Influence of Pore hERG Mutation on Dofetilide Proarrhythmic Risk

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Abstract

The aim of this work was to study the influence of pore KCNH2 mutation on the effects of dofetilide. Markovian models of G604S/WT mutation and dofetilide have been introduced in guinea pig ventricular cellular model. The effects of this pore mutation affecting this channel were analyzed. The G604S/WT mutation accelerates the inactivation and recovery from inactivation. Using this mutated cellular model we have studied the effects of dofetilide concentrations (I_{Kr} blocker).

The results showed that the reduction of G_{Kr} is the main factor in the APD prolongation in the case of G604S/WT mutation. The shift of the inactivation curve and the recovery from inactivation to the left accelerates the transition between the open and inactivated states. The action of dofetilide prolongs the APD in the G604S/WT epicardial and endocardial cells and even provokes EADs development in endocardial cells. In addition, exposure of G604S/WT to this drug amplifies the amplitude of the EADs generated in midmyocardial cells by the mutation alone.

In conclusion, the heterozygous G604S hERG mutation increases the proarrhythmic risk of dofetilide prolonging the APD and enhancing the dispersion of repolarization.

1. Introduction

Acquired long QT syndrome (LQTS) 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 Several drugs have been withdrawn from the market or their approved use was severely restricted when it was discovered that they caused arrhythmia or unexplained sudden death. In order to detect which drugs can induce these side effects, pharmaceutical companies have developed rigorous screening technology. However, some drugs will only cause complications in a subpopulation of sensitive patients. It is well known that genetic factors may underlie the susceptibility to drug-induce adverse reactions such as a LQTS and Torsade de Pointes (TdP).

Hereditary LQTS is characterized by prolonged ventricular repolarization and a variable clinical course with arrhythmia-related syncope and sudden death. Nowadays, twelve ion-channel genes are known to cause this syndrome with numerous LQTS mutations identified in these genes [1].

Mutations involving the KCNH2 gene (hERG, human ether-a-go-go-related gene) [2], 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 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.

The KCNH2 subunits oligomerise to form a tetramer that inserts into the cell membrane to form the functional K^+ channel. Each subunit comprises 6 α-helical transmembrane segments (S1 to S6), where the K^+ selective pore is found between S5 and S6. Within each hERG subunit, the S1–S4 helices form a voltage sensor domain that senses transmembrane potential and is coupled to a central K^+ selective pore domain. The transmembrane segments are flanked by amino (N)- and carboxyl (C)-terminus regions. See figure 1.

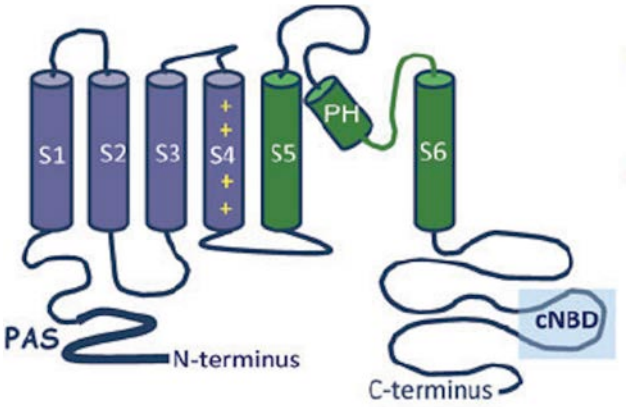

Figure 1. hERG channel structure.

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).

The pore region provides the potassium conductance pathway in channels, and most mutations involving this region are missense mutations with dominant-negative effects on I_{Kr}. In contrast, 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 [3,4]. The long QT syndrome is a disorder characterized by prolonged ventricular repolarization that predisposes carriers to life-threatening arrhythmias, such as TdP.

The aim of this work was to study the influence of pore G604S-hERG mutation on the effects of dofetilide. In the last years, a plethora of genetic information has revealed that genetics may play a critical role in determining arrhythmia susceptibility in efficacy of pharmacological therapy [5].

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 [6] using Kiehn Markov formulation [7], which fits transfected human embrionary kidney (HEK 293) and guinea pig cells experimental data at 37º C.

