

Dofetilide Unmasks Occult Congenital Long QT Syndrome Type 2: A Simulation Study

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Abstract

Accurate diagnosis of long QT syndrome is a key factor for reducing the risk of cardiac arrhythmias. Our goal is to investigate the potential use of dofetilide to unmask latent I_{Kr} mutation carriers.

A modified version of the O'Hara et al. model was used to simulate the electrical activity of isolated cardiac cells. The original I_{Kr} formulation was replaced by the Fink et al. Markov model of the human I_{Kr} channels and our dynamic model of dofetilide was used to simulate drug administration. A sensitivity analysis was performed to study the effect of I_{Kr} transition rate alterations on AP duration (APD) prolongation in the absence and in the presence of dofetilide.

Our results show that acceleration of the rate transition from open to the last closed state ($\beta\beta$) produced the shortest prolongation of the APD in the absence of the drug. However, $\beta\beta$ acceleration provoked the highest additional APD prolongation under dofetilide exposure related to the APD prolongation observed before the drug application. In addition, this I_{Kr} alteration was the transition rate modification that most increased the rate of deactivation.

In conclusion, our observations indicate that dofetilide could potentially be used to unmask I_{Kr} mutations accelerating the deactivation process.

1. Introduction

Drug-induced or acquired long-QT syndrome (LQTS) is a pathology characterized by prolonged ventricular repolarization subsequent to drug administration [1]. This disorder can lead to potentially mortal arrhythmias, such as torsade de pointes [1].

In the last decades, it has been shown that genetic factors may increase the risk of drug-induced LQTS and arrhythmias [1-3]. Gene mutations have been identified in 10% to 15% of subjects with drug-induced LQTS [3].

Accurate diagnosis of LQTS is essential for reducing the risk of cardiac arrhythmias [4]. Unfortunately,

diagnosis based on the QT prolongation is not completely reliable [5] and genetic testing has some disadvantages, such as its difficulty, cost and accessibility [6]. Recently, drug-induced provocative tests have been used to reveal some kinds of latent mutation carriers. In these tests, the QT interval of the subject is measured after the addition of a specific drug. The drug enhances the functional effects of the presumed defective ionic ion channel, which helps to discriminate between normal and mutation carrier subjects.

To investigate the potential use of dofetilide to unmask latent I_{Kr} mutation carriers a sensitivity analysis of the AP duration (APD) in the absence and in the presence of dofetilide to changes in the I_{Kr} transition rates was performed. Moreover, the effects of the I_{Kr} transition rates on the electrophysiological characteristics of I_{Kr} were investigated.

2. Methods

The five-state Markov model defined by Fink et al. [7] was used to simulate the human ventricular I_{Kr} . This Markov chain is composed of three closed states (C3, C2 and C1), a conducting open state and (O) an inactivation state (I), as depicted in Figure 1A. This I_{Kr} model was incorporated into the O'Hara et al. human ventricular action potential (AP) model [8] and it was scaled to yield the same I_{Kr} peak value as the original O'Hara model at 1Hz. Our dynamic model of dofetilide [9] was used to simulate drug administration (Figure 1B).

A sensitivity analysis of the AP duration (APD) in the absence and in the presence of 10 nM dofetilide to changes in the I_{Kr} transition rates was carried out. It was performed by multiplying or dividing by 5 one I_{Kr} transition rate at a time. Steady-state APDs at 90 % repolarization were calculated in virtual isolated endocardial cells at 1Hz, although in some cases it was also computed in mid-myocardial cells.

The effects of changes in the I_{Kr} transition rates on the electrophysiological characteristics of this current were investigated by applying the voltage-clamp protocols defined in [10].

