Alleviating Effects of Long-QT Syndrome Type 2 by Allele-Specific Inhibition of the *KCNH2* Mutant Allele

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Abstract

It has recently been demonstrated that the effects of long-QT syndrome type 1 can be alleviated by allele-specific 40–60% inhibition of the mutant KCNQ1 gene. We investigated whether the effects of long-QT syndrome type 2 (LQTS2) can similarly be alleviated by downregulation of the mutant KCNH2 allele, using the O'Hara–Rudy human ventricular cardiomyocyte model, updated with the rapid delayed rectifier potassium current (I_{Kr}) Markov model from Li et al. (2017; PMID: 28202629).

If wild-type and mutant KCNH2 proteins are equally expressed and co-assemble randomly, only 6.25% of the tetrameric I_{Kr} channels will completely consist of wild-type subunits. Inhibition of the mutant KCNH2 allele by 60% decreases the overall number of I_{Kr} channels by 30%. However, if only I_{Kr} channels entirely built of wild-type subunits are conductive, as in severe LQTS2 mutations, the amount of conductive I_{Kr} channels increases almost three-fold, reducing the mutation-induced prolongation of the APD_{90} at 1 Hz stimulation from 357 ms (+135%) to 236 ms (+89%). An even higher effect is obtained if the mutant KCNH2 allele can be inhibited by as much as 80%.

We conclude that allele-specific inhibition of the KCNH2 mutant allele in case of LQTS2 reduces the mutation-induced action potential prolongation and thus may alleviate the disease.

1. Introduction

Long-QT syndrome type 1 (LQTS1) is caused by loss-of-function mutations in the *KCNQ1* gene, which encodes the pore-forming Kv7.1 α-subunit of the ion channel carrying the slow delayed rectifier potassium current (I_{Ks}) [1]. Patients heterozygous for such a mutation co-assemble wild-type and mutant *KCNQ1*-encoded α-subunits into tetrameric Kv7.1 potassium channels. Similarly, long-QT syndrome type 2 (LQTS2) patients are heterozygous for loss-of-function mutations in the *KCNH2* gene, which encodes the pore-forming Kv11.1 α-subunit of the ion channel carrying the rapid delayed rectifier potassium current (I_{Kr}) [2]. It has been shown that mutant and wild-type Kv channel subunits randomly co-assemble into functional tetramers [3]. Thus, these tetramers contain 0 to 4 mutant subunits according to a binomial distribution with parameters $n = 5$ and $p = 0.5$, representing all possible combinations of a total of four wild-type or mutant subunits. This explains the common dominant-negative nature of LQTS1 and LQTS2 loss-of-function mutations in the *KCNQ1* (*KvLQT1*) and *KCNH2 (HERG)* genes [4,5].

An example of the dominant-negative nature of LQTS2 mutations are the A561P, A561T, and A561V mutations at position 561 of the *KCNH2* gene. Bellocq et al. [6], who used African green monkey kidney cells (COS-7 cells) as their transfection system, observed an I_{Kr} tail amplitude of ≈30% in COS-7 cells co-injected with wild-type *KCNH2* cDNA plus A561P, A561T, or A561V *KCNH2* cDNA as compared to COS-7 cells injected with cDNA coding for wild-type *KCNH2*, suggesting that only channels with one mutant subunit max, constituting 5 of all 16 configurations possible in case of co-assembly of four wild-type or mutant subunits into a tetrameric ion channel (Fig. 1A), are conductive.

Recently, Cócera-Ortega et al. [7] demonstrated, both in vitro and in silico, that the effects of LQTS1 can be alleviated by allele-specific up to 60% inhibition of mutant *KCNQ1*. They investigated whether this allele-specific inhibition, by targeting a common single nucleotide polymorphism (SNP) on the mutant allele, can shift the balance of I_{Ks} channel subunits towards increased incorporation of the wild-type allele to alleviate the disease. Suppression of the mutant *KCNQ1* allele by 60% decreased the occurrence of arrhythmic events in human-induced pluripotent stem-cell-derived cardiomyocytes (hiPSC-CMs) derived from two patients with a pathogenic LQTS1 mutation (R243C) in the *KCNQ1* gene, whereas suppression of the wild-type allele increased the occurrence of arrhythmic events. Computer simulations revealed that 60% suppression of the mutant *KCNQ1* allele is particularly effective if only I_{Ks} channels without a mutant Kv7.1 subunit or with a single mutant Kv7.1 subunit are conductive and I_{Ks} channels with more mutant subunits are not.

