Deactivation of Per-Arnt-Sim Domain Mutation Increases the Proarrhythmic Risk of Dofetilide

Rodolfo Gonzalez[1], Edgar Cardenas[1], Alain Manzo[1], Fernando Martinez[1], Julio Gomis[2], Javier Saiz[2]

[1] Instituto Tecnológico de Morelia, Mexico
[2] Universidad Politécnica de Valencia, Spain

Abstract

The aim of this work was to study the influence of PAS hERG R56Q mutation on the effects of dofetilide. The R56Q/WT mutation acts to increase the rate of deactivation. Markovian models of R56Q/WT mutation and dofetilide have been introduced in mammalian (modified Faber-Rudy) ventricular cellular model. Using this mutated ventricular cellular model we have studied the effects of dofetilide concentrations (I_{Kr} blocker).

The results showed that increased rates of deactivation produce a rightward shift in the voltage dependence of activation and rectification. Deactivation occurs earlier, resulting in less repolarizing current late in the action potential where I_{Kr} usually plays a major role in repolarization and determining APD. Morever, the action of dofetilide increases the APD in the R56Q/WT epicardial and endocardial cells, enhancing the difference in APD between both cell types. In addition, dofetilide amplifies the amplitude of the EADs that the R56Q/WT mutation provokes in midmyocardial cells.

In conclusion, the heterozygous R56Q hERG mutation increases the proarrhythmic risk of dofetilide prolonging the APD and enhancing the dispersion of repolarization.

1. Introduction

Mutations involving the KCNH2 gene (hERG, human ether-a-go-go-related gene) [1], which codes for the pore-forming α-subunit of a cardiac K^+ channel, have been linked to the type 2 LQTS, the second most common variant of long QT syndrome (LQTS). KCNH2 mutations lead to a reduction in the rapid component of the delayed rectifier repolarizing current (I_{Kr}), which contributes to lengthening of the QT interval, the electrocardiographic phenotype in LQT2 patients. Congenital LQTS is characterized by prolonged ventricular repolarization and a variable clinical course with arrhythmia-related syncope and sudden death. Acquired long QT syndrome can be induced by the action of different drugs.

Several drugs have been withdrawn from the market or their approved use was severely restricted when it was discovered that they caused arrhythmia or unexplained sudden death [2].

Native hERG channels are proposed to be hetero-tetramers arising from the assembly of 1a and 1b α-subunits encoded by alternate transcripts of the hERG gene [3]. hERG 1a and 1b subunits have an identical core containing six transmembrane spanning helices (S1–S6) and long conserved carboxyl terminal domains. hERG 1a and 1b subunits differ in the length of their amino terminus: 396 residues in the hERG 1a subunit and 56 residues in the hERG 1b subunit. The crystal structure of the first 135 residues in the hERG 1a subunit has been determined and has been shown to contain a conserved Per-Arnt-Sim (PAS) domain [4]. See figure 1. The functional role of the hERG PAS domain is not known but it is thought to participate in regulation of channel function. hERG exhibits characteristic slow closing (deactivation) kinetics that are regulated by an N-terminal PAS domain, which help to specialize the channels for their role in the heart [5-7]. Loss of hERG function, and thus, loss of I_{Kr} [8], can occur through a number of mechanisms, including defects in channel opening and closing (gating), ion permeation, or protein trafficking [9]. In the hERG channel, removal of the entire PAS domain or the presence of LQT2-associated missense mutations in the PAS domain region of the hERG channel has been shown to accelerate the rate of deactivation of the channel [10]. In other proteins PAS domains have been shown to sense environmental stimuli (light, ligands, and redox potentials) and regulate a variety of biochemical processes in both eukaryotic and prokaryotic systems [11].

