Dynamic Dofetilide-hERG Channel Model Considering Preferential State Binding and Trapping Properties

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Abstract

Assessment of cardiotoxicity is crucial in the development of new compounds and understanding binding dynamics is important to study drug effects. We previously developed computational pipeline for generating Markovian drug-hERG channel models that reproduce binding properties. We have tested our methodology with the real I_{Kr} blocker dofetilide.

Experiments were conducted using hERG transfected HEK cells and the Nanion SyncroPatch 384i. We applied three voltage clamp protocols, named P0, P40 and P-80, to generate the model. A unique IC_{50} value was also estimated using the CiPA ramp protocol and the model was tested with the Milnes protocol.

The model accurately replicates the experimental data recordings with our protocols and the IC_{50} value is in accordance with the available literature. The model also resembles the behavior observed with the Milnes protocol.

We show an experimental proof of concept of a novel methodology for generating dynamic drug models obtained with simple voltage clamp protocols that may constitute a marked improvement in cardiac safety assessment.

1. Introduction

The human ether-á-go-go related gene channel (hERG) is responsible for the rapid delayed inward rectifier current (I_{Kr}), one of the key regulators of action potential duration. Block of this current is usually associated with the apparition of life threatening arrythmia and, therefore, potency of block of this channel by a compound is one of the most common tests to evaluate its safety [1, 2].

Recent publications have proved that considering drug dynamics is important to correctly assess cardiotoxicity and improves its early assessment [3, 4, 5]. Preferential state-dependent binding and unbinding, time course of block and trapping properties are characteristics that have been reported to play a crucial role in studying drug-channel interactions [5, 6, 7]. We have previously developed a computational pipeline to generate dynamic drug models that consider these properties based on simple voltage clamp protocols and with low computational requirements [8], patent pending P202230300. In this study, we present a dofetilide model using this methodology, which constitutes an experimental proof of concept. This model realistically reproduces the experimental data with the P0, P40 and P-80 protocols and resembles the experiments obtained with the Milnes one.

2. Methods

2.1. Experiments

Experiments were performed using the SyncroPatch 384i high throughput platform from Nanion Technologies. HEK cell lines with stably transfected hERG channel expression were kindly provided by SB DD. Internal solutions contained 10 NaCL, 110 KF, 10 KCl, 10 HEPES and 10 EGTA and three filling solutions were used, first a solution containing 140 NaCL, 10 HEPES and 4 KCl, then a seal enhancer containing 60 NMDG, 10 HEPES, 4 KCl, 10 Ca2Cl and 1 Mg2Cl to introduce divalent ions in the external solution and finally a reference solution containing 60 NMDG, 10 HEPES, 4 KCl, 2 Ca2Cl and 1 Mg2Cl. pH was adjusted to 7.4 and experiments were conducted at 22ºC. Dofetilide was supplied by Nanion Technologies.

We used our three previously developed voltage clamp protocols, named P0, P40 and P80, which enhance the probability of the channel to occupy the open, inactive and closed states, respectively [5] (Figure 1A). All recordings were conducted in one day and using the same cell line and solutions.