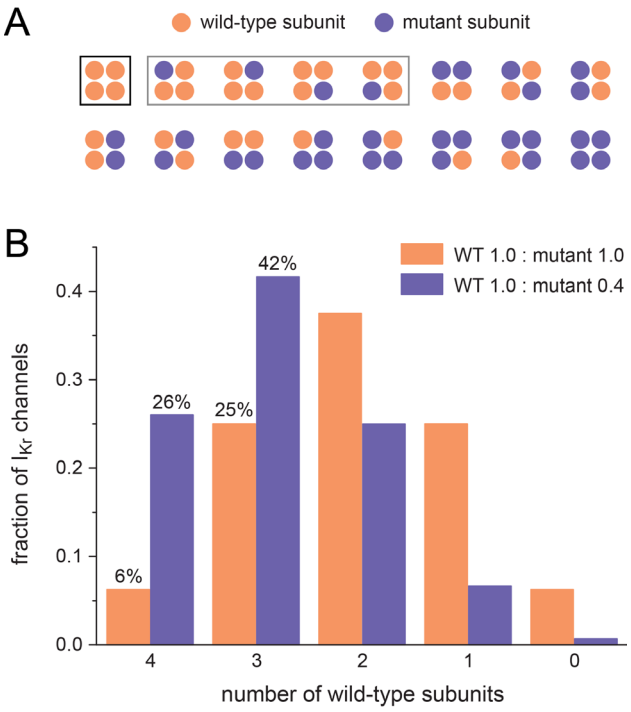

Figure 1. (A) All 16 configurations possible in case of co-assembly of four wild-type or mutant subunits into a single tetrameric ion channel. The black square and gray rectangle indicate channels containing exactly four or three wild-type subunits, respectively. (B) Fraction of rapid delayed rectifier potassium current (I_{Kr}) channels with 0–4 wild-type (WT) subunits in case of equal expression of wild-type and mutant Kv11.1 subunits (WT 1.0 : mutant 1.0) and in case of 60% suppression of mutant Kv11.1 subunits (WT 1.0 : mutant 0.4), assuming random co-assembly of subunits into tetrameric channels.

Given the encouraging results by Cócera-Ortega et al. [7] regarding LQTS1, we questioned whether the effects of LQTS2 can similarly be alleviated by allele-specific downregulation of mutant *KCNH2*. To this end, we carried out computer simulations with the O'Hara–Rudy human ventricular cardiomyocyte model [8], as updated with the Markov-type I_{Kr} formulation by Li et al. [9].

2. Methods

The allele-specific downregulation of mutant *KCNH2* was implemented in the CellML code [10] of the O'Hara–Rudy human ventricular cardiomyocyte model [8], as updated with the Markov-type I_{Kr} formulation by Li et al. [9], which is available at the CellML Model Repository and known as the "Comprehensive in vitro Proarrhythmia Assay (CiPA) 1.0 ORd model", "I_{Kr}-dynamic ORd model", or simply "ORd 2017 model".

The CellML code was edited and run in the Cellular Open Resource (COR) environment by Garny et al. [11], version 0.9.31.1409. All simulations were run for a sufficiently long time to reach steady-state behaviour.

3. Results

First, we assessed the extreme case of a dominant-negative LQTS2 mutation in which only I_{Kr} channels built of four wild-type subunits are conductive (Fig. 1A, black square). We assumed that wild-type and mutant Kv11.1 proteins are equally expressed in a 1:1 ratio and co-assemble randomly, thus creating a symmetrical binomial distribution of I_{Kr} channels built of 0–4 wild-type subunits (Fig. 1B, orange bars). Accordingly, only $1/16^{th}$ (6.25%) of the I_{Kr} channels are conductive, reducing the fully-activated I_{Kr} conductance to only 6.25% of its control value. At 1 Hz stimulation, the action potential duration at 90% repolarization (APD_{90}) increased by as much as 357 ms from its control value of 264 to 620 ms (+135%), as shown in Fig. 2A (arrow), as a result of the dramatic decrease in I_{Kr} (Fig. 2B).

