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# In-silico study of the effect of Ritonavir on Human Ventricle Cells

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## Introduction

The evaluation of cardiotoxicity is a crucial aspect of drug development. Current regulatory guidelines for drug safety require the measurement of IKr channel block in vitro and QT interval prolongation in vivo to assess the arrhythmic risk of a drug. However, it has become evident that these markers alone are insufficient to predict cardiotoxic behavior accurately. A new paradigm has been proposed, which combines in vitro studies that measure the drug's effect on different ionic channels and in-silico models of cardiac myocyte electrophysiology. This report evaluates the cardiotoxicity of Ritonavir using the MATLAB program provided.

The findings of this study will contribute to the existing knowledge on the pharmacodynamics of the drug under investigation and enhance our understanding of its impact on cardiac electrophysiology. Ultimately, this information can aid in the development of safer and more effective drug therapies, as well as guide regulatory decision-making regarding drug dosage and usage.

## Dataset

The dataset provided for this project involves an in-silico study of the potential cardiotoxicity of a drug on human ventricle cells using a combination of in vitro studies and in-silico models of cardiac myocyte electrophysiology. Specifically, the project focuses on evaluating the effect of Ritonavir on the different ionic channels using a pore block model that reduces the channel conductance based on the available half-maximal inhibitor concentration, IC50, and Hill coefficient, n.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Channels | | FB | | | | | | |
| 1x | | 2x | | 10x | | 100x |
| GNa | Hill Coeff. | 0.984 | 0.969 | | 0.864 | | 0.390 | |
| IC50 (μM) |
| GNaL | Hill Coeff. | 0.876 | 0.813 | | 0.585 | | 0.220 | |
| IC50 (μM) |
| GKr | Hill Coeff. | 0.921 | 0.855 | | 0.541 | | 0.105 | |
| IC50 (μM) |
| GCaL | Hill Coeff. | 0.978 | 0.948 | | 0.694 | | 0.102 | |
| IC50 (μM) |
| EFTPCmax (μM) | | 0.4369 | | | | | | |

The electrophysiological variability inherent to the cardiac tissue is considered by means of a population of models approach using a file called "mask.mat".

The evaluation of the cardiotoxic effect is carried out by analyzing the effect of the drug on 10 different models of ventricular myocytes for four different concentrations of the drug (1x, 2x, 10x, 100x [EFTPC]). The stimulation protocol involves stimulating with a current 1.5x the stimulation threshold 500 times with a BCL=800ms and analyzing the last three beats, with pro-arrhythmic behavior considered if certain conditions are met.

### Ritonavir

Ritonavir is a medication primarily used in the treatment of HIV/AIDS. It is a protease inhibitor that works by blocking the enzyme responsible for the replication of the virus. Common side effects of ritonavir include nausea, vomiting, diarrhea, headache, and fatigue. It may also cause changes in blood sugar levels and lipid metabolism. In the medical field, ritonavir is often used in combination with other antiretroviral drugs to suppress the HIV virus and prevent progression to AIDS. Ritonavir is not typically considered an inductive arrhythmic drug. In fact, it is not commonly associated with significant cardiovascular side effects. However, it has been associated with an increased risk of cardiac events such as heart attack and arrhythmias in some patients, particularly those with pre-existing heart conditions. Therefore, the cardiotoxicity of ritonavir and its potential to cause pro-arrhythmic effects in the heart have to be considered in its use[1].

## Methods

For each of the ten models, the effect of Ritonavir was evaluated for four different concentrations: 1x, 2x, 10x, and 100x [EFTCP] (a total of 40 simulations). The stimulation protocol consisted of stimulating with a current 1.5x the stimulation threshold 500 times with a BCL=800ms and analyzing the last three beats. A pro-arrhythmic behavior was considered if:

* alternans in the APD90 were observed.
* abnormal repolarizations appeared.
* APD prolongation of more than 25% (with respect to control).

### APD90

The APD90 is the interval of time between the point of maximum variation of the transmembrane potential and the 90% repolarization point. Fig. 1 displays an example of the three action potentials of the respective APD90 time windows. As calculated for the subject 1 the APD90 was calculated for all the other subjects.

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Figure 1: Example of the three APD90 intervals, and their fiducial points: the point of maximum inclination, the maximum depolarization point and the 90% point of repolarization.

