Computational Modeling Supports Induced Pluripotent Stem Cell-derived Cardiomyocytes Reliability as a Model for Human LQT3

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Abstract

Long QT 3 (LQT3) is a specific LQT syndrome, induced by defects in the SCN5A gene, encoding for the Na^+ channels. Its effect is a Na^+ current (I_{Na}) gain-of-function, resulting in a sustained late current and in an action potential (AP) duration (APD) prolongation.

In this paper we aim to develop a control and a LQT3 patient in silico action potential model of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs), based on experimental electrophysiological data. We aim to study how in vitro and in silico hiPSC-CMs can model this syndrome. We also provide a comparison with one state-of-the-art model of adult cardiac cell.

The control model showed (simulations vs experiments) (i) AP amplitude: 94.0 vs 86.0+/-1.4 mV, (ii) maximum diastolic potential: -64.0 vs -61.4+/-1.4 mV, (iii) APD90: 458.3 vs 434.0+/-31.1 ms and (iv) rate of spontaneous beating: 67.6 vs 69.1+/-11.3 bpm. In simulations, the LQT3 I_{Na} induced the experimentally observed APD prolongation (APD_{90} +32.3%) and rate slowdown (-33.0%). By simulating the administration of 50 μM of mexiletine in the patient model, the effect of the mutation was partially compensated, resulting in an APD_{90} shortening (-16.0%), in agreement with the experiments (~-20%). Finally, by simulating a 5-fold I_{Na} late increment in the O'Hara-Rudy adult model we got APD prolongations similar to those reproduced by our LQT3 model, APD_{30} (+30.9%), APD_{50} (+33.6%), APD_{70} (+34.1%) and APD_{90} (+30.2%).

Our results show that hiPSC-CMs and computational models derived from their electrophysiological traces represent in vitro and in silico models comparable to adult cardiomyocytes for LQT3, suitable for personalized studies on this pathology.

1. Introduction

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) are increasingly proving to be valuable *in vitro* model for specific pathologies: until now they have been used to reproduce many long QT (LQT) syndromes, namely LQT1, LQT2 and LQT3, as well as other pathologies such as catecholaminergic polymorphic ventricular tachycardia (CPVT) [1]. The focus of this work is the LQT3 syndrome, caused by the mutation of the SCN5A gene, encoding for the Na^+ channels. Its macroscopic effect includes gain-of-function of the Na^+ current (I_{Na}), which results in a sustained late Na^+ current (I_{NaL}) and a slower repolarization of the action potential (AP). Consequently, the AP duration (APD) and the ST segment of the ECG are prolonged. The aim of this work is developing an *in silico* model of control and patient hiPSC-CM based on the data reported in [2] to assess how this syndrome affects the hiPSC-CM AP and to compare it to the adult AP. Moreover, we validate the patient model against experiments of administration of mexiletine and the consequent reduction of the APD.

2. Methods

To develop the control and the LQT3 models we used the same approach detailed in [3]: (i) formulating the control and the mutated I_{Na} according to the experimental data in [2]; (ii) replacing the control I_{Na} in the ventricular-like control Paci2013 model [4]; (iii) tuning the other ionic currents to reproduce the control ventricular-like AP in [2] and finally (iv) replacing the control I_{Na} by the mutated one for testing the effects of the LQT3 I_{Na}. Thus as in our LQT1 study [3] the control and the LQT3 models differ only for the formulation of I_{Na}.

2.1. Experimental and simulated I_{Na}

The available I_{Na} data reported in [2] include: (i) I_{Na} peak current (Fig. 1); (ii) the steady state activation (Fig. 2) and deactivation (Fig. 3) curves; (iii) the recovery from inactivation curve (Fig. 4) and finally (iv) the quantification of I_{NaL}, defined as the sustained component of I_{Na} recorded at 200 ms. The aforementioned figures also include our curve fittings. A significantly large I_{NaL} is distinctive of the patient hiPSC-CMs (control *vs* patient:

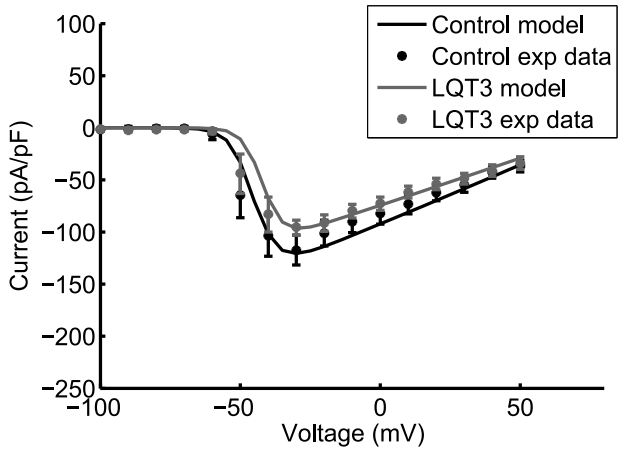

Figure 1. Experimental data (circles) and curve fitting (solid lines) of peak I_{Na}, in control and patient hiPSC-CMs. No significant differences were found between the I_{Na} peak currents from control and patient. Experimental data reproduced from Ma *et al.* [2].

