Computational Modelling of LQT1 in Human Induced Pluripotent Stem Cell Derived Cardiomyocytes

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Abstract

The production of disease-specific lines of cardiomyocytes derived from human induced pluripotent stem cells (hiPSC-CMs) opened new opportunities to study genetic cardiac disorders such as Long QT (LQT) syndrome. We focused on the computational modelling of hiPSC-CMs with LQT1 syndrome which reduces the slow delayed rectifying, I_{Ks}, current.

Both control and LQT1 I_{Ks} formulations are based on recently published I_{Ks} data, which were integrated into our previous model of hiPSC-CM action potential (AP).

The control model reproduced the automaticity and the shape of the experimental spontaneous APs. In simulations, LQT1 mutation induced a marked prolongation of the action potential duration (APD_{90} +27.5%). By simulating the application of isoproterenol in the LQT1 model, the mutation effects were exacerbated (APD_{90} further increased by 23.5%) due to the impaired rate adaptation, as shown by the 11.8% increment of the ratio APD_{90}/Cycle Length.

Our in-silico analysis confirmed that in hiPSC-CMs I_{Ks} plays a more important role in AP repolarization than in adult cardiomyocytes. Our model explains this behavior by a reduced repolarization reserve. This is manifested by the fact that I_{Kr} and I_{K1} were reduced compared to the adult AP in order to reproduce the control hiPSC-CMs AP shape.

1. Introduction

The opportunity of differentiating cardiomyocytes (CMs) from human induced pluripotent stem cells (hiPSCs) opened new promising perspectives, such as the availability of patient-specific cell sources for regenerative medicine or for new *in-vitro* disease-specific models for pharmacological studies.

This last aspect is of particular interest since it has been shown that human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) carry the same genetic mutations identified in the donors and which are responsible of cardiac diseases such as Long QT (LQT) syndromes [1,2]. Thus this could result in some years in the development of patient-specific personalized therapies.

In this work we focus on the LQT1 syndrome induced by the mutation of the KCNQ1 gene, which encodes for the slow rectifying K^+ current, I_{Ks}.

We developed two new models of the action potential (AP) of healthy and LQT1 hiPSC-CMs aimed to show the effect of the LQT1 mutation on the AP shape and to explain the more important role that I_{Ks} seems to play in the repolarization of these cells with respect to adult CMs.

2. Methods

To develop the healthy/LQT1 hiPSC-CM models we (i) formulated a new version of the healthy/LQT1 I_{Ks} model based on the specific data published by Moretti *et al.* [1], (ii) replaced the control I_{Ks} in our ventricular-like hiPSC-CM model [3], (iii) tuned the other ion currents to reproduce the AP shape of the control ventricular-like AP of [1], (iv) introduced the LQT1 I_{Ks} to obtain the LQT1 model. Thus, the control and the LQT1 models differ only for the I_{Ks} formulation.

2.1. I_{Ks} experimental data

We based our I_{Ks} formulations on the experimental data reported by Moretti *et al.* [1]: (i) the deactivation time constants (Fig. 1), (ii) the steady-state activation curves (Fig. 2), (iii) the I/V curves for the step (Fig. 3) and tail (Fig. 4) currents and (iv) the observed I_{Ks} density reduction of 75%.

Compared to controls, LQT1 hiPSC-CMs showed a slower deactivation time constant, a shift of the activation curve towards positive potentials and smaller step and tail currents.

It is worth to note that the I_{Ks} step current in [1] is way greater than the original I_{Ks} in our hiPSC-CM model

based on Ma *et al.* [4] data (Fig. 3), also in the LQT1 case. In fact our previous hiPSC-CM model was almost insensitive to I_{Ks} blockade up to 90%, in particular the action potential duration (APD) was not prolonged.

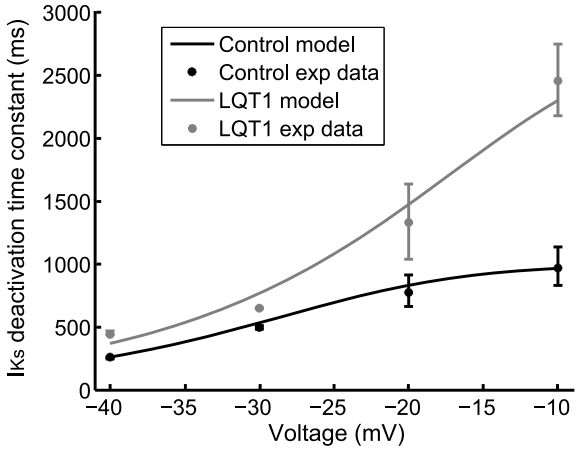

Figure 1. Voltage dependence of I_{Ks} deactivation kinetics. Experimental data reproduced from Moretti *et al.* [1].