There are two proposed molecular mechanisms that may account for reduced I_{Kr} current in patients with hERG mutations: (1) coassembly or trafficking abnormalities, in which mutant subunits either do not coassemble with normal subunits, or if they do, are not transported to the cell membrane (in either case, the net effect can result in a 50% reduction in the number of

functional channels, haplotype insufficiency); and (2) formation of defective channels involving mutant subunits, with the altered channel protein transported to the cell membrane (the dysfunctional channel can result in more than 50% reduction in channel function, a so-called dominant-negative effect).

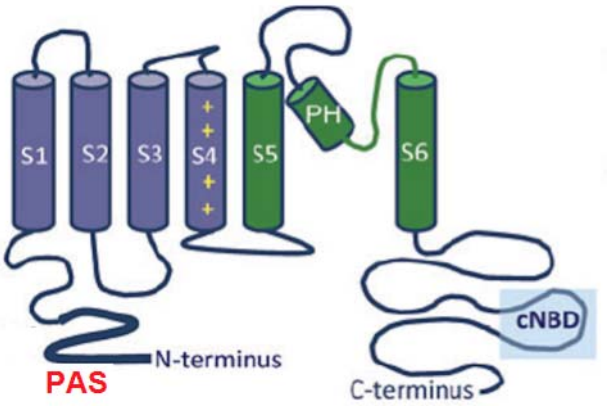


Figure 1. hERG channel structure.

Most mutations in the nonpore regions of hERG are associated with coassembly or trafficking abnormalities resulting in haplotype insufficiency.

Dofetilide is a specific and potent blocker of the rapid component of I_{Kr} with an IC_{50} in the nanomolar range (3.9–31 nM) for ventricular myocytes. Dofetilide is classified as a pure class-III antiarrhythmic agent and provokes a prolongation of APD without any effect on the resting membrane potential, AP amplitude, or maximum rate of depolarization. The efficacy of class-III drugs as antiarrhythmic agents is associated to the prolongation of refractoriness resulting from the APD prolongation, however, their effects on the increment of spatial dispersion of APD within the ventricular myocardium seem to be related to a form of acquired long-QT syndrome [12,13].

The aim of this work was to study the influence of PAS hERG R56Q mutation on the effects of dofetilide. A plethora of genetic information has revealed that genetics may play a critical role in determining arrhythmia susceptibility in efficacy of pharmacological therapy.

2. Methods

The model used to describe the guinea pig WT hERG cardiac channels is the Markov model (see figure 2) developed by Clancy-Rudy [14] using Kiehn Markov formulation [15], which fits transfected human embrionary kidney (HEK 293) and guinea pig cells experimental data at 37º C.

Mutations in the PAS domain of the amino terminal of hERG act to increase the rate of deactivation [10]. We focussed on the R56Q mutation since this mutation can increase the rate of deactivation most profoundly. This alteration in current kinetics reduces outward current through HERG channels during repolarisation from the plateau phase of the cardiac action potential producing a prolongation of the QTc interval, and is the likely cause of the increased risk of torsades de pointes arrhythmia in affected individuals.

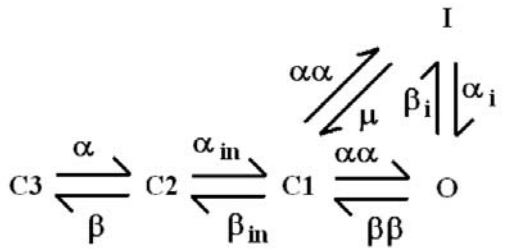


Figure 2. Markov hERG model developed by Clancy-Rudy using Kiehn Markov formulation.

This mutation was simulated by increasing the O ➔ C1 transition rate by a factor of 6.3 and the C2 ➔ C3 transition rate by a factor of 10.5 [14]. These increased rates of deactivation result in a rightward shift in the voltage dependence of activation and rectification, due to the strong coupling between the discrete states.

In order to analyze the effects provoked by the heterozygous R56Q hERG mutation at the cellular level, a R56Q/WT Markov models were incorporated into the guinea pig ventricular AP model of Faber Rudy 2000 [16], and the original Hogdkin-Huxley formulation of I_{Kr} was removed.

