

***In Silico* Trials for Drug Safety and Efficacy Assessment Using a Novel Human Purkinje Fibre Model**

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Abstract

In silico trials using multiscale and biophysically-detailed human models have proven to be a new powerful tool for proarrhythmic risk prediction. Our goal is to evaluate the consistency between in silico results using a novel human Purkinje model and experimental data obtained from rabbit Purkinje fibres. The effects of Diltiazem, Dofetilide, Risperidone, and Verapamil were evaluated through AP biomarkers, at 1 and 0.2 Hz. Drug-induced effects on the ion channels were simulated through a simple pore-block model using IC₅₀ values and Hill coefficients. Simulation results quantitatively reproduced the drug-induced effects experimentally observed for both Dofetilide and Risperidone. Simulations of Diltiazem and Verapamil correctly reproduced drug-induced effects observed experimentally in the early phase of repolarisation, but they showed AP prolongation instead of shortening. The interplay between Ca²⁺ and K⁺ current inhibition was identified as the leading cause of the discrepancy. The experimentally observed AP shortening was quantitatively matched when not considering the effects on K⁺ current. In silico trials are a powerful tool for drug safety and efficacy assessment, and their performance requires careful evaluation of data describing drug-ionic current interactions since they critically affect the simulation results.

1. Introduction

Preclinical assessment of drug-induced safety and efficacy is still a challenge for pharmaceutical industries and regulators, which require new approaches [1]. *In vitro*, *in vivo*, and *ex-vivo* animal models are widely used for preclinical drug assessment, though they do not always translate to humans and represent a hefty cost for the pharmaceutical industry [2]. Cardiac Purkinje fibres, obtained often from dog or rabbit hearts, are one of the ICH S7B-recommended *in vitro* models for preclinical drug screening, especially for cardiotoxicity [3]. *In silico* trials using multiscale biophysically-detailed human-based models have proven to be a promising technology for cardiac safety prediction, achieving high level of accuracy

for a large variety of compounds [4,5]. The productive integration of *in silico* trials for drug testing requires their evaluation against currently-used experiments. Our goal is to compare *in silico* simulations using a novel human Purkinje model with experimental data from rabbit Purkinje fibres and evaluate their consistency. We analysed the effects of four different compounds having different ionic profiles and different risk of inducing Torsade de Pointes (TdP) arrhythmia. We hypothesised that computational simulations could quantitatively predict drug-induced effects on cardiac Purkinje cells, and the model response needs to be interpreted considering the following elements: i) consistency of the IC₅₀ values, especially for multi-channel blockers; ii) electrophysiological inter-species differences; iii) computational model characteristics, e.g. the balance of ionic currents and their dynamics to compensate the drug-induced blocks.

2. Methods

2.1. Experimental data

The effects of Diltiazem, Dofetilide, Risperidone, and Verapamil were investigated in Purkinje fibres (n=3) obtained from left ventricles of female rabbits (N=3) anaesthetised with sodium pentobarbital (0.9 to 1.0 mL/kg, intravenous) and maintained at 36 ± 0.5°C in a 5 mL-bath. Using the intracellular microelectrode technique, fibres were electrically stimulated at 1 Hz and superfused with Tyrode's solution, DMSO 0.1%, and increasing concentrations of drugs, as reported in Table 1, for 30 minutes and washout with vehicle for 40 minutes. For each solution, after 1 Hz, fibres were stimulated at 0.2 Hz for at least 3 min (to facilitate the occurrence of electrical abnormalities), followed by a 5-min recovery to 1 Hz. The last ten action potential (AP) recordings for each concentration were used to compute the following biomarkers: resting membrane potential (RMP), AP durations at 40%, 50%, 70% and 90% of repolarisation (APD₄₀, APD₅₀, APD₇₀, and APD₉₀ respectively), AP triangulation (APD₉₀₋₄₀), AP amplitude (APA) and maximal rate of depolarisation (dV/dt_{max}).

Based on the classification by CredibleMeds® [9], we considered Dofetilide and Risperidone as drugs with risk of inducing TdP, and Diltiazem and Verapamil as safe drugs (Table 1).