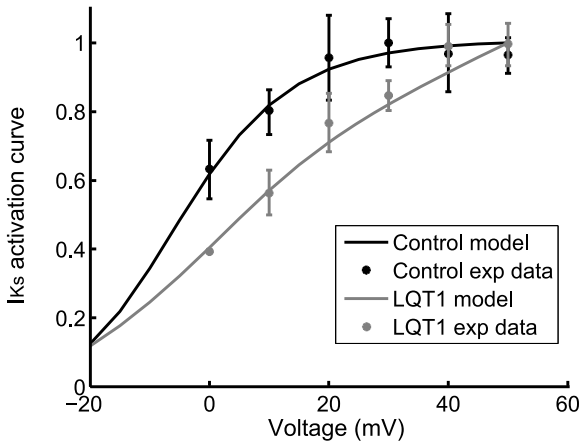

Figure 2. Voltage dependence of I_{Ks} activation. Experimental data reproduced from Moretti *et al.* [1].

2.2. Ion current tuning

Our original ventricular-like hiPSC-CM model [3] reproduces the AP morphology of the experimental APs reported in [4], which are fundamentally different from those reported in [1]. A comparison is reported in Table 1 and the main differences are:

- higher rate of spontaneous beating (F);
- less negative maximum diastolic potential (MDP) and greater peak voltage (Peak);
- smaller maximum upstroke velocity (Vmax).

According to this information we tuned the ion currents in

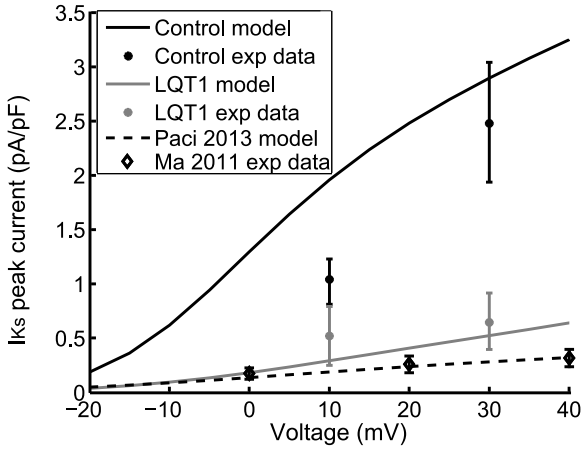

Figure 3. I_{Ks} step current. Experimental data reproduced from Moretti *et al.* [1] (circles) and from Ma *et al.* [4] (diamonds).

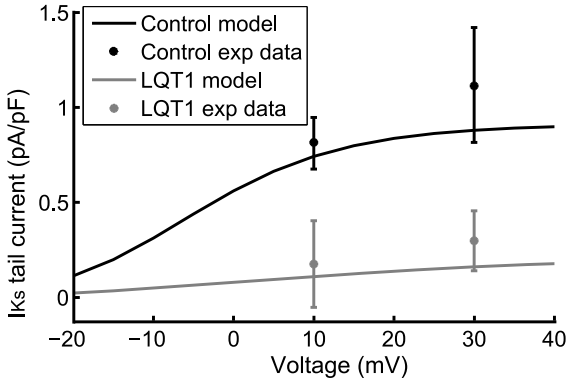

Figure 4. I_{Ks} tail current. Experimental data reproduced from Moretti *et al.* [1]

our original hiPSC-CM model (Table 2):

- I_{K1} conductance reduction and slight shift of its I/V curve towards positive potentials to depolarize MDP;
- due to the depolarized MDP, I_{Na} activation was compromised, thus we increased its maximum conductance to get closer to Moretti's Vmax;
- I_f and I_{pCa} increment to increase the rate of spontaneous beating;
- I_{Kr} reduction to strengthen I_{Ks} role in repolarization;
- I_{CaL} increment to increase the APD, which was reduced by the MDP depolarization and the

increased rate of spontaneous beating;

- I_{NaK} and I_{NaCa} consequently tuned to balance Na^+ and Ca^{++} concentrations in the cytoplasm and in the sarcoplasmic reticulum.

2.3. Isoproterenol simulation

To simulate the effects of the application of isoproterenol on the LQT1 hiPSC-CM model we referred to Severi *et al.* [5], where the β-adrenergic stimulation was shown to have a strong effect on the I_{Ks} amplitude. In particular a significant difference of 266±34% of the control I_{Ks} was reported in response to the application of 100 nM of isoproterenol. In our model we scaled the I_{Ks} maximum conductance by a factor 2, assuming also the non-fully functional response to the β-adrenergic stimulation in immature cells like hiPSC-CMs. As reported in [5], both the L-type Ca^{++} current I_{CaL} and the uptake by the sarcoplasmic reticulum SERCA pump I_{up} were scaled by the factor 1.5.

Table 1. AP morphological features from Ma *et al.* [4] and Moretti *et al.* [1] for ventricular-like hiPSC-CMs. Mean values ± SE are reported.

AP feature	Ma [4]	Moretti [1]
F (bpm)	35±2	68±3
MDP (mV)	-76±1	-64±2
Vmax (V/s)	27.8±4.8	9.0±0.2
APD_{90} (ms)	415±22	381±35
APA (mV)	104±1	108±2
Peak (mV)	28±1	44±2

Table 2. Parameter changes with respect to our original hiPSC-CM model [3].

