Systems Biology in Drug Safety Assessment: Use of a Recalibrated Hund-Rudy Model to Predict the Effect of Novel Drug Compounds on Action Potential Duration

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Abstract

Emerging practice in drug safety assessment delivers relatively early data on the potency of compounds at a range of cardiac ion channels, but there is little consensus on how to use this data to understand human risk, particularly when a compound modifies a range of mutually compensatory electrophysiological processes. In-silico physiological modelling of the processes involved offers a method to achieve this understanding but the models in the literature are not yet widely validated in the presence of a range of pharmacological profiles.

We have used a model of canine ventricular myocytes and retrained it to data from a series of action potential (AP) recordings on isolated canine myocytes. By representing inter-animal variability by using an ensemble of parameters sets, we are able to evaluate the likely impact of novel compounds on a series of virtual dogs.

1. Introduction

The use of hERG IC_{50} has become a tool for making drug discovery and development decisions and there have been extensive studies supporting an adequate safety margin of 30-fold between the hERG IC_{50} and the free C_{max} at the efficacious dose [for example 1]. However, rejecting compounds based on hERG IC_{50} alone potentially risks rejecting valuable compounds for treating disease. Indeed, one recent study of 92 hERG active compounds found that only just over half caused action potential duration (APD) prolongation, whilst a sixth showed *shortening* of the AP [2]. Moreover many potentially valuable compounds have been found to have multiple cardiac ion channel activities, and it can be unclear how to interpret these more complex profiles.

Whilst there is clear evidence that many drugs that are hERG blockers cause Torsades de Pointes [3], there is growing evidence that effects on other ion channel types can affect APD and therefore potentially carry a proarrhythmic risk. AstraZeneca has therefore begun to screen compounds for their effect at other key cardiac ion channel types early in drug discovery e.g. [4,5]. To understand the integrated effect of a compound ultimately requires recording an AP from an isolated cardiac myocyte [6]. However, as discussed in [7] these assays are much more limited in their throughput and demand on primary tissue, and so are carried out later in drug discovery, with a correspondingly higher cost of compound failure. Thus, in addition to the mechanistic understanding that an integrative model can bring, there would be considerable value in being able to supplement the early ion channel potencies with model-based simulations of APs that take those potencies as input and produce simulations of APD changes that can be used to prioritise compounds likely not to fail in the later stage cell-based assays. These models would not be intended to replace cell based assays but to ensure a more robust decision-making process for which compounds should reach them.

2. Methods

We reviewed a number of these models with the purpose of assessing their applicability to this study, specifically their similarity to the AP morphology seen within the sharp electrode (SE) recordings at 1Hz pacing. For a brief summary of each model we direct the reader to the review by Fenton and Cherry [8]. The models under consideration are: 1) Winslow (W99) model [9]; 2) Fox (F02) [10]; 3) Hund-Rudy (HR04) [11]; 4) Greenstein (G06) [12] and 5) Flaim (F06) [13]. This is not the full set of canine ventricular myocyte models within the literature but does include all those that we were able to obtain computational code for through on-line repositories and personal web-pages.

In order to compare the models to the SE traces the extracellular ion concentrations were set to match with

the standard myocyte Tyrode solution concentrations in our laboratory. In addition, intracellular ion concentrations were set to the same value in each of the models. These values were chosen to ensure that the resting membrane potential was in accord with the mean resting membrane potential from SE data. The comparison in modelled AP between these different models and experiments can be seen in Figure 1. From this figure we can see that the HR04 model compares most favourably out of all models to the mean of the SE data. In addition to this quantitative/qualitative observation a recent review also commented on the suitability of the HR04 model as a good approximation to a mid-myocardial cell [14]. Therefore, we chose the HR04 model, as further modified to represent the canine midmyocardial myocyte [15], as the initial basis for this study.

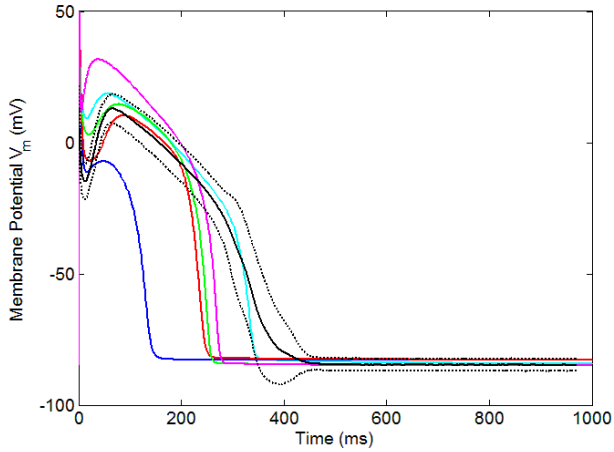

Figure 1: Plot showing the mean (solid black), mean ± standard deviation (dashed black) of the 19 SE AP experimental traces and model predictions at 1Hz pacing: 1) W99 (solid red); 2) F02 (solid blue); 3) HR04 (solid cyan); 4) W06 (solid green); 5) F06 (solid magenta).