Table 1. Drugs, concentrations, TdP risk, and references to the IC₅₀ values and Hill coefficients used in this study

Drug	Concentrations ($\mu\text{mol/L}$)	TdP risk	IC ₅₀
Diltiazem	0.1; 1; 3; 10	No	[6,7]
Dofetilide	0.001; 0.003; 0.01; 0.03	Yes	[4,6,7]
Risperidone	0.1; 0.3; 1; 3	No	[6]
Verapamil	0.1; 0.3; 1; 3	Yes	[7,8]

TdP risk categories from CredibleMeds® [9]

2.2. *In silico* drug trials

In silico drug trials were performed for the same drugs and concentrations, using the Trovato2020 model, a recently published computational model of human cardiac Purkinje cellular electrophysiology [10].

Drug-induced ion current inhibition was simulated using a simple pore-block model, with IC₅₀ and Hill coefficient from literature (Table 1) for: i) fast Na⁺ current (I_{Na}), ii) L-type Ca²⁺ current (I_{CaL}), iii) rapid delayed K⁺ inward rectifier (I_{Kr}). Extracellular concentrations were set as in the corresponding experiments. For Diltiazem, Dofetilide, and Verapamil more IC₅₀ datasets were available as reported in Table 1. Simulations were repeated for each set of IC₅₀ individually. Starting from steady state, the model was paced for 150 beats at either 1 or 0.2 Hz. The same AP biomarkers considered for the experimental recordings were computed on the last beat.

All the simulations presented in this study were conducted using Virtual Assay [11] (v.3.2 © 2014 Oxford University Innovation Ltd. Oxford, UK), a user-friendly software package based on C++, and specifically designed for *in silico* drug assays. Further analysis of the results was performed in Matlab (Mathworks Inc. Natick, MA, USA).

3. Results

3.1. Drug-induced effects at 1 Hz

Figure 1 shows simulated APs traces at 1 Hz for each of the drugs investigated in the present study. Dofetilide is a well-known Class III antiarrhythmic, selectively blocking I_{Kr}. In all the simulations (Figure 1A), Dofetilide induced concentration-dependent AP prolongation, with no relevant effects on RMP, APA, and dV/dt_{max}. The degree of AP prolongation varied depending on the IC₅₀ values considered [4,6,7], leading to an increase in the APD₉₀ of

81%, 40%, and 105%, respectively, at the maximum tested concentration (0.03 $\mu\text{mol/L}$). For all tested concentrations (Table 1), the first set of IC₅₀ values led to AP prolongation consistent with the experimental observations in rabbit Purkinje fibre, whereas the second and third under and overestimated the magnitude of the AP prolongation, respectively.

Risperidone is an antipsychotic which is associated with QT prolongation and TdP arrhythmia. The simulated effects of Risperidone on the AP are reported in Figure 1B. Risperidone induced dose-dependent AP prolongations, with small effects on RMP, APA, and dV/dt_{max}. Simulations showed a good agreement with the experiments on the rabbit Purkinje *in vitro* model. At higher concentrations, 1 and 3 $\mu\text{mol/L}$, the APD₉₀ increased by 79% and 102% in line with the experimental observations. Decreasing the concentrations of one order of magnitude to 0.1 and 0.3 $\mu\text{mol/L}$, led to a smaller APD₉₀ increase of 18% and 41%, respectively, which overestimate the AP prolongations observed experimentally (10% and 13%, respectively). Finally, a small decrease (<10% at the maximum concentration tested) in the dV/dt_{max} was observed in the experiments, which was not captured by the simulations.

Verapamil is a Ca²⁺ antagonist with different applications in the clinical setting, including treatment of arrhythmia. Two different experimental datasets were produced to investigate the effects of this drug. For the first dataset, rabbit Purkinje preparations were perfused for 7 minutes at each tested concentration (Table 1), whereas, preparations in the second dataset were perfused for 30 minutes. For both datasets, Verapamil showed mild effects on the RMP, APA and dV/dt_{max}. However, in the first dataset, AP was shortened by Verapamil, whereas, in the second dataset AP was significantly prolonged. Simulations of Verapamil-induced effects are reported in Figure 1C for the two different sets of IC₅₀ values [7,8]. In both cases, simulations yielded minor effects on the RMP and APA, and dose-dependent prolongation of the APs. At the maximum tested concentration (3 $\mu\text{mol/L}$), the APD₉₀ increased by 51% and 59%, consistently with the prolongation observed in the second experimental dataset. For all tested concentrations, simulations using the first IC₅₀ set showed a better match to the experimental recordings than the second IC₅₀ set.

In any case, simulations including the IC₅₀ values for all the ionic currents were unable to reproduce the AP shortening observed in the first experimental dataset, due to a dominant role of I_{Kr} compared to I_{CaL} in setting the APD at the later repolarisation phase. In order to reproduce the AP shortening observed in the second set of experiments, additional simulations were performed for Verapamil, without including the effect on hERG. Figure 1E reports the simulated APs for both IC₅₀ sets [7,8].

