BIOINFORMATION
Discovery at the interface of physical and biological sciences

open access www.bioinformation.net Hypothesis

Volume 8(22)

Wild type and K897T polymorphisms of the hERG gene: modeling the APD in Caucasians

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Received September 20, 2012; Accepted October 01, 2012; Published November 13, 2012

Abstract:
The presented study aims to assess the possibility of simulating changes in cardiac cell electrophysiology due to K897T polymorphism in the Caucasian population. In the first part of the experiment, the parameters of the equations describing channel gating were fitted to the experimental data. Then, the action potentials of midmyocardial cells of 100 individuals were simulated in the *in vitro - in vivo* extrapolation system - ToxComp. Mean APD90 for the entire simulated population is 352.05 ms (SD = 21.69 ms). Mean APD90 for the 80 individuals with the WT version of the hERG gene and for the 20 K897T homo- and heterozygotes is respectively 349.08 ms (SD = 21.09 ms) and 363.95ms (SD = 20.41 ms). The ToxComp system can be useful in predicting the impact of genetic variability on drug triggered cardiac cell electrophysiology interference.

Background:
The KCNH2 (*human ether-a-go-go related gene*, hERG) gene encodes the rapid component of the delayed rectifier potassium current, whose mutations are linked to QT prolongation in the ECG. Long QT syndrome predisposes to life-threatening arrhythmias, and therefore, it seems important to identify individuals in the population who are more vulnerable to this effect **[1, 2].** The *in vitro* patch clamp methods are considered to be the "gold standard" for assessing the difference between the currents encoded by the WT version of the gene and its mutations **[3].**

K897T – an amino acid polymorphism of the hERG channel, in which basic lysine (K) is changed into neutral threonine (T) at position 897 counting from the N-terminus, which changes the electrical charge of the protein. A study carried out by Bezzina and colleagues with use of different mathematical model indicates that individuals who are homozygous for the above-mentioned polymorphism exhibit shorter QTc **[4],** although other study indicates its prolongation **[5].**

The aim of this study was to assess the possibility of simulating the changes in cell electrophysiology, which arise due to the genetic mutations in the background of the entire population of healthy Caucasians.

Methodology:
The frequency of mutation – population data
The available literature on the frequency of mutations in the hERG gene have been reviewed, which showed that K897T is one of the most commonly occurring polymorphisms in the Caucasian population. Nordic populations were excluded as a separate subpopulation in terms of frequency of the K897T polymorphism. The remaining data was used to calculate the percentage of homozygotes present in the Caucasian population **[4, 5-12].**

Model for the i_{Kr} current (WT and K897T)
The equation 1 **(see supplementary material for description)** describes the rapid delayed rectifier current was extracted from the ten Tusscher model of a single cardiac cell **[13].** Then, the parameters of the equations describing channel gating were fitted to the experimental data obtained from the experiment carried out by Bezzina and colleagues, after graphs digitation with the use of the GetData software **Table 1 (see supplementary material).** The fitting procedure was done with the use of the BerkleyMadonna™ system **[4, 13].** The population data and parameters of the equations describing the action of the hERG channel have been combined and implemented to the *in vitro – in vivo* extrapolation system ToxComp **[14].**

Simulations

In the next part of the experiment, 10 groups with 10 healthy Caucasians in each of them, aged 18-65 years, have been simulated using the ToxComp system **[14].** 50% of individuals were female. The result of the simulations was APD90 for the midmyocardial cells of each individual. For each person, the data was generated for the situation before and after the administration of moxifloxacin. The operational concentration of the drug was set to 5.73 µM. This concentration was chosen on the basis of the clinical study conducted by Hulhoven and colleagues **[15].** The IC50 value (the concentration of an agent blocking the i_{Kr} current at 50%) for moxifloxacin, determined by Chen and coworkers, was 35.7 µM **[16].** Moxifloxacin was chosen as a positive control due to its effect on the ionic currents responsible for evoking the AP, especially on the rapid component of the delayed rectifier potassium current. The drug influence on the i_{Kr} ionic current was simulated as the dose dependent maximal conductance reduction (Hill equation, n=1). The concentrations of potassium, sodium and calcium were respectively 5, 140 and 2 mM, the simulation time was 10 000 ms. to apply for inter individual variability; the coefficient of variation around the mean value was set to 10% **[16-20].** The chosen time of the day when the moxifloxacin effect was simulated was set at 8.00 am **[14].**

The purpose of the simulation was to assess the duration of the action potential of the single cardiac cell, depending on the genetic constitution.

Statistical analysis

In the present study the mean APD90 of two groups were compared: 1) individuals with the WT version of the hERG gene and 2) homo- and heterozygotes with the K897T polymorphism. Statistical analysis was performed using the Student's t test. The significance level was set to 0.05.

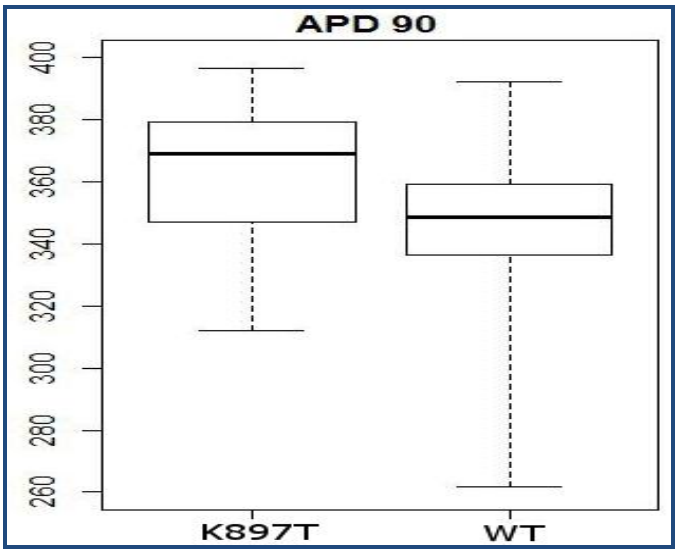

Figure 1: Results plot for the K897T/WT comparison: median, lower and upper quartile of the distribution, min and max value of APD90.