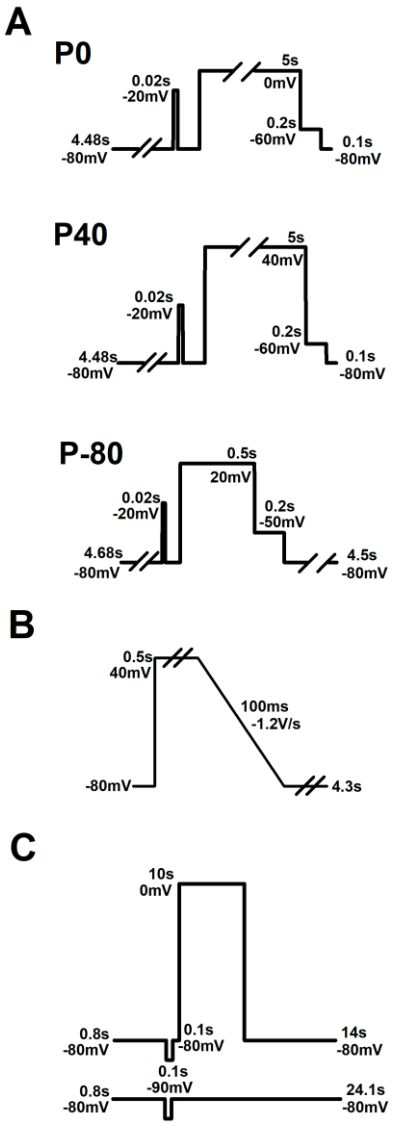

Figure 1. Protocols used for experiments (A). CiPA proposed ramp protocol used for simulation of IC50 (B).

We used the Fink et al. dynamic hERG model [9]. The model has five closed states, one open state and one inactivated state, named C3, C2, C1, O and I respectively. We added five drug-bound states to simulate compound effects named C3d, C2d, C1d, O and I respectively, as displayed in Figure 2. We considered a wide range of binding states combinations and trapping properties [8].

Figure 2 shows the full scheme of the methodology used to generate the dynamic dofetilide-hERG channel interaction model. We first used our proposed pipeline [8] to classify the compound based on the preferential binding states and trapping properties and to obtain the transition rates for the dynamic model. Then, we simulated our newly generated model using the CiPA initiative ramp protocol (Figure 1B) to obtain a unique IC_{50} for our developed model [10]. Finally, we simulated the model with the Milnes protocol (Figure 1C) [3, 11] to compare the results with the data available from the FDA [3].

3. Results

3.1. Dofetilide dynamic model

To generate the dynamic model of dofetilide we obtained the IC_{50} for each of the experimental protocols tested and measured the onset of I_{Kr} block. Then we used our previously developed computational tool [8] to elucidate the binding properties and transition rates of the compound.

Dofetilide was fitted to an InactiveO compound with trapped properties, meaning that the drug binds to the inactive and open states with higher affinity for the former and that the model was allowed to change its conformational state while drug bounded. Figure 3A shows the experimental hill-plots for P0, P40 and P-80 (solid red, green and blue lines, respectively) and the hill-plots obtained with our dynamic model for each protocol.

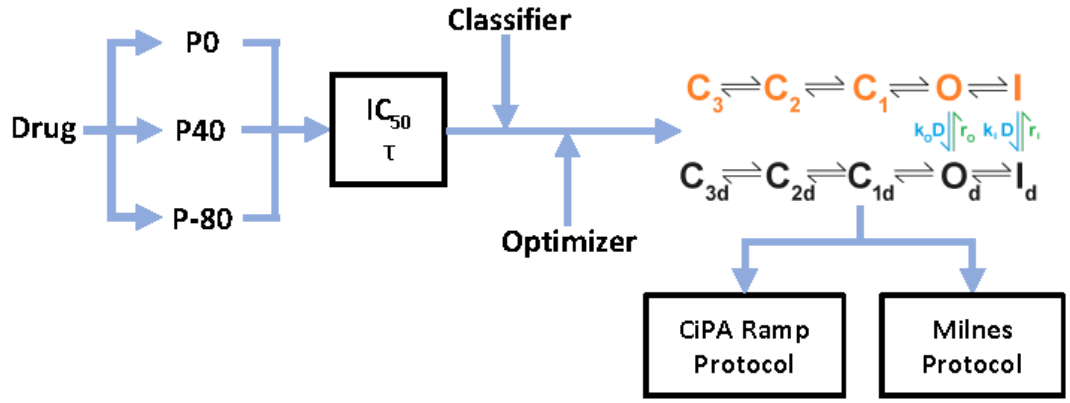

Figure 2. Full scheme of the methodology used for generating the dynamic drug model and studying its behavior.