Our group has developed a dynamic model of dofetilide-I_{Kr} interaction [17]. This model is illustrated in figure 3, in which dofetilide binds in both open and inactivated states, the association rate constants (k_O and k_I) and the dissociation rate constants (r_O and r_I) being k_O =0.459 $\mu mol^{-1}s^{-1}$, k_I = 0.511 $\mu mol^{-1}s^{-1}$, r_O =0.003675 s^{-1} and r_I = 0.003606 s^{-1}. This model has been validated at the ionic level and at the cellular level using experimental data.

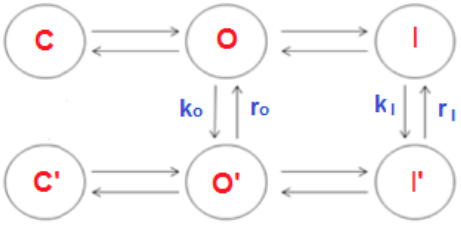


Figure 3. State diagram with dofetilide-I_{Kr} interaction. C=closed, O=open and I=inactivated.

To investigate the influence of R56Q mutation on the proarrhyhmic effects of dofetilide, the model of dofetilide-I_{Kr} interaction was included in the guinea pig hERG formulation and left ventricular endocardial, midmyocardial and epicardial action potentials were simulated and increment of APD_{90} epicardial, midmyocardial and endocardial cells paced at 1 Hz was analyzed.

3. Results

Figure 4 shows the steady state AP, I_{Kr} and state probabilities (O, I, C3, C2 and C1) waveforms for a WT (thick line) and for a heterozygous R56Q hERG mutated (thin line) cell of the guinea pig epicardium at 1Hz.

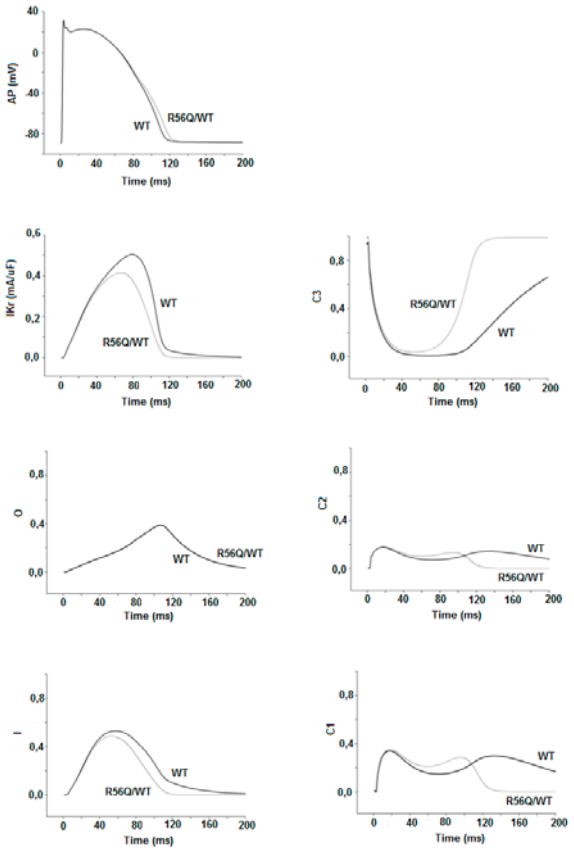


Figure 4. Steady state AP, I_{Kr}, and probabilities of residence in the states (O, I, C3, C2, and C1) waveforms of a WT (thick line) cell and R56Q /WT (thin line) epicardial cell paced at 1Hz.

The simulated APD_{90} for WT and for R56Q/WT cells is 114 ms and 120 ms, respectively. Therefore, epicardial mutated cells show a 6 ms prolongation of the APD, which corresponds to a 5.3 % of the APD_{90}.

