Prediction of Drug-Induced Arrhythmogenic Risk Using *In Silico* Populations of Models

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Abstract

In silico *tools hold potential to improve drug cardiotoxicity predictions. However, computational models do not usually consider inter-individual variability, which may be crucial when predicting rare adverse events such as drug-induced Torsade de Pointes (TdP). In this study we analyze the effect of incorporating inter-individual variability in the prediction of drug-induced TdP-risk. Specifically, the effects of the 12 training CiPA drugs were simulated on a single baseline model and on an electrophysiologically calibrated population of 848 models. Ternary classifiers based on support vector machines and logistic regression, were built using biomarkers obtained from simulation results. Classifiers were validated using the 16 validation CiPA drugs as an external data set. The classification accuracy increased to 80.1% when using the population of models, with respect to an accuracy of 62.4% obtained using the baseline model. Simulations with population of models allowed to identify individuals more prone to develop TdP. The methodology presented provides new opportunities to assess drug-induced TdP, taking into account inter-individual variability and may be helpful to improve current cardiac safety screening methods.*

1. Introduction

Drug-induced Torsade de Pointes (TdP) is a specific form of polymorphic ventricular tachycardia. It is one of the most feared adverse drug reactions since it can precipitate ventricular fibrillation and cause sudden death. Although it is a very rare adverse event, accounting for less than one case out of 100,000 exposures, several compounds, including antidepressants, pain medications, antihistamines, etc., have been withdrawn from the market because of their risk of inducing TdP.

Over the last years, new paradigms for the assessment of TdP-risk of drugs have been proposed with the aim of improving current regulatory guidelines. One remarkable example is the Comprehensive In Vitro Proarrhythmia Assay (CiPA) initiative, which considers that *in silico* simulations of proarrhythmic effects for different compounds are essential to improve arrhythmogenicity prediction.

Most of the mathematical and biophysical cardiac models used in *in silico* studies typically represent the average behavior of a group of cells characterized experimentally. Therefore, these models do not take into account inter-individual variability. However, it is well-known that identical pharmacological interventions produce different responses between individuals. As mentioned previously, the majority of individuals may not suffer any side effects while some undergo TdP. For this reason, accounting for electrophysiological variability may help better estimate drug-proarrhythmicity. A useful strategy to take into account variability in *in silico* models are population of models [1]. Other limitations of some *in silico* classifications tools that have been published are that: i) they usually are two-class categorization systems (TdP+ and TdP-), while the CiPA initiative recommends a ternary classification (high, intermediate, and low risk) [2]; ii) they use cross-validation methods (e.g., leave-one-out-cross-validation), in which the data used to train the model are also used to validate the model [2]. A validation of the tool with a "hidden" test data set, with data not used during the training phase, provides higher confidence on the performance of the tool.

The aim of this study was to analyze the role of incorporating inter-individual variability in the prediction of drug-induced arrhythmogenic risk. Specifically, the effects of the 12 training CiPA drugs were simulated on a single baseline model and on an electrophysiologically calibrated population of 848 models. Ternary classifiers were built using biomarkers extracted from simulation results and validated with result simulations using the 16 validation CiPA drugs.

2. Materials and methods

2.1. Electrophysiological *In Silico Model* and Population of Models

The electrophysiological characteristics of human

ventricular cells were simulated using a modified version of the human endocardial ventricular action potential (AP) model published by O'Hara et al. [3], as described in [4]. Briefly, model modifications include modulation of five channel conductances that were scaled as follows: I_{Kr} by 1.119, I_{Ks} by 1.648, I_{K1} by 1.414, I_{CaL} by 1.018, and I_{NaL} by 2.274; reformulation of the activation and inactivation gates of I_{Na}, and a reduction of its conductance by 60%.

To account for inter-individual variability, a population of models was built. First, an initial population of 1,000 models was generated. These models were obtained by randomly and simultaneously modifying the conductances of the 15 ionic currents of the AP model. These scale factors modifying channel conductances were randomly sampled from a normal distribution with mean 1 and standard deviation 0.2, thus assuring that the majority of the population (>99%) was in a range between ±60% with respect to the baseline model.

After running this initial population in control conditions, a calibration was performed. Those models with physiological features not fulfilling the calibration requirements were discarded. Plausible electrophysiological properties were defined according to acceptable ranges, found in the literature, for 15 AP characteristics related to AP duration, amplitude of membrane potential, and calcium dynamics [3], [5]–[8].

2.2. Drug Effects Simulation

Drug effects on the AP were simulated via the simple pore block model. Thus, the block produced on each current was simulated by scaling the channel's maximal conductance (g_i). This scaling factor was calculated using the standard Hill equation:

$$g_{i,drug} = g_i \left[1 + \left(\frac{D}{IC_{50,i}}\right)^h\right]^{-1}$$

where $g_{i,drug}$ is the maximal conductance of channel i in the presence of the drug, D is the drug concentration, $IC_{50,i}$ is the half-maximal response dose for that drug and current through channel I, and h is the Hill coefficient indicating the number of molecules of the drug that are assumed to be sufficient to block one ion channel.