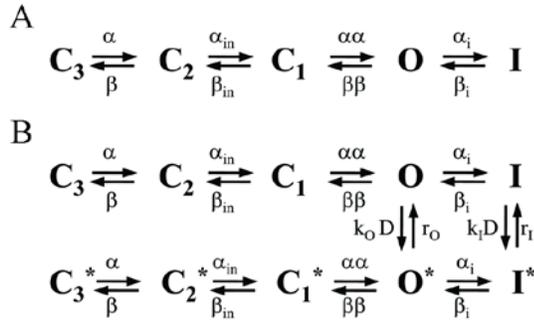


Figure 1. Markov chain of the human I_{Kr} channels in the absence (A) and in the presence of dofetilide (B).

3. Results and discussion

The simulated effects of changes in the I_{Kr} transition rates on the simulated human ventricular AP at 1 Hz are shown in Figure 2. This figure depicts the time course of the simulated endocardial AP under control conditions (thick lines) and when each I_{Kr} transition rate is five-folded (thin lines). Our results show that the transition rates between the open and inactivated states (α_i and β_i) exert a critical influence on the AP (Figure 2D), followed by the transition rate from the leftmost to the intermediate closed state (β_{in}) (Figure 2B). As expected, acceleration of the transition rate from the open to the inactivated state (α_i) and the transition rate from the rightmost to the intermediate closed state (β_{in}) prolonged the AP. This effect is due to the fact that these alterations reduce the probability of the conducting open state and, therefore, I_{Kr} . Conversely, acceleration of the transition rate from the inactivated to the open state (β_i) shortened the AP (Figure 2D), as it decreases the probability of the open state. The five-fold increase of the remaining transition rates had a minimal impact on the simulated AP (Figure 2).

Addition of 10 nM of dofetilide, which is in line with its therapeutic dose [11], to WT and mutated cells with one transition rate multiplied or divided by five was simulated to investigate the differential effect of

dofetilide in these cells. Figure 3 illustrates the APD_{90} prolongation relative to WT of the simulated mutated cells with longer APD than wild-type (WT) under drug-free conditions (black) and during 10 nM dofetilide exposure (gray). This figure shows that the APD_{90} differences between WT and mutated cells observed under drug-free conditions are specially enhanced during 10 nM dofetilide exposure in the cells whose transition rate from the open to the inactivated state (α_i) is accelerated and those whose transition rate from the inactivated to the open state (β_i) is slowed. This effect is also observed in those cells whose transition rate from the open to the last closed state ($\beta\beta$) is accelerated or whose transition rate from the last closed state to the open state ($\alpha\alpha$) or the transition rate from the intermediate to the rightmost closed state (α_{in}) is decreased. Comparison of the additional increments of the APD observed in the mutated cells in the presence and in the absence of dofetilide reveals that this drug preferentially

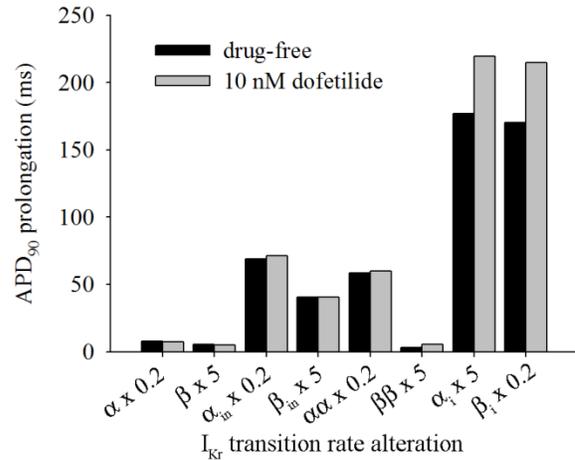


Figure 3. Influence of I_{Kr} transition rates on the endocardial APD under drug-free conditions (black) and during 10 nM dofetilide exposure (gray). The y-axis represents the APD_{90} prolongation relative to WT. Only those I_{Kr} transition rate alterations that prolonged the APD are represented.