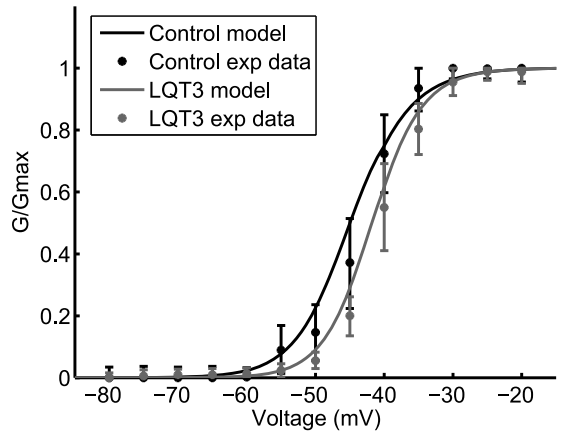

Figure 2. Experimental data (circles) and curve fitting (solid lines) of I_{Na} steady-state activation curve in control and patient hiPSC-CMs. No significant differences were found in V_h and slope of the activation. Experimental data reproduced from Ma *et al.* [2].

0.65±0.11 vs 3.16±0.27 pA/pF). Moreover, significant differences were found in the half-maximal potential (V_h) of the steady state inactivation and in the time constant of the recovery from inactivation. No significant differences were found in the peak I_{Na} and in the steady state activation. Since no I_{NaL} was included in the Paci2013 model [4], we took its basic formulation from the O'Hara-Rudy model (ORd) and tuned it to reproduce the I_{NaL} current measurements reported in Fig. 5 (first and second column).

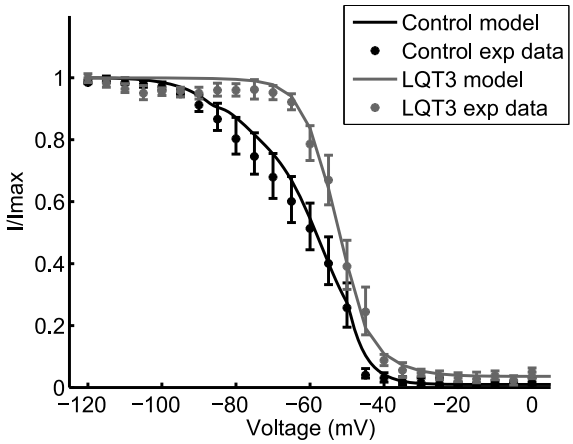

Figure 3. Experimental data (circles) and curve fitting (solid lines) of I_{Na} steady-state inactivation curve in control and patient hiPSC-CMs. V_h is significantly shifted towards positive potentials in patient hiPSC-CMs (control *vs* patient: -61 *vs* -45 mV). Experimental data reproduced from Ma *et al.* [2].

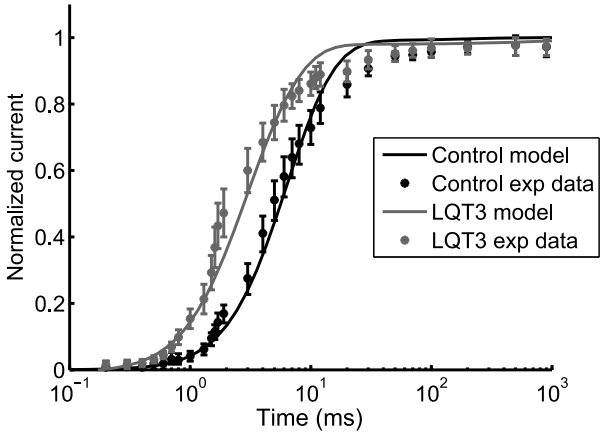

Figure 4. Experimental data (circles) and curve fitting (solid lines) of I_{Na} recovery from inactivation curve in control and patient hiPSC-CMs. Recovery was observed to be significantly faster in patient hiPSC-CMs (fast component in control *vs* patient: 6.4 *vs* 3.0 ms). Experimental data reproduced from Ma *et al.* [2].

2.2. Tuning the other ionic currents in the control model

To obtain a control model representative of the ventricular-like data in [2] (Table 1) and to compensate the different rate of spontaneous electrical activity (Rate) and maximum diastolic potential (MDP), we needed to tune our original model [4] in a similar way to [3]. All the model changes are reported in Table 2.

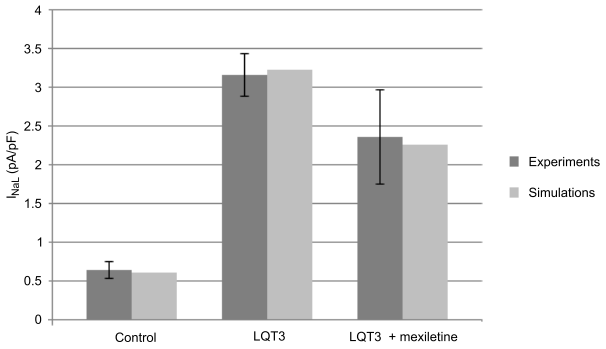

Figure 5. Experimental and simulated quantification of I_{NaL}. Experimental data reproduced from Ma *et al.* [2].