Discussion:

Population data – calculations

Based on the publication it was calculated that the percentage of homozygotes in the Caucasian population is 5.9 **[4-12, 14].**

Fitted parameters of the ten Tusscher's equations

The activation gate ($xr1$) is described by the steady-state activation (x_{r1inf}) and activation time constants - activation (a_{xr1}) and slow deactivation (β_{xr1}). Whereas the inactivation gate ($xr2$) is described by the steady-state inactivation (x_{r2inf}) and inactivation time constants - recovery from inactivation (a_{xr2}) and inactivation (β_{xr1}).

Simulation results

The mean APD90 for the entire population is 352.05 ms (SD = 21.69 ms). The mean APD90 for the 80 individuals with the WT version of the hERG gene and for the 20 K897T homo- and heterozygotes is, respectively, 349.08 ms (SD = 21.09 ms) and 363.95ms (SD = 20.41 ms).

Statistical analysis

The Student's t-test analysis was used to test the significance of the differences between WT and K897T groups (with a confidence interval of 95%). The alternative hypothesis stated that the true difference in means is not equal to 0. The mean difference was 14.87 (4.38; 25.37). It has been proven that there is a statistically significant difference among the groups (p-value = 0.006995). **Figure 1** presents the results plot for the K897T and WT comparison: median, lower and upper quartile of the distribution, minimal and maximal value of APD90.

The data on the frequency of mutations in the gene encoding the hERG channel, in the Caucasian population, and the data on the effects of the K897T polymorphism on the ionic channel behavior, were collected and merged in the ToxComp system. Subsequently, the software was used to simulate a group of 100 individuals randomly selected from the Caucasian population **[14].**

A significant prolongation of APD90 for the K897T group in comparison with the WT group was observed. The APD90 prolongation is linked to the long QT in the ECG, and therefore the presented results may be applied to the clinical study carried out by Pietilä and co-workers, which shows a QTc prolongation in homozygous individuals **[5].** The potential sources of errors come from two main areas. One of them is the graphs digitization process and the second one is the fitting procedure. To minimize the human influence factor on data taking, the digitization procedure was repeated twice, independently by both authors. There were no differences regarding the final values.

The proposed methodology of modeling the impact of changes in the genes on APD90 is obviously a simplification. The data used comes from studies done on the whole cell patch clamp experiment on HEK-293 cells, which have been transfected with the previously prepared genetic constructs, isolated from humans with WT or the K897T version of the gene. Such settings do not fully mimic human cardiomyocytes in the *in vivo* situation. Another problem is that the studies were conducted *in vitro*, which does not allow observation of the impact of all factors on the results. However, there is no available data from studies carried out on human cardiac cells derived from individuals with a specific mutation in the gene encoding the hERG channel, and therefore we have decided to apply the described methods.

Conclusion:
The presented results indicate that the *in vitro - in vivo* extrapolation system ToxComp can be used to predict the impact of drugs on cardiac cell electrophysiology, also taking into account the genetic variation in the population.

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Edited by P Kangueane
Citation: Glinka & Polak, Bioinformation 8(22): 1062-1065 (2012)

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Supplementary material:

$$i_{Kr} = g_{Kr} \cdot \sqrt{\frac{K_o}{5.4}} \cdot xr1 \cdot xr2 \cdot (V - E_K)$$

Eq. 1: Equation describing the rapid delayed rectifier current.
i_{Kr} – rapid delayed rectifier current
g_{Kr} – maximal i_{Kr} conductance
K_o – extracellular K^+ concentration

xtracellulare
*xr*1 – activation gate
*xr*2 – inactivation gate
V – membrane potential
E_K – reversal potential

Table 1: Parameters of equations for the WT and K897T versions of the gene fitted to the experimental data. The parameters of the equations describing channel gating were fitted to the experimental data obtained from the experiment carried out by Bezzina and colleagues (Bezzina *et al*. 2003).

Equation	Effect	Parameter (WT)			Parameter (K897T)		
		$V_{1/2}$	*k*	*A*	$V_{1/2}$	*k*	*A*
$x_{r1\inf} = \frac{1}{1+e^{(-V_{1/2}-V)k}}$	Steady-state activation gate	26	7	-	33	6	-
$\alpha_{xr1} = \frac{A}{1+e^{(-V_{1/2}-V)/k}}$	Activation time constant	45	10	450	34.37	8.93	657.25
$\beta_{xr1} = \frac{A}{1+e^{(V+V_{1/2})/k}}$	Deactivation time constant	30	11.5	6	55.11	12.8	11.04
$x_{r2\inf} = \frac{1}{1+e^{(-V_{1/2}-V)k}}$	Steady-state inactivation gate	-88	24	-	-90	23	-
$\alpha_{xr2} = \frac{A}{1+e^{(-V_{1/2}-V)/k}}$	Recovery from inactivation	60	20	3	58	20	3
$\beta_{xr2} = \frac{A}{1+e^{(V+V_{1/2})/k}}$	Inactivation	-60	20	1.12	-59	22	1.05

A – constant
$V_{1/2}$ – half-maximum activation voltage
k – slope factor