### **First condition**

Our analysis revealed a significant criterion for identifying pro-arrhythmic behavior within each subject at different concentrations. We focused on the absolute values of the last three consecutive differences between the ADP90 values. When these differences exceeded 10 milliseconds, it served as a clear indicator of the presence of pro-arrhythmic behavior in the subject.[2]

By employing this approach, we were able to reliably determine and categorize subjects exhibiting such behavior across all concentrations studied.

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| Figure 2: Observation of alternans in the ADP90 |

The results indicate that at dose 100, dose 10, dose 1, and dose 2, the probability of encountering pro-arrhythmic behavior is 0.00%. This is an encouraging outcome, suggesting a favorable safety profile within the scope of our investigation. The absence of pro-arrhythmic tendencies at varying concentrations highlights the potential efficacy and low-risk nature of the subjects under study. These results provide reassurance and support the hypothesis that the tested doses do not induce pro-arrhythmic behavior in the subjects examined.

### **Second condition**

Based on the plots, our findings reveal a consistent absence of abnormal repolarization across all subjects, irrespective of varying concentrations.

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| Figure 3: Observation of abnormal repolarization |

The exemplary plot presented here for the first subject vividly exemplifies this trend, wherein no instances of abnormal repolarization were detected. Encouragingly, our observations extend beyond this subject, as we meticulously examined all subjects across a range of concentrations, consistently revealing a lack of abnormal repolarizations.

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| Figure 4: First subject’s AP repolarization plot |

### **Third condition**

For the third condition analysis, we made a criterion to identify pro-arrhythmic behavior in each subject at varying concentrations. By focusing on the absolute value of the difference between the last (third) ADP90 measurement and the corresponding normal value, we found that when this difference exceeded 25% of the normal value, it served as a clear indication of pro-arrhythmic behavior within the subject. This stringent evaluation allowed us to effectively identify and categorize subjects exhibiting such behavior across all concentrations examined. The utilization of this criterion contributed to a precise and reliable assessment of pro-arrhythmic tendencies in our study participants.

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| Figure 5: Observation of APD90 prolongation |

Impressive findings have emerged from our analysis, demonstrating the varying probabilities of pro-arrhythmic behavior across different concentrations. Notably, for dose 100, we observed a substantial likelihood of 90.00% for pro-arrhythmic behavior, indicating a higher risk within this particular concentration. Moving to dose 10, the probability decreased to 40.00%, suggesting a moderate association with pro-arrhythmic behavior. Encouragingly, no instances of pro-arrhythmic behavior were detected at doses 1 and 2, resulting in probabilities of 0.00% for both concentrations. These results emphasize the significant impact of dosage on the likelihood of experiencing pro-arrhythmic effects, underscoring the importance of dosage considerations in understanding the potential risks associated with these concentrations.

### Discussion

The effects of the Ritonavir can be explained by looking at the functionalities of the channel it modifies:

* GNa (Sodium Channel):

GNa refers to the conductance or permeability of the sodium ion channels. These channels are responsible for the depolarization phase of the action potential. When GNa is activated, sodium ions (Na+) rapidly enter the cell, leading to a rapid influx of positive charge, which results in membrane depolarization and the initiation of the action potential.

* GNaL (Persistent Sodium Channel):

GNaL refers to the conductance or permeability of the persistent or late sodium channels. These channels exhibit a prolonged opening compared to the fast sodium channels (GNa). GNaL contributes to the maintenance of depolarization during the action potential plateau phase. Increased GNaL can lead to an enhancement of the action potential duration and prolonged depolarization.

* GKr (Delayed Rectifier Potassium Channel):

GKr represents the conductance or permeability of the delayed rectifier potassium channels. These channels are responsible for the repolarization phase of the action potential. When GKr is activated, potassium ions (K+) flow out of the cell, leading to membrane repolarization. By activating slowly, GKr contributes to the lengthening of the action potential duration.

* GCaL (L-Type Calcium Channel):

GCaL refers to the conductance or permeability of the L-type calcium channels. These channels play a crucial role in the upstroke and plateau phase of the action potential. When GCaL is activated, calcium ions (Ca2+) enter the cell, which contributes to depolarization and the maintenance of the action potential plateau phase. GCaL also participates in triggering various intracellular processes, such as muscle contraction and neurotransmitter release.