The HR04 model was then trained to reproduce the shape and morphology of 19 DMSO SE traces obtained from separate animals to give an ensemble of 19 different parameter sets (variants) designed to represent inter-animal variability.

We assumed that the variation in AP morphology seen between the experimental traces is due to biological variability in processes captured by parameters within the model. Specifically, we assumed that this inter-dog variability is due to the variation in 12 model parameters including the membrane densities of 5 ion channels (IKr, IKs, Ito1, INa and ICaL) and that this density influenced total conductivity through each channel. Thus, we fitted each individual AP trace by allowing the conductances of these ion channels to be scaled from their values in the original model.

In order to fit the model to each of the 19 DMSO SE traces we performed an optimisation procedure that minimized the root-mean-square-deviation between model and experimental APs in the range t=0ms and t=100ms (capturing the notch and height of the dome) and t=250ms and t=500ms (capturing the end of the AP). We did not impose any upper bounds on the scaling values. A lower bound of zero was set for all except INa, for which the lower bound was 0.5 to ensure we obtained a significant upstroke. In addition to fitting to the morphology of the DMSO SE traces we also included within the optimization function, fitting to the dose-response curves for both dofetilide and diltiazem. Equal weights were assigned to each of the 3 constituents of the objective function. The result of this optimisation can be seen in figure 2.

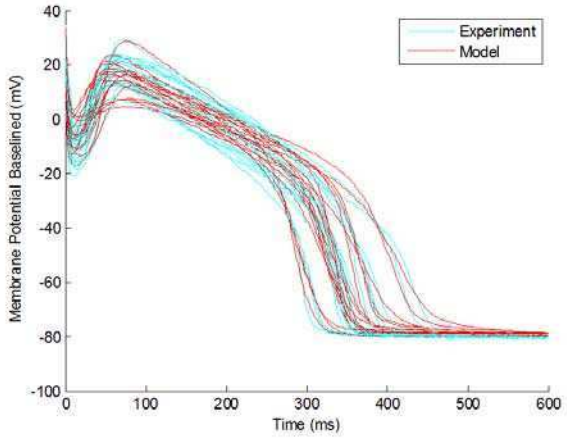

Figure 2. Plot showing the comparison between model (red) and experimental SE (cyan) traces following model training.

When simulating the effect of compounds known to have IonWorks™ IC_{50} values against any of the five ion channels IKr, IKs, Ito1, INa and ICaL we modelled the effect at a concentration [D] by scaling the conductance of those channels according to equation (1).

$$B = 1 + \frac{I_{\max}}{I_0} \frac{[D]^{\eta}}{IC_{50}^{\eta} + [D]^{\eta}}. \qquad (1)$$

We assumed that compounds with a known effect on INa in IonWorks™ assays blockaded only the peak (INa_{peak}) current and not the late sodium current (INa_{late}) which are separately represented in the model. However we found considerably better performance of the model in prediction when the same IC_{50} was applied to both channels, this is consistent with at least some instances of

drugs were a measurement of both peak and late current have been made [16]. We chose to compare the marker, %ΔAPD90, defined to be the percentage change in APD90 value between DMSO and compound, between model and experiment to assess the model's predictivity. The effect on %ΔAPD90 for the 'fitted' compounds: dofetilide and diltiazem for the ensemble of models can be seen in Figure 3.

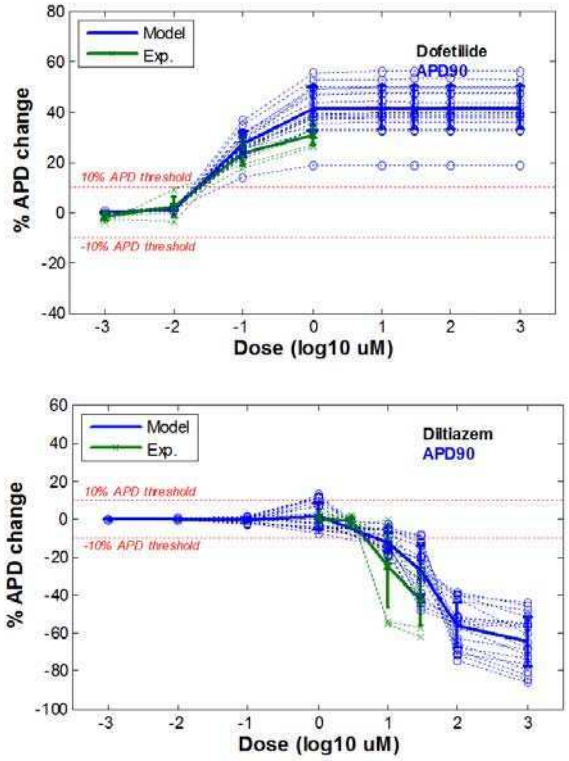

Figure 3. Plot showing mean %ΔAPD90 for both model and experiment (bold line) as a function of drug dose (log10 μM) for dofetilide and diltiazem. The mean data point for the model corresponds to the mean of the %ΔAPD90 from the 19 models. The mean data point for experiment is the mean of the %ΔAPD90 for each cell from the same dog. Error bars represent standard deviation across the 19 different parameter sets in the case of model and typically 4-5 cells in the experimental case. Dotted lines represent individual model predictions (blue) and individual cell recordings (green).