2.2. Simulation

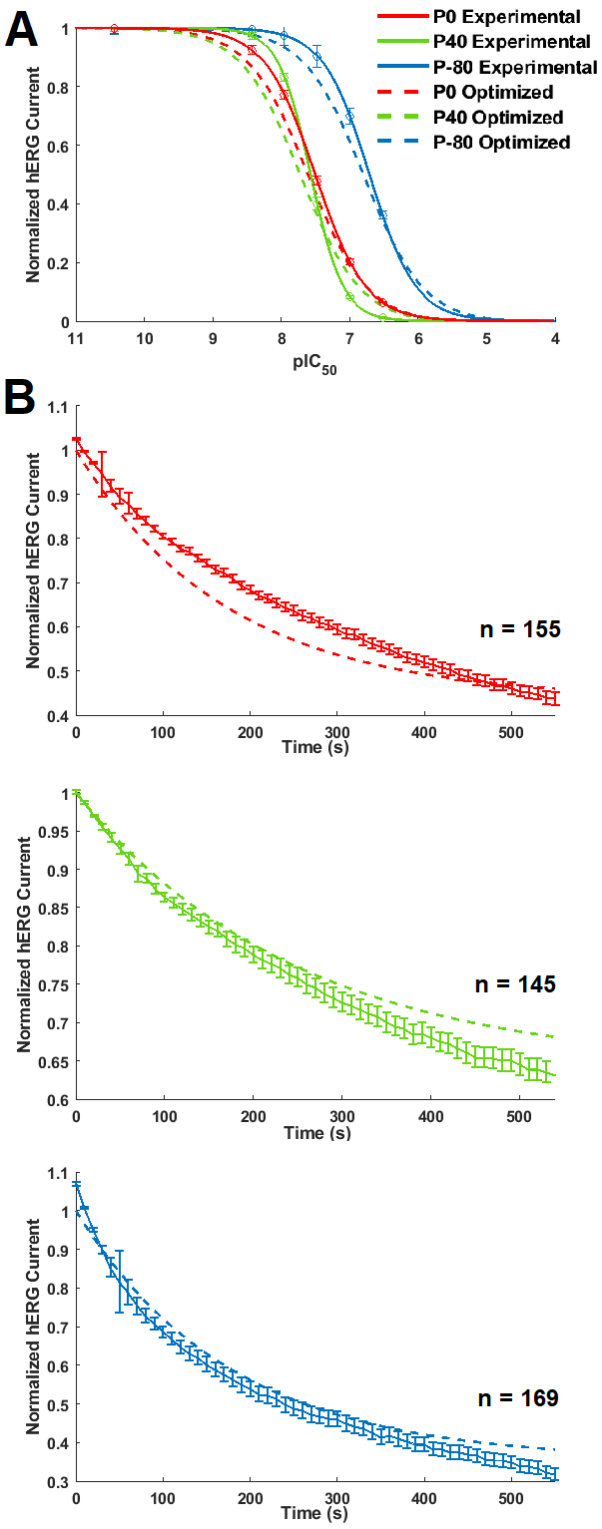

Figure 1. Hill-plots (A) and onset of I_{Kr} blocks for the experimental and optimized P0 (solid and dashed red respectively), P40 (solid and dashed green, respectively) and P-80 (solid and dashed blue, respectively) (B). N is the number of cells used.

The evolution of the normalized hERG tail peak for the experiments and for the simulations with our model is also displayed in panel B. Our dofetilide dynamic model closely reproduces the experimental data for both, the hill-plots and the onset of I_{Kr} block.

Then, we simulated our dofetilide dynamic model with the CiPA ramp protocol [10] and we obtained an IC_{50} of 112 µM.

