Electrotonic Coupling Effect on Pharmacological Cardiotoxicity Assessment in Atrial Tissue

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Abstract

In the last decade, considerable efforts have been dedicated to changing the cardiac proarrhythmia safety paradigm. Novel electrophysiological models are developed to accurately describe the pharmacological response, aiming at assessing pharmacological cardiotoxicity in silico. This opens the potentiality of using mathematical models at the early stages of the manufacturing of new pharmacological compounds for an initial and effective cardiotoxic screening. The Courtemanche model has been leveraged to create a population of stable action potential models which later on have been clustered according to the most relevant anatomical atrial regions. Thus, pharmacological cardiotoxicity assessment has been performed going to simulate the electrophysiological behaviour under the pharmacological effect; firstly, ten drugs have been investigated on a model of isolated cells and then the same drugs have been employed in a model of atrial tissue introducing the electrotonic coupling. In this way, by making use of a metric, the aritmic risk score (ARS Risk Score), the pro-arrhythmicity of the chosen drugs has been assessed. Results show that the electrotonic coupling lowers the Aritmic Risk Score of all the drugs investigated, opening a new scenario of the pharmacological cardiotoxicity assessment.

1. Introduction

In 2013, a new cardiac proarrhythmia safety paradigm was proposed to overcome the issues concerning the methodology to assess the cardiotoxicity of new pharmacological compounds. At the time, the approach was that of focusing just on the predictive link between drug-induced in vitro hERG channel blockade and in vivo/clinical QT interval prolongation and torsades de pointes (TdP) [1].
This paradigm was too stringent; in fact, clinical evidence pointed out how drugs such as Amiodarone, one of the major anti-arrhythmic drugs available on the market [2], causes marked QTc prolongation (even >550 ms) nevertheless rarely inducing TdP [1]. Thus, the need for a more comprehensive paradigm gave birth to the Comprehensive In Vitro Proarrhythmia Assay (CiPA) which consists of mixing experimental electrophysiological data with *In Silico* cellular simulations aiming to generate a Proarrhythmia score based on Repolarization Abnormalities (RA), i.e. Early After Depolarisations (EADs). [1,3] In this work a step forward has been made; to evaluate how to deepen the grade of complexity of simulations to describe the electrophysiological nature of the heart under pharmacological effect, the electrotonic coupling has been accounted for, enabling the cells to mutually interact once stimulated.

2. Methods

This work provides a comparison in terms of pharmacological cardiotoxicity assessment between the isolated cell model and tissue model aiming to evaluate how the Aritmic Risk Score could be affected. The Courtemanche-Ramirez-Nattel [4] has been employed to describe the behavior of atrial action potentials and to generate several stable action potentials to be investigated under the pharmacological effect. On the other hand, the same analysis has been carried out on tissue preparation.

2.1. Isolated Cell Model

The Courtemanche model [4] is an electrophysiological detail action potential model of the atria (see Figure 1).

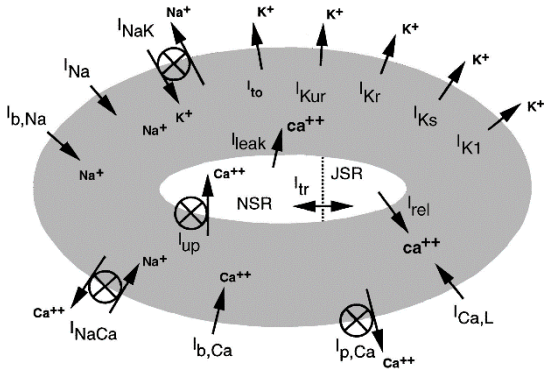

Fig. 1 Here is depicted a schematic representation of currents, pump and exchangers related to the Courtemanche model [4]