Current	Parameter change	
I_{Na}	G_{Na}	× 1.4
I_f	G_f	× 1.2
I_{CaL}	G_{CaL}	× 1.3
I_{Kr}	G_{Kr}	× 0.6
I_{K1}	G_{K1}	× 0.5
	$shift_{K1}$	=10 (mV)
I_{NaCa}	k_{NaCa}	× 0.3
I_{NaK}	k_{NaK}	× 0.8
I_{pCa}	G_{pCa}	× 2

3. Results

Simulated spontaneous control and LQT1 APs are reported in Fig. 5: a comparison between the control experimental and simulated AP morphological features shows that the control model reproduces Moretti's APs better than our original ventricular-like model [3] (see Table 1 and 3). Moreover the control model reproduces a ventricular-like AP shape according to the following criterion: $1.10 \leq APD_{90}/APD_{50} = 1.29 \leq 1.30$ [1].

The main effect of LQT1 mutation is the marked prolongation of the action potential duration (APD), in particular APD_{90} is increased by +27.5%. This is qualitatively in agreement with the experimental APD_{90} prolongation of +95.4%, although less pronounced (see Table 3). The other AP features are affected by the LQT1 mutation in agreement with the experimental data: (i) the rate of spontaneous beating is reduced as a consequence of the prolonged APD, (ii) Vmax is slightly decreased and (iii) Peak and the AP amplitude (APA) are increased.

The simulated application of isoproterenol exacerbated the APD_{90} prolongation (+51% with respect to the healthy control model, see Fig. 6) due to the impaired rate adaptation, as shown by the +11.8% increment of the ratio APD_{90}/Cycle Length, in agreement with the experimentally recorded increment of +15%.

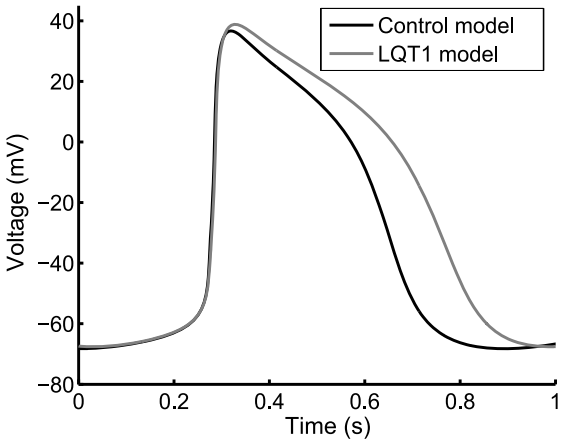

Figure 5.Control and LQT1 simulated spontaneous APs.

Table 3. AP features simulated by the control and LQT hiPSC-CM models.

AP feature	Moretti control data [1]	Control model	LQT1 model
F (bpm)	68±3	67	62
MDP (mV)	-64±2	-68	-67
Vmax (V/s)	9.0±0.2	7.1	6.7
APD_{90} (ms)	381±35	417	532
APA (mV)	108±2	105	107
Peak (mV)	44±2	37	39

4. Discussion and conclusions

The aim of this work was developing a proof of concept model to investigate the role of I_{Ks} in LQT1 hiPSC-CMs. Due to the very different I_{Ks} reported by [4] and by [1], our original ventricular-like hiPSC-CM model was basically insensitive to changes in I_{Ks} maximum conductance. Thus we needed to integrate a new I_{Ks} dataset into our model. Both the control and the LQT1 models qualitatively reproduced the experimental results

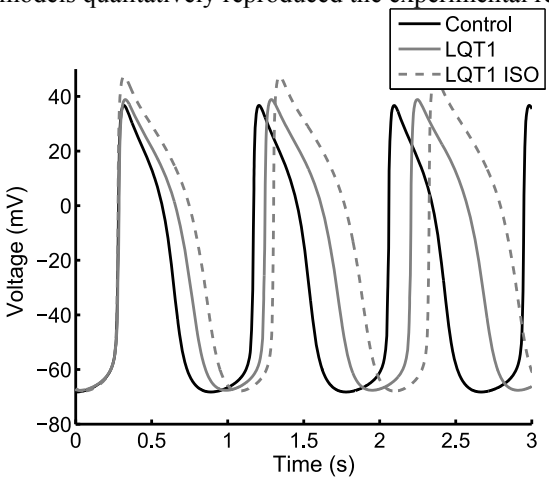

Figure 6. Simulation of the effect of isoproterenol on the LQT1 AP.

by Moretti *et al.* [1], even they suffer of some limitations in reproducing all the experiments. Therefore, even if our present LQT1 model is far from being considered an ultimate hiPSC-CM LQT1 model, it clearly indicates that I_{Ks} plays a more important role in the repolarization of the AP in hiPSC-CMs than in adult ventricular CMs. The greater I_{Ks} contribution in Moretti's hiPSC-CMs can be explained by a reduced repolarization reserve, due to the smaller hiPSC-CM I_{Kr} and I_{K1}, necessary to reproduce the control hiPSC-CM AP features. This consideration also suggests that mutation effect data in hiPSC-CMs deserve careful examinations. To this purpose, the use of computational models, conveniently tuned to take into account the high variability observed in these cells, is a valuable support.

References

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