Allele-specific inhibition of mutant *KCNH2* by 60%, thus creating a 1:0.4 ratio of wild-type and mutant subunits, results in skewing the binomial distribution of I_{Kr} channels towards channels containing three or four wild-type subunits (Fig. 1B; binomial distribution with parameters $n = 5$ and $p = 5/7$). The 60% inhibition makes that 26.0% of the I_{Kr} channels rather than only 6.25% are completely built of wild-type subunits, thus being conductive. This will result in a less dramatic decrease in I_{Kr}. However, at the same time, the 60% inhibition results in a 30% decrease in the total number of channels, so that the net effect of the 60% inhibition of the mutant allele is limited to a reduction of the fully-activated I_{Kr} conductance to 18.2% of its control value, instead of the 6.25% in absence of the inhibition.

At 80% inhibition, the fully-activated I_{Kr} conductance amounts to 28.9% of its control value, representing that, at 80% inhibition, 48.2% of the I_{Kr} channels are completely built of wild-type subunits, whereas the total number of channels is decreased by 40%. The effects of 60% and 80% inhibition are shown in Fig. 2, A and B. At 60% inhibition, APD_{90} amounts to 500 ms, reducing its mutation-induced increase to 236 ms (+89%). At 80% inhibition, the mutation-induced action potential prolongation is further reduced to 177 ms (+67%). Because we assumed that only I_{Kr} channels built of four wild-type subunits are conductive, the maximum effect of the allele-specific inhibition of mutant *KCNH2* is obtained at 100% inhibition, at which the increase in APD_{90} amounts to 103 ms (+39%).

Next, we assessed the case of a more common dominant-negative LQTS2 mutation in which only I_{Kr} channels with one mutant subunit max are conductive. We started with the assumption that wild-type and mutant Kv11.1 proteins are equally expressed in a 1:1 ratio and co-assemble randomly, thus creating the symmetrical