It accounts for the most important sarcolemma ionic currents and is extensively used in modeling the electrophysiology of the atrial cardiomyocyte.
A key element when evaluating cardiotoxicity and improving the accuracy of electrophysiology simulations is to account for the variability of the cardiac

tissue, as has been demonstrated in previous studies [5,6,7]. In this way, the population of model technique has been used to account for the electrophysiological variability of the cardiac tissue. The conductance of nine of the main ionic currents, namely: the fast sodium current, INa, L-type calcium current, ICaL, the transient outward potassium current, Ito, the inward rectifier potassium current, IK1, the rapid delayed rectifier current, IKr, the slow delayed rectifier
current, IKs, the calcium pump, ICa, the sodium-potassium pump, INaK, and the sodium-calcium exchanger, INaCa, were uniformly varied in a range of [5]. Combinations causing action potential alternans, repolarization abnormalities, and self-stimulating behavior were removed from the final population, leading to more than 70000 stable models.
Clusterization into a population with the characteristics of the Right Atria region was performed based on five markers of the action potential morphology namely: Action Potential Duration at 20%, 50% and 90% of repolarization, *APD20*, *APD50*,
and *APD90* respectively, Action Potential Amplitude, APA, and Resting Membrane Potential *RMP*, following the methodology presented in [5]. The mean and standard deviation of the different markers are shown in Table 1 [5].

Table 1. Mean and standard deviations of right atrium action potential biomarkers

Biomarkers	Values
RMP	−78 ± 12
APA	116.6 ± 14
APD20	30 ± 18
APD50	72.2 ± 37
APD90	200 ± 62

2.2. Tissue preparation

To study the effect that electrotonic coupling has on the cardiotoxicity evaluation of a given compound, a tissue preparation consisting on a parallelepiped 1.8x1.8x18 mm^3, with fibers oriented along the longitudinal direction (y-axis) as shown in Figure 2 was considered.

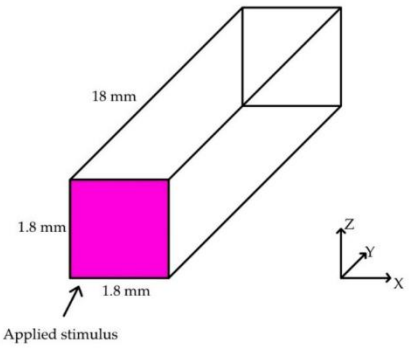

Figure 2. Tissue preparation. A parallelepiped 1.8x1.8x18 mm3 with fibers along the y direction, stimulated with a planar wave.

Electric propagation was simulated using the monodomain model

$$\nabla \cdot (\mathbf{D}\nabla V) = \left(C_m \frac{\partial V}{\partial t} + I_{ion}(V,u)\right) \text{ in } \Omega \quad (3)$$

$$\mathbf{n} \cdot (\mathbf{D}\nabla V) = 0 \quad \text{in } \partial\Omega \quad (4)$$

where **D** is the effective conductivity tensor, C_m is the capacitance of the cellular membrane, I_{ion} the transmembrane ionic current, Ω and $\partial\Omega$ are the domain of interest and its boundary respectively, with **n** the outward boundary of $\partial\Omega$.
The computational domain was discretized with 2160 trilinear hexahedral elements 0.3 mm in size for a total of 2989 nodes. The tissue was stimulated at the base (see Figure 2) with a rectangular stimulus 2 ms in duration two times the stimulation threshold. A train of 500 stimuli delivered at a frequency of 75 beats per minute (BCL =800ms) associated with a physiological sinus rhythm was considered.
Computations were performed by means of the software Elvira [15] using a constant time step of 0.02 ms.

2.3. Pharmacological assessment

Ten different compounds have been selected for the study grouped in three different families: pro-arrhythmic, safe compounds, and borderline compounds. In particular: *Bepridil, Dofetilide, Flecainide, Moxifloxacin, Quinidine* identified as Pro-arrhythmic, *Diltiazem and Mibefradil* identified as safe compounds, and *Amiodarone, Sotalol* and *Verapamil* identified as Borderline Drugs, whose Aritmic risk is uncertain [9].
In order to simulate the effect of the pharmacological compound on the different ionic channels, the Pore Block Model has been used, where the Ionic Current Block under the drug effect, B_k, is given as [9]

$$B_k = \frac{1}{1+\left(\frac{[C]}{IC_{50}}\right)^n} \quad (5)$$

where [C] is the concentration of the compound in plasma, n represents the Hill Coefficient and IC_{50} represents the half maximal inhibitory concentration. Table 2 gives the value of n, IC_{50}, together with the effective free therapeutic plasmatic concentration (EFTPC) for three of the analyzed compounds. The data for the remaining compounds can be found in [10,11]. Furthermore, all ten compounds have been tested at five different concentrations 1X, 3X, 10X, 30X and 100X the EFTPC to obtain insights precluded to animal studies [3].
To assess the overall Torsadogenic Risk related to the concentrations tested, the Aritmic Risk Score (ARS) provided in [9] was used

$$ARS = \frac{\sum_c (W_c \cdot nRA_c)}{N \cdot \sum_c W_c} \quad (6)$$

where $\sum_c$ is the sum on all concentration, [C] is the concentration under consideration, $W_c = EFTPC/[C]$, N is the total number of models in the population, and nRA_c is the number of models showing Repolarization Abnormalities i.e. Early