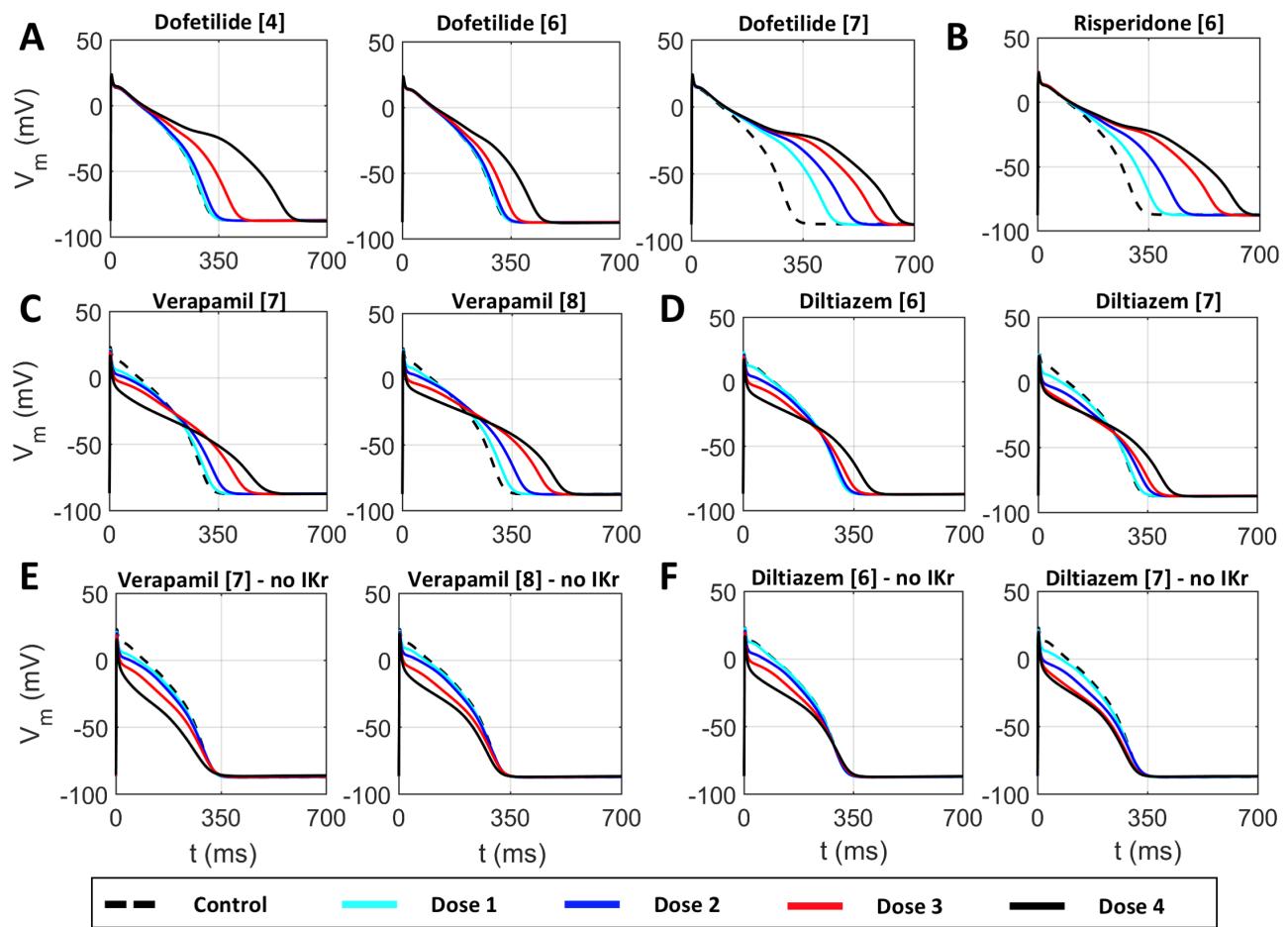


Figure 1. Simulated AP for 12 *in silico* trials at 1 Hz for four different drugs. Each plot shows drug-induced effects on the AP at four concentrations of the same drugs and same IC_{50} and Hill coefficients, as reported in Table 1 and the plot title. A) Dofetilide; B) Risperidone; C) Verapamil; D) Diltiazem; E) Verapamil, without simulating of the inhibition of I_{Kr} ; F) Diltiazem, without simulation of the effects on I_{Kr} . The legend, common for all plots, is reported at the bottom of the figure and refers to the concentrations listed on in Table 1.