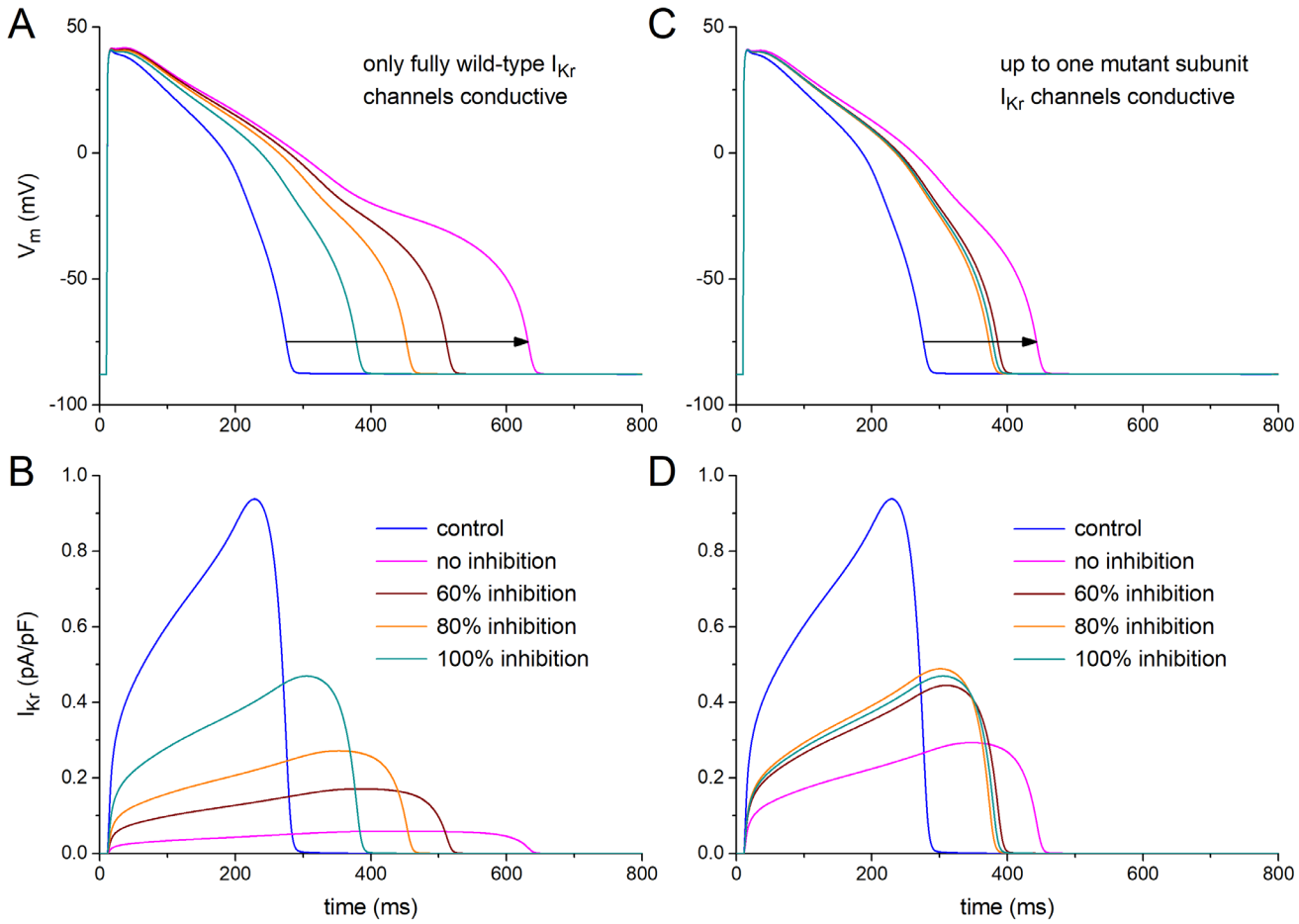

Figure 2. Effects of dominant-negative LQTS2 mutations on the electrophysiology of a human ventricular cardiomyocyte. Membrane potential (V_m) and associated rapid delayed rectifier potassium current (I_{Kr}) of the O'Hara–Rudy human ventricular cardiomyocyte model [7] as updated with the Markov-type I_{Kr} formulation by Li et al. [8] (ORd 2017 model) at 1 Hz stimulation. (A) V_m and (B) I_{Kr} under control conditions and under the assumption that only I_{Kr} channels built of four wild-type subunits are conductive, at a 1:1 ratio of wild-type and mutant subunits (no inhibition of the mutant allele), at a 1:0.4 ratio (60% inhibition of the mutant allele), at a 1:0.2 ratio (80% inhibition of the mutant allele), and upon complete inhibition of the mutant allele (100% inhibition). (C) V_m and (D) I_{Kr} under control conditions and under the assumption that only I_{Kr} channels with one mutant subunit max are conductive, at a 1:1 ratio of wild-type and mutant subunits (no inhibition of the mutant allele), at a 1:0.4 ratio (60% inhibition of the mutant allele), at a 1:0.2 ratio (80% inhibition of the mutant allele), and upon complete inhibition of the mutant allele (100% inhibition). Horizontal arrows in the top panels indicate the increase in action potential duration at 90% repolarization in absence of inhibition of the mutant allele.

binomial distribution of I_{Kr} channels of Fig. 1B (orange bars). Under this assumption, $5/16^{th}$ (31.25%) of the channels are conductive (Fig. 1A). Simulations with the fully-activated I_{Kr} conductance set to 31.25% of its control value show an increase in APD_{90} by 168 ms from its control value of 264 to 432 ms (+64%) and an I_{Kr} amplitude of approximately one-third of its control value (Fig. 2, C and D). The increase in APD_{90} is reduced to 111 ms (+42%) upon allele-specific inhibition of mutant *KCNH2* by 60%, associated with an increase in I_{Kr} amplitude from about one-third to nearly one-half of its control value (Fig. 2, C and D). The increase in APD_{90} is further reduced to 98 ms (+37%) upon 80% inhibition, associated with an increase in I_{Kr} amplitude to over one-half of its control value (Fig. 2, C and D).

Of note, unlike the extreme case in which only I_{Kr} channels built of four wild-type subunits are conductive (Fig. 2, A and B), the maximum effect of the allele-specific inhibition of mutant *KCNH2* is not obtained at 100% inhibition. Whereas the increase in APD_{90} was reduced to 98 ms (+37%) upon 80% inhibition, it amounts to 103 ms (+39%) at 100% inhibition (Fig. 2, C and D). At this degree of inhibition, the V_m and I_{Kr} traces of Fig. 2, A and B, and those of Fig. 2, C and D, are identical.

4. Conclusions

Selective inhibition of the mutant *KCNH2* allele in case of a dominant-negative LQTS2-causing mutation, e.g., by targeting a common single nucleotide polymorphism (SNP) on the mutant allele, reduces the mutation-induced action potential prolongation and thus may alleviate the disease and reduce arrhythmias. Such inhibition may also turn out effective in case of short-QT syndrome type 1 patients, who are heterozygous for a gain-of-function mutation in the *KCNH2* gene.

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