As we se in the Fig. 3, all the APs of the control population reaches higher repolarization points than the 100x dose subjects. This can be reconducted to the fact the Ritonavir modify the activation of the Na channel

which are responsible to the first repolarization phase.

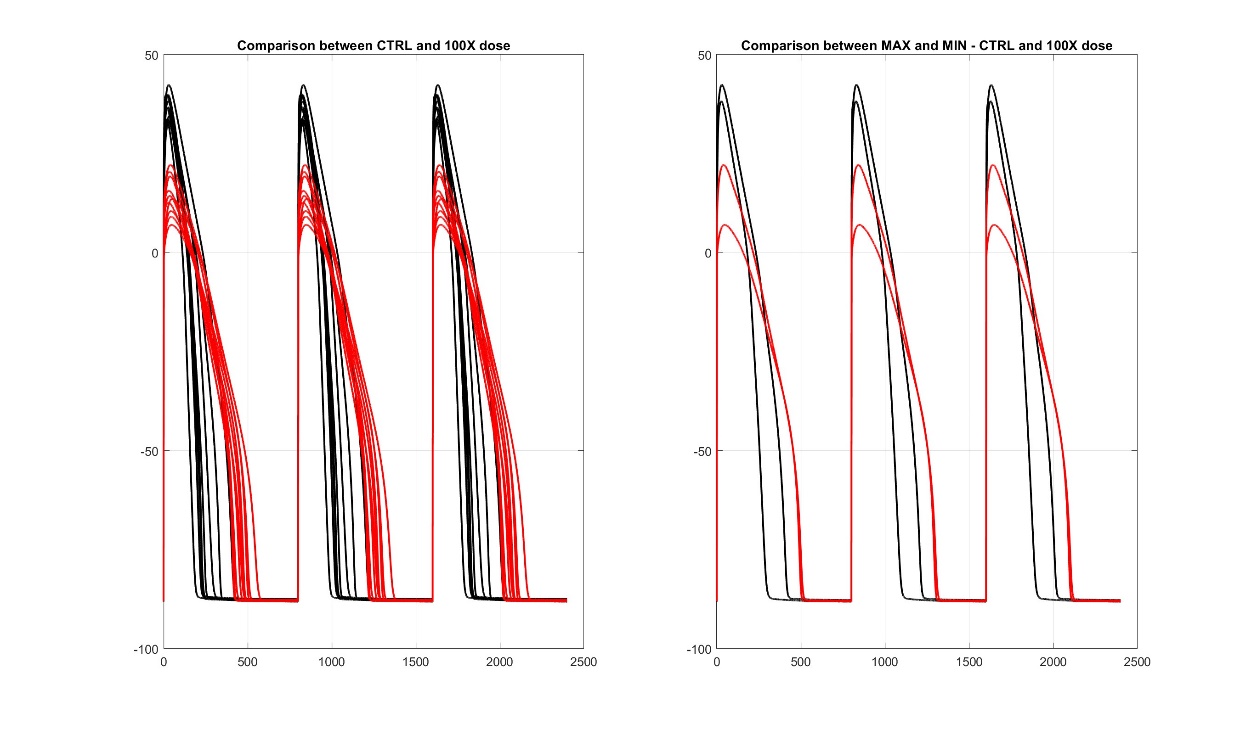


Figure 3: (a) Comparison between the control and the 100x population. (b) Comparison between the max and min points of repolarization between the control and the 100x population.

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Figure : a) APD90 Boxplot of all subjects, b) APD90 boxplots of non-arrhythmic subjects

## Results

By plotting the average APD90 as a function of the drug concentration, we will observe the overall trend and ascertain whether there is a concentration-dependent effect on cardiac repolarization. Additionally, incorporating the standard deviation in the plot will provide insights into the data dispersion, highlighting the variability across the drug concentrations.

## Discussion

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Figure : Comparison between boxplots of all subjects and non-arrhythmic subjects.

## References

[1] “Ritonavir | C37H48N6O5S2 - PubChem.” https://pubchem.ncbi.nlm.nih.gov/compound/Ritonavir (accessed May 14, 2023).

[2] (Kenji Hiromoto, et al., Discordant Repolarization Alternans-Induced Atrial Fibrillation is Suppressed by Verapamil, 2005)