In this work we considered drug effects on the seven ionic currents selected by the Ion Channel Working Group of the CiPA initiative. These currents play the most important role in the generation of the AP and cardiac arrhythmias (I_{Na}, I_{NaL}, I_{Kr}, I_{to}, I_{CaL}, I_{K1}, and I_{Ks})[9].

All simulations were carried out at a basic cycle length (BCL) of 1000 ms and a stimulus of 1.5-fold the diastolic threshold of amplitude and a duration of 0.5 ms. All drugs were simulated at 10 times the effective free therapeutic plasma concentration (EFTPC).

Here, we study and assess the proarrhythmic risk of the 28 CiPA drugs [9]. For each drug, IC_{50} values, Hill coefficients (h) and human EFTPC were taken from [4], which collects data from public databases and from the scientific literature. IC_{50} and h values for I_{Kr} channel were updated with more recent data from [10].

2.3. Drug-Induced TdP Risk Assessment

First, the 12 training CiPA drugs were simulated using both: the baseline model and the population of models. In each simulation, 9 biomarkers related to TdP-induction risk were measured: action potential duration at 90% repolarization (APD_{90}), triangulation 90-30, triangulation 90-50, net charge along the AP beat (q_{net}) [11], systolic and diastolic intracellular calcium, calcium transient duration at 90% and 50% repolarization (CTD_{90} and CTD_{50}), and the electromechanical window (EMw), defined as CTD_{90}-APD_{90}. When a model developed an early afterdepolarization (EAD), this model was considered as high TdP-risk in the classification.

Using these biomarkers as input, two ternary classifiers (high, intermediate, and low risk) were built: one for the baseline model and the other for the population of models. In both cases, a Support Vector Machine with a 30% hold out was trained. Leave-p-out cross-validation was performed with 50 bootstrap repetitions, i.e., the training phase was repeated 50 times to avoid the influence of data partitioning. This way, the output of the SVM is the risk (low, intermediate, or high) of a given model to suffer a TdP. In the case of the population of models the percentage of models that are classified as high, as intermediate or as low risk was calculated. Therefore, to determine the risk category of the drug, a logistic regression was applied on these percentages. This way, the classification tool built using the population of models produces as output the TdP-risk category of the drug, associated with the percentage of models in each category. Figure 1 shows a representation of the workflow of the ternary classifier using population of models.

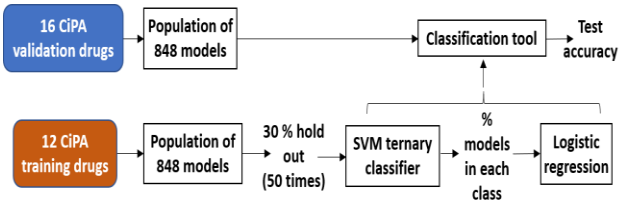

Figure 1. Schematic representation of the overall method of classification.

Once the classifiers were built, the validation of both classifiers was evaluated using the 16 validation CiPA drugs, thus being an external data set that had not been seen by the classifier during the training phase. Accuracy metrics (number of drugs correctly classified over the total of drugs) were averaged over the 50 repetitions.

3. Results

After calibration, 848 models presented a plausible electrophysiological behavior according to experimental data. AP traces of the population are shown in Figure 2. As shown in the Figure the population of models presents electrophysiological variability. For example, the APD_{90} of the baseline model yields 263 ms, and the population of models presents APD_{90}s varying between 188 and 417 ms. This inter-individual variability can also be observed when simulating drug effects. As shown in Figure 2, the same pharmacological intervention, in this case 10 times EFTPC of vandetanib, have very different effects, with some individuals developing EADs while others prolong its APD slightly with respect to individuals under control conditions. These differences cannot be captured with the baseline model.

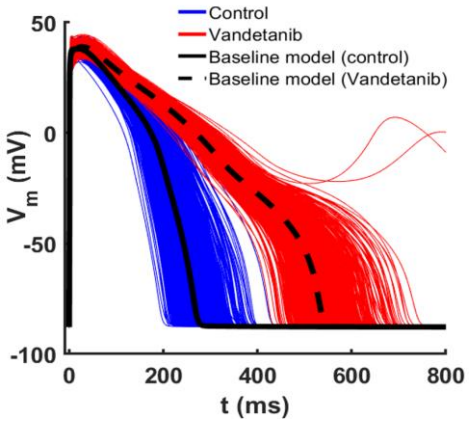

Figure 2. Action potential traces of the calibrated population in control conditions – no drug – (blue lines) and under vandetanib effect, a high TdP-risk drug (red lines). Baseline model results are plotted in black lines (continuous for control, discontinuous for vandetanib).

Those models of the population more prone to develop EADs under the effect of different drugs were analyzed. The most susceptible models presented on average lower conductances of I_{Kr}, I_{Ks} and I_{NaK} and higher conductances of I_{CaL}, I_{NaL} and I_{NCX}. This information could be useful to further investigate and establish clusters of patients in which the dose of proarrhythmogenic drugs should be avoided or reduced.