In this case, simulations showed AP shortening with a decrease in the APD_{90} of 7% and 5% at the maximum tested concentration, quantitatively consistent with the second set of experiments.

Diltiazem is a Ca channel blocker, and it is used to treat hypertension and cardiac arrhythmias. Figure 1D shows simulated APs for Diltiazem, whose effects were represented using two different IC_{50} datasets [6,7]. In both cases, simulations showed mild effects on RMP, APA, lowered AP plateau, and AP prolongations. The latter was not observed in the experiments.

Similarly to Verapamil, additional simulations were performed in order to match the AP shortening observed experimentally for Diltiazem, not including the IC_{50} for I_{Kr} inhibition. In this case, simulations showed a better agreement with the experiments, leading to AP shortening for one set of IC_{50} [7], but not for the other [6].

3.2. Drug-induced effects at 0.2 Hz

Figure 2 reports simulated APs at 0.2 Hz for Dofetilide, Risperidone, Verapamil, and Diltiazem. Both Dofetilide and Risperidone induced early afterdepolarisations at the higher concentrations tested (Table 1), in line with their known TdP risk and experiments in rabbits. Verapamil and Diltiazem induced AP prolongation and no repolarisation abnormalities for all the tested concentrations, consistently with their safety profiles. At slow pacing, both experiments with Verapamil showed AP prolongation, whereas, AP shortening was observed for Diltiazem.

4. Discussion and Conclusion

In this combined experimental and computational study,

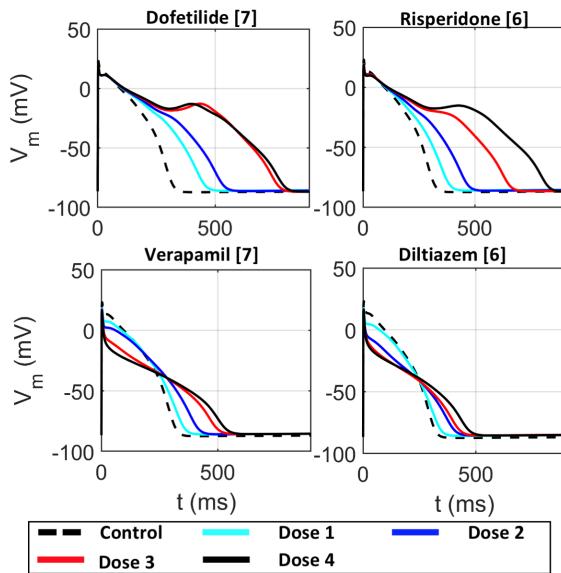


Figure 2. Simulated AP for *in silico* trials at 0.2 Hz for four different drugs. Each plot shows drug-induced effects on the AP at four concentrations of the same drugs and same IC₅₀s, as reported in Table 1 and in the plot title. The legend for all plots is reported at the bottom of the figure and refers to the concentrations listed in Table 1.

we compared the electrophysiological changes induced by 4 drugs (Diltiazem, Dofetilide, Risperidone, and Verapamil) on rabbit Purkinje fibre *in vitro* models and a human Purkinje *in silico* model.

Simulations of Dofetilide and Risperidone showed a quantitatively good agreement between experimental and simulations, though *in silico* predictions were strongly dependent on the set of IC₅₀ values chose to simulate the ionic current inhibitions. This was even more important for Verapamil and Diltiazem, due to the inhibition of both L-type Ca²⁺ channel and hERG. In particular, experimental recordings with Verapamil showed two different behaviours, which could be reproduced by the model only including I_{CaL} and I_{Kr} inhibition, selectively. The main difference between the 2 sets of experiments was the drug-exposure time, which suggest a different timing for the block of the L-type Ca²⁺ channel and hERG. Ultimately, IC₅₀ values are recorded from heterologous expression systems and are static, capturing a specific time frame of the drug actions, and potentially missing more complex dynamic, especially when acting on multiple ion channels. Finally, species-differences between rabbit and human Purkinje fibres have been reported for the expression of the delayed rectifier K⁺ channels subunits [12] though it is not yet clear how these differences affect the current dynamics, especially under drug action.

In silico drug trials are a powerful technology for drug safety assessment, and caution needs to be taken to the IC₅₀ values that describe the drug behaviour since they critically affect the *in silico* model response.

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