3. Results

The model has been trained on canine APs both in the absence of pharmacological intervention and in the presence of compounds modelled as pure IKr and ICal blockers. We observe a good agreement both qualitatively and quantitatively to both the DMSO SE traces and the dose-response relationships for both compounds.

Thus an immediate but undemanding form of validation is to test its performance on two different selective compounds. We used the IKr selective blocker almokalant, and the ICal selective blocker isradipine, to see if the data supports the hypothesis that these compounds act identically to the training compounds dofetilide and diltiazem save only through concentration-dependent effects explained solely by IC_{50} differences, see first 2 graphs of Figure 4. A considerably more stringent validation is to evaluate the predictions of the model for a compound which has activities against multiple ion channels. We tested the multichannel blocker, pimozide and we show the results of this comparison in the final graph of Figure 4.

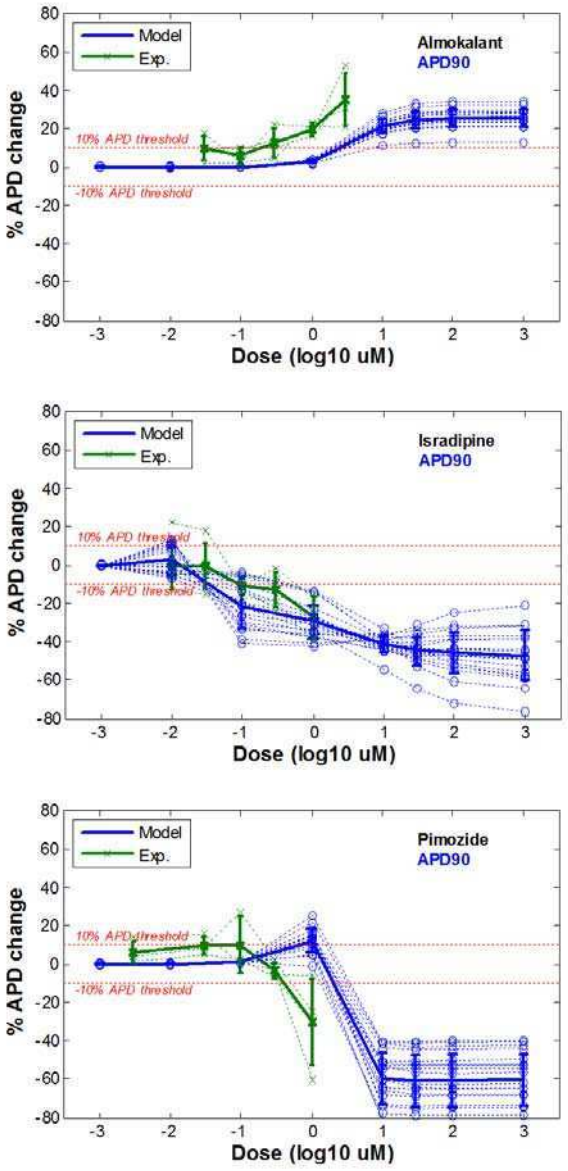

Figure 4. Plot showing mean %ΔAPD90 for both model and experiment as a function of drug dose (log10 μM) for A) almokalant, B) isradipine and C) pimozide.

4. Conclusions

Changes in cardiac AP are undesirable and early detection in drug discovery is critical. Although this issue has prompted drug discovery programs to "molecularise" the AP using automated electrophysiology, there is still the need to test compound effects on the AP in an integrated system. This has been partially addressed by using novel experimental assays that use epifluorescence recording of the cardiac AP rather than conventional electrophysiology, by reducing the technical challenges involved in SE recordings. However, this assay is still dependent upon primary tissue for assaying and hence limits its throughput. Therefore this study goes some way to provide a predictive tool to interpret results from a panel of IonWorks™ assays across multiple ion channels at a much earlier stage of the drug discovery process.

To the best of the author's knowledge, this is the first study to report a model trained to canine midmyocardial myocyte AP which has been subsequently validated using compounds blockading multiple ion channels, and which incorporates observed inter-dog variation through the use of a parameter ensemble. This use of an ensemble of model variants allows us to observe that even when a majority of model variants demonstrated APD prolongation for a given drug treatment, not all of them did. This addresses some of the concerns raised in [7] where a lack of APD effect measured experimentally may be due to balance in e.g. hERG and L-type calcium channel inhibition. Potentially, this may also provide an explanation for the rarity of some toxicities, and provides a methodology to explore this further by identifying ion channel heterogeneities which would show an increased sensitivity to compound challenge.

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