Table 2. Parameters of the pore block model for the different compounds, IC_{50}, in μM, and Hill coefficient, n, in parenthesis, together with the EFTPC in μM. Green color indicates safe, red color indicates pro-arrhythmic, and yellow color indicates borderline

	I_{Na}	I_{CaL}	I_{to}	I_{Kr}	I_{Ks}	I_{K1}	I_{NaCa}	I_{NaK}	EFTPC
Amiodarone	4.577 (0.7)	1.281 (0.6)	3.758 (0.4)	0.941 (0.6)	13.390 (1)	∞ (1)	∞ (1)	∞ (1)	0.0007
Bepridil	2.929 (1.2)	2.806 (0.6)	∞ (1)	0.149 (0.9)	∞ (1)	∞ (1)	∞ (1)	∞ (1)	0.0315
Diltiazem	∞ (1)	0.112 (0.7)	∞ (1)	6.569 (0.8)	∞ (1)	∞ (1)	∞ (1)	∞ (1)	0.1275
Dofetilide	∞ (1)	∞ (1)	0.018 (1)	0.001 (0.6)	∞ (1)	∞ (1)	∞ (1)	∞ (1)	0.0021
Flecainide	6.677 (1.9)	25.599 (1.4)	9.266 (0.7)	0.692 (0.8)	∞ (1)	∞ (1)	∞ (1)	∞ (1)	0.7529
Mibefradil	5.866 (1)	0.652 (1.1)	∞ (1)	0.307 (0.9)	∞ (1)	33.802 (1)	∞ (1)	∞ (1)	0.0106
Moxifloxacin	922.727 (1)	∞ (1)	∞ (1)	93.041 (0.6)	50.321 (1)	∞ (1)	∞ (1)	∞ (1)	3.5625
Quinidine	18.815 (1)	∞ (1)	3.847 (1.3)	0.343 (1)	4.899 (1.4)	∞ (1)	∞ (1)	∞ (1)	0.8429
Sotalol	∞ (1)	5976.923 (1)	∞ (1)	86.369 (0.9)	4762.745 (1)	3340.415 (1)	∞ (1)	∞ (1)	14.6864
Verapamil	∞ (1)	0.202 (1.1)	∞ (1)	0.499 (1.1)	∞ (1)	∞ (1)	∞ (1)	∞ (1)	0.045

After Depolarizations, repolarization failures, or action potential alternans. The original metric proposed in [9] was slightly modified to incorporate alternans, which are precursors of Ventricular Tachycardia (VT) such as bigeminal premature ventricular contraction (PVC) or indicators of Supraventricular Tachycardia (SVT) [13] and thus an important index to be accounted when calculating the arrhythmic risk.

The *ARS* was calculated for both, the single-cell and tissue simulations. In addition, to make a faire comparison between single cell and tissue, the same population of models consisting of 2989 different action potential models (coincident with the number of nodes in the tissue preparation) was used for both the single cell and tissue preparation simulations.

3. Results

As far as the isolated model is concerned, results are reported in Figure 3.

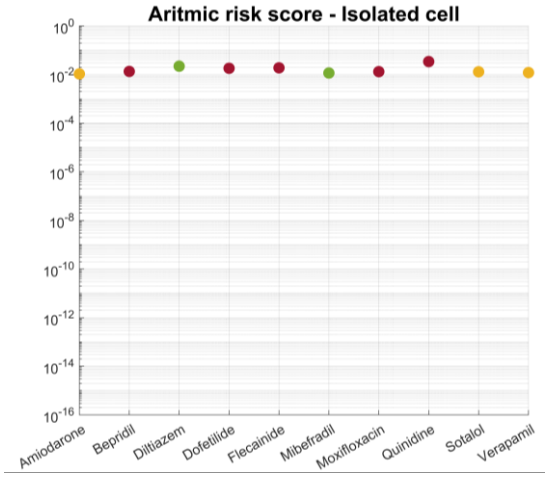

Figure 3 – *ARS* for the evaluated compounds. Amiodarone exhibits the safest behaviour, on the other hand Quinidine is the most pro-arrhythmic drug among all. Green color is related to Anti-arrhythmic drug, red to Pro-arrhythmic and yellow to Borderline one.