Next, the 12 training CiPA drugs were simulated and two classifiers were constructed: one using the baseline model and the other classifier using the population of models to account for inter-individual variability. Then the classification tool was tested using the 16 validation CiPA drugs. Figure 3 shows the percentage of models of the population that the SVM classifies in each of the three classes. For almost all the drugs (except ibutilide and nifedipine), the different individuals of the population are assigned to the three risk categories. This is due to the inter-individual variability. However, depending on the actual category of the drug, the proportions of models predicted in each class change. These percentages were the inputs of the logistic regression that predicts the overall risk class of the drug.

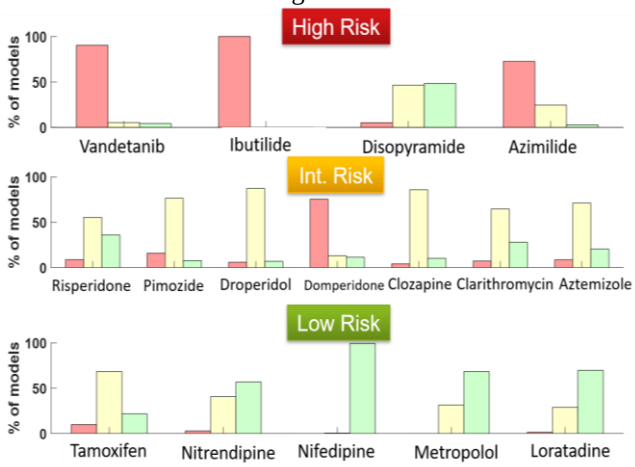

Figure 3. Percentage of models predicted as high, intermediate, or low TdP-risk for the 16 validation CiPA drugs with the SVM using population of models. High risk CiPA drugs are represented at the top, intermediate CiPA drugs in the middle, and low risk drugs at the bottom. For each drug, the red bar indicates the percentage of models predicted as high risk, the yellow bar the percentage of models predicted as intermediate risk , and the green bar the percentage models predicted as low risk.

As shown in table 1, when comparing the results of the predictions of both classifiers (baseline and population), the accuracy improves almost 20 percentual points. This increase in accuracy is due to the fact that there are two low-risk drugs (nifedipine and metropolol) and one high-risk drug (azimilide) which are correctly classified only if the population of models is used. These results reflect the importance of taking into account inter-individual variability when simulating the effects of drugs. Misclassified drugs with both classifiers are disopyramide, domperidone, and tamoxifen.

Table 1. Confusion matrices of the two classifiers for the 16 validation CiPA drugs. Baseline model (top) and population of 848 models (bottom).

Baseline	**High**	**Intermediate**	**Low**
Pred. High	2	1	0
Pred. Intermediate	1	6	3
Pred. Low	1	0	2
Mean accuracy:		62.4 %	

Population of models	**High**	**Intermediate**	**Low**
Pred. High	3	1	0
Pred. Intermediate	1	6	1
Pred. Low	0	0	4
Mean accuracy:		80.1 %	

4. Discussion and conclusion

In this work, we calibrated a population of 848 models to take into account inter-individual variability. The use of a population of models allows to obtain different AP responses under the same pharmacological intervention. Also, a ternary classifier was built based on SVM and logistic regression. This classifier was blinded validated using the 16 validation CiPA drugs as described in [2]. The classification accuracy when using the population of models was 80.1%, whereas using the baseline model the classification tool obtained an accuracy of 62.4%. Taken together, the results outline the benefits of using population of models when predicting TdP risk and evidence that inter-individual variability needs to be considered.

We also identified that individuals with lower conductances of I_{Kr}, I_{Ks} and I_{NaK} channels and higher conductances of I_{CaL}, I_{NaL} and I_{NCX} channels are more prone to develop TdP. This is in closely agreement with other studies: Britton et al. [7] suggested that decreased I_{NaK}, combined with low I_{Kr}, can increase pro-arrhythmic risk of drugs; Passini's [12] group showed that individuals with increased I_{CaL}, I_{NaL} and I_{NCX} and reduced I_{NaK} were highly vulnerable to drug-induced repolarization abnormalities; Lacerda et al. [13] stated that enhancement of I_{NaL} plays a relevant role in increased risk of TdP; similarly, the role of I_{Kr}, I_{Ks} and I_{CaL} in the precipitation of EAD is widely known [9].

The present results could be extended in the future by including uncertainty quantification and propagation of pharmacological data, validating the system with other new drugs or representing the intrinsic beat-to-beat fluctuation of action potential duration in a single cell.

The methodology presented in this study provides new opportunities to assess drug-induced TdP, taking into account inter-individual variability . The use of such *in silico* tools as screening methods could be helpful to accelerate the development of new drugs and reduce the costs of cardiac safety screening in preclinical phases.

Acknowledgments

This work was partially supported by the the European Union's Horizon 2020 research and innovation programme under grant agreement No 101016496 (SimCardioTest) and by Generalitat Valenciana, Prometeo programme 2020/43. Jordi Llopis-Lorente is funded by the Ministerio de Ciencia, Innovación y Universidades for the "Formación de Profesorado Universitario" (Grant Reference: FPU18/01659).

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