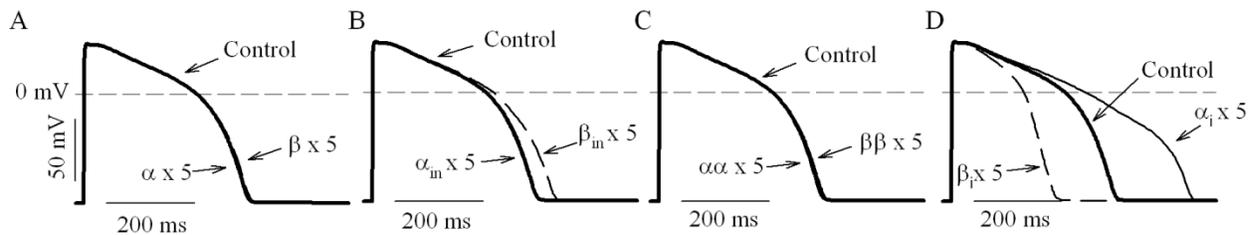


Figure 2. Time course of the simulated endocardial AP under control conditions (thick lines) and when each I_{Kr} transition rate is five-folded (thin lines), namely, α (solid line) and β (dashed line) (A); α_{in} (solid line) and β_{in} (dashed line) (B); $\alpha\alpha$ (solid line) and $\beta\beta$ (dashed line) (C); α_i (solid line) and β_i (dashed line) (D).

enhances the functional effects of the acceleration of the transition rate from the open to the rightmost closed state ($\beta\beta$). Specifically, the APD difference between endocardial WT cells and those affected by the acceleration of the transition rate from the open to the rightmost closed state ($\beta\beta$) (3ms) was doubled (6 ms) when 10 nM dofetilide was added (Figure 3 and Figures 4A and 4B). When 30 nM dofetilide was applied it increased five-fold (15 ms). As the effect of this rate on the APD under drug-free conditions is very small, our results suggest that dofetilide could be used to unmask this kind of genetic defect. As mid-myocardial cells are more sensitive to defective I_{Kr} , the effect of the five-fold increase of the transition rate from the open to the rightmost closed state ($\beta\beta$) was also investigated in this type of cells. Our simulations show that the APD difference between mid-myocardial WT cells and those affected by the acceleration of the transition rate from the open to the rightmost closed state ($\beta\beta$) (4 ms) was increased seven-fold (28 ms) under 10 nM dofetilide (Figures 4C and 4D).

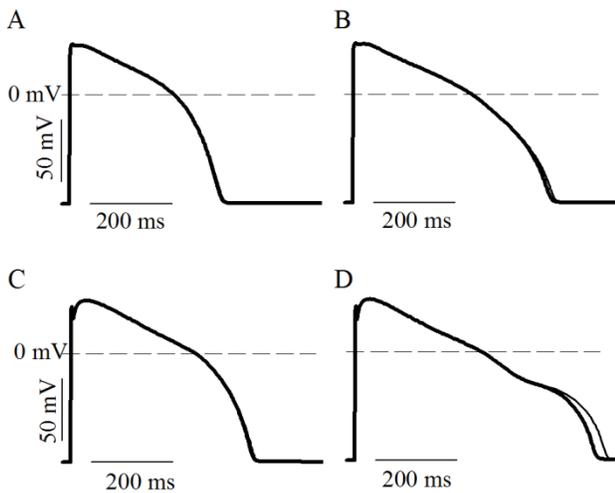


Figure 4. Steady-state AP time-course of WT (thick lines) and mutated cells with the transition rate from the open to the rightmost closed state rate five-folded (thin lines) in isolated endocardial (top row) and mid-myocardial (bottom row) cells in the absence (left column) and in the presence of 10 nM dofetilide (right column).