As indicated in Fig. 3, *Amiodarone* scored the lowest ARS Risk Score, whereas Quinidine scored the highest. The other pro-arrhythmic classified compounds scored, in general, higher values of the *ARS*, except Diltiazem, a drug classified as safe, that score the second highest value of *ARS*. Concerning the borderline compounds, Sotalol and Verapamil were found to score intermediate values as compared to other proarrhythmic drugs as Bepridil and Moxifloxacin (which score slightly higher) and safe drugs as Mibefradil. It is interesting that any of the safe drugs' score have an *ARS* of zero for the range of concentrations investigated.

Figure 4 shows the ARS for the tissue simulations. The plot has been depicted in a logarithmic scale to better appreciate the difference in the level of torsadogenic risk.

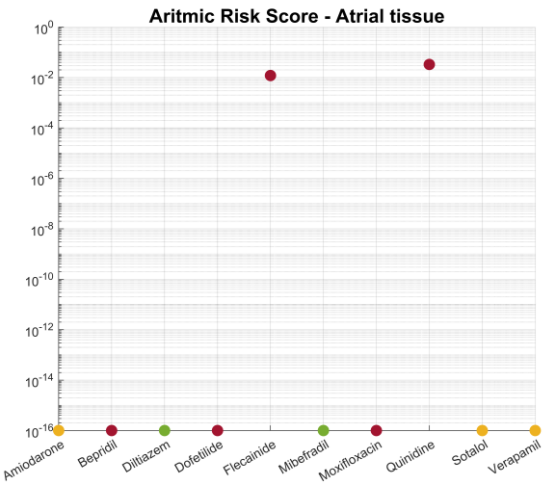

Figure 4 – *ARS* for the evaluated compounds in tissue simulations. Green color is related to Anti-arrhythmic drug, red to Pro-arrhythmic and yellow to Borderline one.

As seen in Fig. 4, all the ARS Risk Scores are decreased with respect to the isolated cell simulations. When considering the electrotonic coupling, *Flecainide* and *Quinidine* show a *ARS* different to zero. Furthermore, no EADs were found in any of the tissue simulations, with AP alternans being the only repolarization abnormality contributing to the *ARS*.

4. Discussion

Results related to the isolated cell simulations do show a similar trend to previous in-silico studies [9], with the exception of Diltiazem that results pro-arrhythmic in the case of the Courtemanche model. However, previous results, as those in [9], have been obtained using a ventricular Action Potential model [14], and no specific study has been conducted for atrial cardiomyocytes. Despite the fact that some pharmacological compounds may be less arrhythmogenic at the atrial level, an aspect to be further demonstrated by in-vitro experiments, our results clearly indicate a dependence on the sensitivity of the *ARS* on mathematical model used to performed the evaluation. On the other hand, undoubtedly the atrial tissue model provides promising results. It is evident how the electrotonic coupling decreases the ARS Risk Score enabling the cells to communicate one another and to mutually interact once stimulated. Moreover, electrotonic coupling eliminated the occurrence of EADs or repolarization failure in our simulations. For this reason, when evaluating repolarization abnormalities in tissue, the Aritmic Risk Score introduced in [9] needs to be extended to include other markers of repolarization abnormalities, as for instance action potential alternans, to increase the sensitivity and efficacy of the metric to evaluate pharmacological cardiotoxicity.

5. Conclusion and future developments

This works aims at presenting a comparison between isolated cell model and atrial tissue model in the context of the pharmacological cardiotoxicity assessment, highlighting how the electrotonic coupling decreases the ARS. The results are promising and pave the way for future developments; in particular, keeping on focusing at the atrial level, could be undoubtably helpful to include in future analysis the fibrosis due to atrial remodeling i.e., post myocardial infarction. The fibrotic tissue represents a physical barrier to cells, which prevent them to mutually interact in the syncytium, mitigating the electrotonic coupling effect introduced in this paper.

Last but not least, since O'Hara model is thought to be sensitive to pharmacological effects, it would be of great interest to carry out the same analysis at the ventricular level, where fatal arrhythmias, i.e., TdP and Ventricular Fibrillation (VF) originate.

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