3.2. Comparison with the CiPA model

Next, we simulated the dofetilide dynamic model proposed by the CiPA initiative [3] using our three protocols to and we generated the hill-plots, which are shown in Figure 4. Direct comparison between our IC_{50} values and the ones obtained with the CiPA model is not pursued since the CiPA dynamic model was fitted to data obtained at 37ºC and our experiments were performed at 22ºC. However, when looking just at the relationship among the IC_{50} values obtained with our three protocols, we can see that the CiPA dynamic model predicts similar IC_{50} values for P0 and P-80 with P40 having a higher value, Therefore, the CiPA dynamic model of dofetilide does not resemble the tendencies observed in our experiments as P0 and P40 have similar IC_{50} values while P-80 has a higher value, which is correctly by our dynamic dofetilide model (Figure 3A).

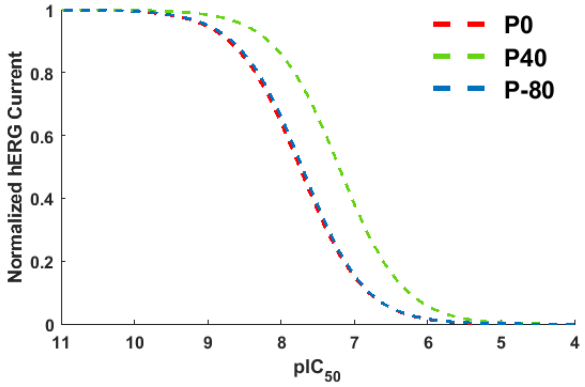

Figure 2. Hill plots obtained with the experiments and the CiPA dynamic model for P0, P40 and P-80 (dashed red, green and blue traces, respectively).

Finally, we wanted to test whether our new dynamic model could reproduce the data the CiPA dynamic model was fitted to. For this, we simulated our dofetilide model using the Milnes protocol. Figure 5 represents the remaining current for the first (solid) and last sweep (dashed) of the Milnes protocol and it shows that our dofetilide model reproduces the significantly reduced peak current at the tenth sweep compared with the first.

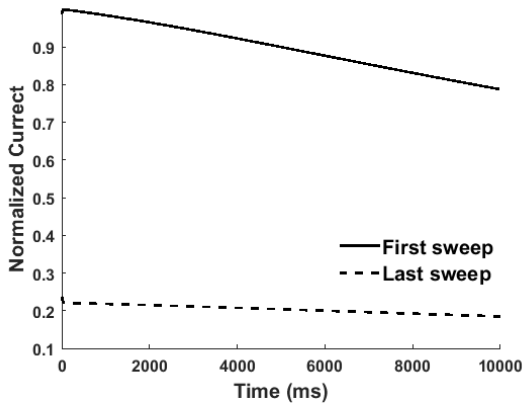

Figure 3. Normalized current for the first and last sweep of the Milnes protocol obtained with our generated dofetilide dynamic model.

3. Discussion

In this study, we have developed a new dofetilide dynamic model using our previously developed pipeline [8]. Our model closely reproduces the experimental results obtained with our three simple voltage clamp protocols [5].

Dofetilide have been previously reported to bind to the open and inactivated states with up to 70-fold times higher affinity for the later and trapping properties [7, 12, 13]. All of these features are correctly reproduced by our newly generated model which is an InactiveO model with trapping dynamics and 100 times higher affinity for the inactive state.

Our model has predicted an IC_{50} of 112 µM when using the CiPA ramp protocol, which is in line with previous results using automated patch clamp at room temperature [14] but is significantly different than the ones obtained at physiological temperature [3]. In fact, temperature has been proved to be an important factor when measuring drug dynamics and kinetics [15]. Therefore, the differences observed with our model could be explained by this fact.

Our dofetilide model resembles the experimental behavior observed with the Milnes protocol [3]. However, the CiPA model does not reproduce the tendencies observed with our protocols. This suggests that the Milnes protocol may not be sufficient to correctly elucidate preferential binding state properties [16]. Overall, we consider that our proposed methodology advances the CiPA initiative and is a step further in the assessment of drug cardiotoxicity and early risk prediction.

Acknowledgments

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