While the initial current elicited during the upstroke of the AP is comparable between the WT and R56Q mutant cells, the late current in the mutant cell is decreased due to increased probability of the O □ C1 transition in the mutant cell. Deactivation occurs earlier, resulting in less repolarising current late in the AP where I_{Kr} usually plays a major role in AP repolarisation and in the mutant APD.

Figure 5 depicts the action potential time courses of R56Q/WT epicardial, midmyocardial and endocardial cells paced at 1 Hz (panels A, B and C respectively) with control and applications of dofetilide of 10 nM, 30 nM and 100 nM and figure 6 shows the increment of APD_{90} versus R56Q/WT epicardial, midmyocardial and endocardial cells paced at 1 Hz with applications of dofetilide.

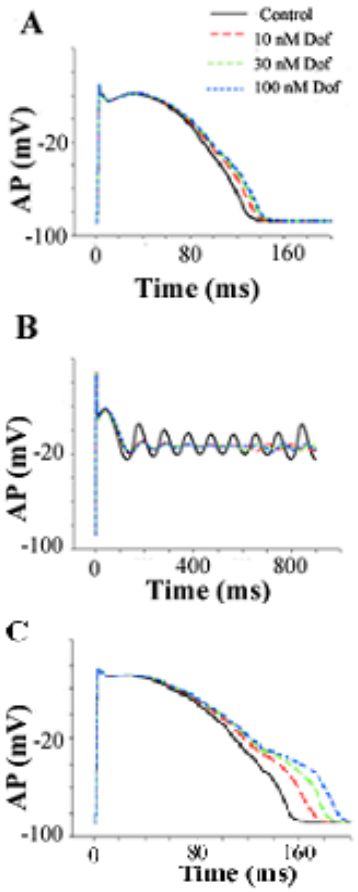
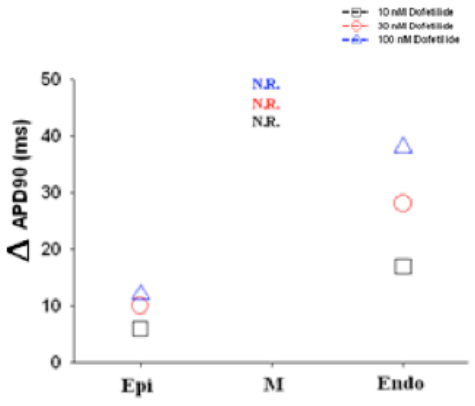


Figure 5. Action potentials of R56Q/WT epicardial (A), midmyocardial (B) and endocardial (C) cells paced at 1 Hz with applications of dofetilide.

Our results show that dofetilide increases the APD in the R56Q/WT epicardial and endocardial cells up to 12 ms and 38 ms, respectively, enhancing the difference in APD between both cell types. In addition, dofetilide

amplifies the amplitude of the EADs that the R56Q/WT mutation provokes in midmyocardial cells. Therefore, dofetilide increases the risk of developing cardiac arrhythmias in R56Q/WT mutated cells.



Note.- N.R. means not repolarization

Figure 6. Increment of APD_{90} versus R56Q/WT epicardial, midmyocardial and endocardial cells paced at 1 Hz with applications of dofetilide.

4. Discussion and conclusions

The influence of R56Q hERG mutation on the effects of dofetilide was analyzed.

The in vivo clinical findings of increased cardiac events in patients with PAS mutations are consistent with the known in vitro electrophysiological effects of the reported PAS hERG mutations having a decreasing I_{Kr} current as is shown in this study.

In conclusion, the heterozygous R56Q hERG mutation increases the proarrhythmic risk of dofetilide prolonging the APD and enhancing the dispersion of repolarization.

Acknowledgements

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Address for correspondence.

Rodolfo Gonzalez
Departamento de Ingeniería Electrónica, Instituto Tecnológico de Morelia, Morelia, México.
rodogon21@yahoo.com