The G604S-hERG mutation exhibited strong dominant-negative current suppression resulting in decreased current density and altered gating properties of the WT-hERG channel, as well as interference with the trafficking of WT-hERG channel protein [8]. The G604S mutation is located between the S5 transmembrane segment and the pore in hERG channels and accelerates the inactivation and recovery from inactivation.

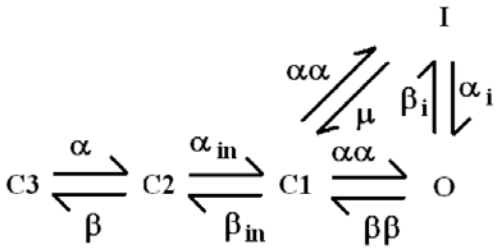

Figure 2. Markov hERG model developed by Clancy-Rudy using Kiehn Markov formulation.

This mutation was simulated by altering the voltage dependence of the I ➔ O transition rate ((v+25) factor was replaced by (v+35)) and decreasing the maximal conductance G_{Kr} to 70.4% [9]. These increased rates of inactivation and recovery from inactivation result in a leftward shift in the voltage dependence of inactivation and recovery from inactivation of transfected cells. These cells showed currents with similar waveforms but in which cells expressing G604S-hERG channels showed reduced current amplitudes compared to those expressing WT-hERG channels alone.

In order to analyze the effects provoked by the heterozygous G604S hERG mutation at the cellular level, a G604S/WT Markov models were incorporated into the guinea pig ventricular AP model of Faber Rudy 2000 [10], and the original Hogdkin-Huxley formulation of I_{Kr} was removed.

Our group has developed a dynamic model of dofetilide-I_{Kr} interaction [11]. 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.

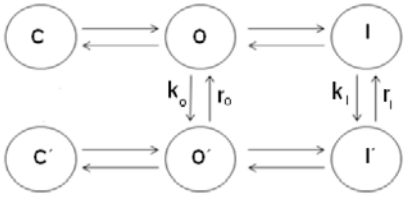

Figure 3. State diagram with dofetilide-I_{Kr} interaction. C=closed, O=open and I=inactivated.

In order to investigate the influence of G604S 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 G604S hERG mutated (thin line) cell of the guinea pig epicardium at 1Hz.

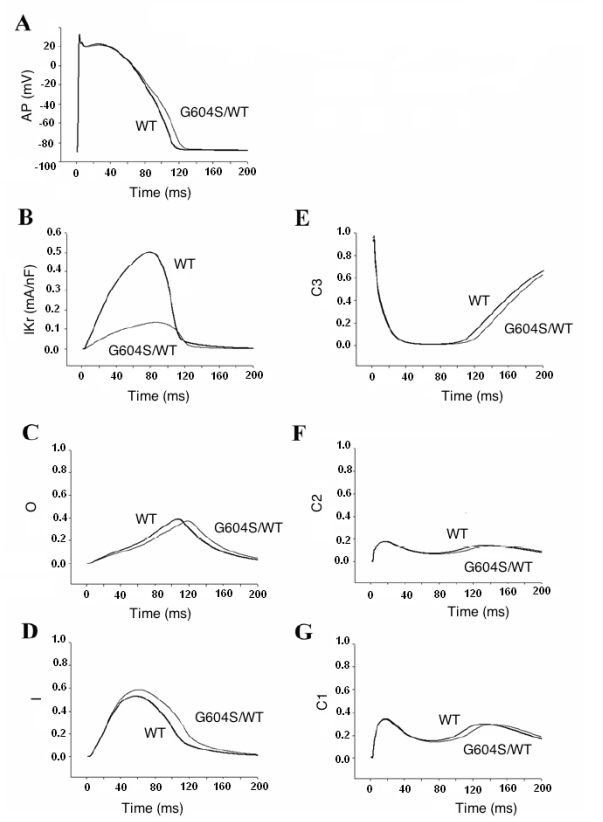

Figure 4. Steady state AP, I_{Kr}, and probabilities of residence in the states (O, I, C3, C2, and C1) waveforms of a WT (thick line) cell and G604S /WT (thin line) epicardial cell paced at 1Hz.