Figure 5 shows the characterization of WT and mutant I_{Kr} channels with a five-fold increase of the transition rate from the open to the rightmost closed state ($\beta\beta$). This rate alteration significantly reduced the deactivation time constant (Figure 5C). Specifically, this time constant was approximately divided by five at very negative membrane potentials. It also caused an 8.7 mV leftward shift of the steady-state activation curve (Figure 5A) as well as a 7.4 mV rightward shift and a 10 % decrease of the slope of the steady-state inactivation curve (Figure 5B). A sensitivity study of the electrophysiological

characteristics of I_{Kr} to changes in its transition rates revealed that the transition rate from the open to the rightmost closed state ($\beta\beta$) was the most relevant for the deactivation time constant, followed by the transition rates between the open and inactivated state (α_i and β_i), although their influence was much more smaller. In addition, the impact of the transition rate from the open to the rightmost closed state ($\beta\beta$) on the steady-state activation and inactivation curves was significantly smaller than the influence of other transition rates. Therefore, our observations indicate that dofetilide could potentially be used to unmask I_{Kr} mutations accelerating the deactivation process. This observation is in accordance to the clinical and experimental reports of patients with normal QT interval, but affected by mutations accelerating the deactivation of I_{Kr} , that suffered drug-induced arrhythmia [1,2,12,13].

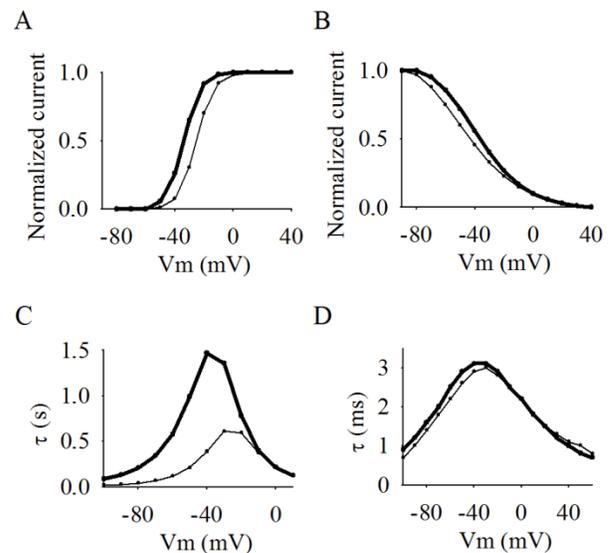


Figure 5. I-V relationships of the tail currents (A), steady-state inactivation curves (B), activation and deactivation time constants (C) and inactivation and recovery from inactivation time constants for WT (thick lines) and mutant (thin lines) I_{Kr} channels with a five-fold increase of the transition rate from the open to the rightmost closed state ($\beta\beta$).

4. Conclusions

Our simulations predict that the transition rates between the open and inactivated state of I_{Kr} are pivotal for APD at 1Hz. In addition, the transition rate from the open to the rightmost closed state has a minimal effect on APD. However, under dofetilide exposure de influence of this rate on the APD is specially amplified. Moreover, it was the transition rate modification that most accelerates the deactivation process. Therefore, our results suggest that dofetilide could potentially be used to unmask I_{Kr}

mutations accelerating the deactivation process.

Acknowledgements

This work was partially supported by the Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica del Ministerio de Ciencia e Innovación of Spain (TEC2008-02090), Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica, Plan Avanza en el marco de la Acción Estratégica de Telecomunicaciones y Sociedad de la Información del Ministerio de Industria Turismo y Comercio of Spain (TSI-020100-2010-469), Programa de Apoyo a la Investigación y Desarrollo (PAID-00-10-3212 to L.R., PAID-06-09-2843 and PAID-06-11-2002) de la Universidad Politécnica de Valencia, Dirección General de Política Científica de la Generalitat Valenciana (GV/2010/078 and PROMETEO/2012/030). The research was also supported by the American Heart Association (GIA, Western States Affiliate), the Alfred P. Sloan Foundation and the National Institutes of Health NHLBI RO1-HL-085592-04 and NHLBI RO1-HL-085592-S1 (to CEC).

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