where is the safety barrier? White lines join the IC_{50} 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.

Secondly, 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 lower safety plot of Figure 2. The reference QT interval corresponds to the pathological situation with no blockade (shown in the bottom right corner of the safety plot). 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. These QT intervals are shown in Figure 3 for the particular cases of Ranolazine and compound A.

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 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.

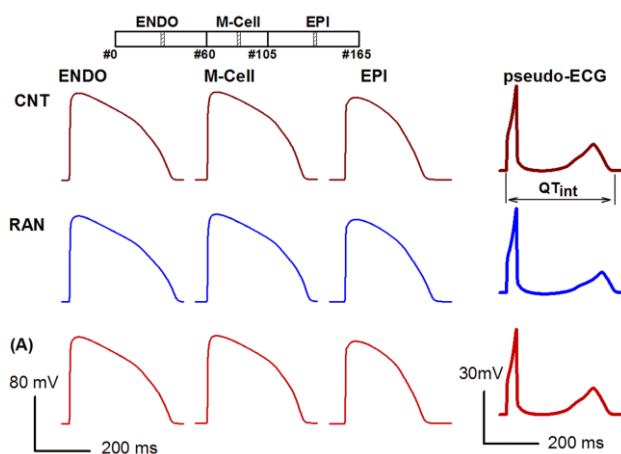


Figure 3. Left: APs in endocardial, M, and epicardial cells. Right: pseudo-ECGs. The results are shown for pathological control (first row), under the effects of 5 μ M Ranolazine and compound A, in the second and third rows, respectively.

4. Conclusions

The present work provides a helpful tool for drug safety assessment. The obtained results suggest 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 Politécnica de Valencia
Camino de Vera s/n Valencia 46022. Spain.
btrenor@eln.upv.es