The simulated APD_{90} for WT and for G604S/WT cells is 114 ms and 126 ms, respectively. Therefore, epicardial mutated cells show a 12 ms prolongation of the APD, which corresponds to a 10.5 % of the APD_{90}.

The reduction of G_{Kr} is the main factor in the APD prolongation in the case of G604S mutation. Alterations in the inactivation and recovery from inactivation kinetics also influence AP, since O and I probabilities are modified by the G604S mutation (see Figure 4, panels C and D). The shift of the inactivation curve and the recovery from inactivation to the left accelerates the transition between the open and inactivated states (see Figure 4, panel D). In this mutation, the reduction of G_{Kr} is the main factor in having a subsidiary I_{Kr} decreased, causing the APD_{90} is longer in comparison to the APD_{90} of WT cells and R56Q mutation – nonpore mutation, located in amino (N)- terminus region, which results are not shown.

Figure 5 depicts the action potential time courses of G604S/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 G604S/WT epicardial, midmyocardial and endocardial cells paced at 1 Hz with applications of dofetilide.

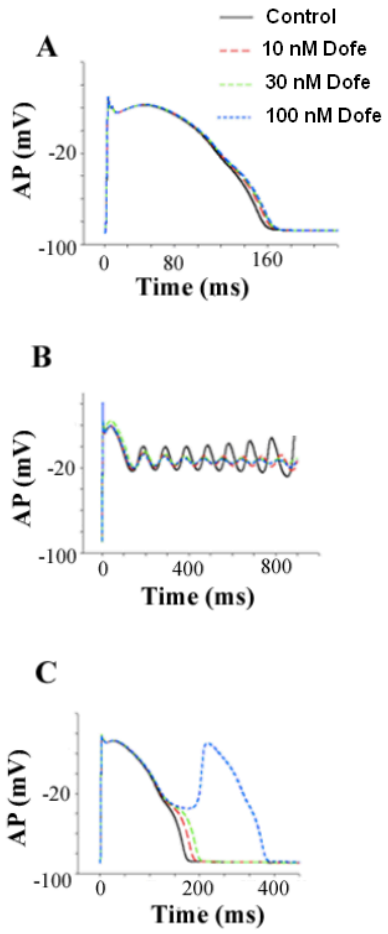

Figure 5. Action potentials of G604S/WT epicardial (A), midmyocardial (B) and endocardial (C) cells paced at 1 Hz with applications of dofetilide.

These figures show that dofetilide prolongs the APD in the G604S/WT epicardial and endocardial cells and even provokes EADs development in endocardial cells with application of 100 nM. In addition, exposure of G604S/WT to this drug amplifies the amplitude of the EADs generated in midmyocardial cells by the mutation alone.

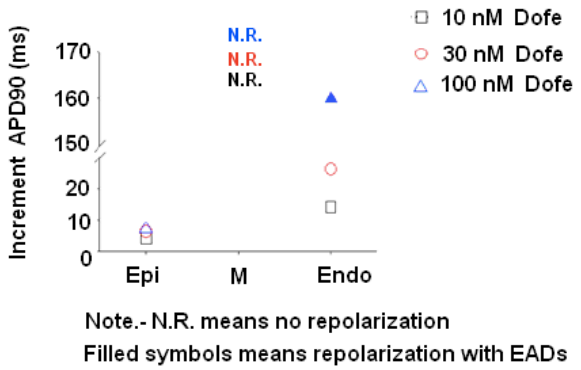

Figure 6. Increment of APD_{90} versus G604S/WT epicardial, midmyocardial and endocardial cells paced at 1 Hz with applications of dofetilide.

4. Discussion and conclusions

The influence of G604S a pore KCNH2 mutation on the effects of dofetilide was analyzed.

The in vivo clinical findings of increased cardiac events in patients with pore mutations are consistent with the known in vitro electrophysiological effects of the reported hERG mutations, with pore mutations having a greater negative effect on I_{Kr} current than nonpore mutations [12] as is shown in this study.

In conclusion, the heterozygous G604S hERG mutation increases the proarrhythmic risk of dofetilide prolonging the APD and enhancing the dispersion of repolarization.

Acknowledgements

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