Table 1. Comparison of the Ma2011 (Ma *et al.* [5]) and the Ma2013 (Ma *et al.* [2]) datasets of AP morphological features for ventricular-like hiPSC-CMs. Mean values ± SE are reported. §:in Ma2013 APA is measured from the AP threshold to the peak voltage, and not from MDP.

AP feature	Ma2011 [5]	Ma2013 [2]
Rate (bpm)	35±2	69±11
MDP (mV)	-76±1	-61±1
Vmax (V/s)	27.8±4.8	13±5
APD_{90} (ms)	415±22	434±31
APA (mV)	104±1	86±1§

Table 2. Parameter changes with respect to the original hiPSC-CM model [4].

Current	Parameter change	
I_f	G_f	× 1.2
I_{CaL}	G_{CaL}	× 1.5
I_{Kr}	G_{Kr}	× 0.9
I_{K1}	G_{K1}	× 0.5
	$shift_{K1}$	= 8 (mV)
I_{NaCa}	k_{NaCa}	× 0.3
I_{NaK}	k_{NaK}	× 0.6
I_{pCa}	G_{pCa}	× 2.5

2.3. Simulation of mexiletine in the patient model

Mexiletine administration was simulated by reducing the maximum conductances of I_{Na} (fast and late) and of the L-type Ca^{2+} current (I_{CaL}) [6]. I_{Na} block was estimated by the experimental data in [2] (I_{NaL}, control *vs* patient: 0.75±0.18 *vs* 2.36±0.61 pA/pF). I_{CaL} block was estimated for 50 µM concentration as 40% from the dose-response curve fitted from the experiments of Ono *et al.* [7] (experimental doses: 10, 30, 100 µM; blockade: 23%, 28.9%, 55.4%). Mexiletine simulation was performed from steady state by reducing I_{Na} and I_{CaL} by 32% and 40%, respectively.

3. Results

Illustrative control and patient spontaneous APs are reported in Fig. 6. In Table 3 we compare the experimental and simulated AP morphological features: both models are able to reproduce all the AP features subjected to the most significant changes due to the LQT3 syndrome. The main effect of the mutation consists in a marked prolongation of the APD (APD_{20}:+22.2%, APD_{50}:+33.4%, APD_{70}:+37.0% and APD_{90}:+32.3%), and a slower Rate (-33.0%). All these changes are in agreement with experiments. Moreover, simulations qualitatively reproduce also the greater APA in patient hiSPC-CMs and the unchanged MDP. Administration of mexiletine (Fig. 7) resulted in a -18% reduction of APD_{90} in the patient model, comparable to the experimentally recorded ~-20%. In Fig. 7 we show how the new models reproduce the experimental I_{NaL} densities in the following 3 conditions: (i) control, (ii) patient and (iii) patient with mexiletine. Finally, we performed additional simulations aiming to compare our results to the response to an I_{NaL} increment in a state-of-the-art *in silico* adult model: the O'Hara-Rudy (ORd) model of human adult ventricular cell [8]. By simulating a 5-fold I_{NaL} increment in the ORd model, we obtained prolongations of APD similar to the ones reproduced by our LQT3 model (APD_{50}:+33.6%, APD_{70}:+34.1% and APD_{90}:+30.2%).

Table 3. Comparison between the control and patient AP features (experiments from Ma *et al.* [2], simulated values in brackets). *:$p < 0.05$.

AP feature	Control exp data (LQT3 exp data)	Control model (LQT3 model)
Rate (bpm) *	69±11 (41±8)	68 (45)
APD_{20} (ms) *	138±22 (229±32)	185 (226)
APD_{50} (ms) *	338±33 (541±55)	344 (459)
APD_{70} (ms) *	388±35 (561±61)	400 (546)
APD_{90} (ms) *	434±31 (645±69)	458 (606)
MDP (mV)	-61±1 (-61±3)	-64 (-65)
Vmax (V/s)	13±5 (10±2)	7.6 (8.2)
APA (mV)	86±1 (91±3)	94 (98)

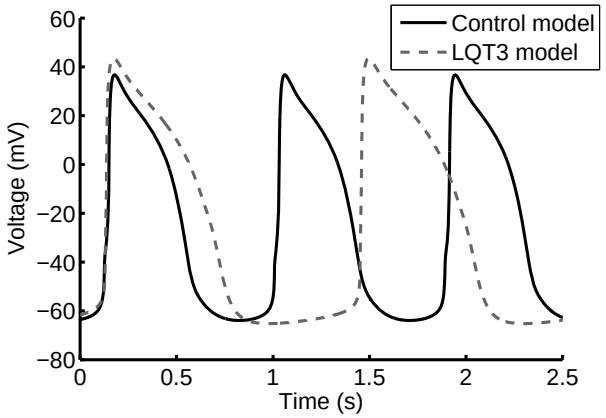

Figure 6. Simulated control and patient APs. Patient APs are characterized by slower spontaneous electrical activity and prolonged APD, compared to control APs.

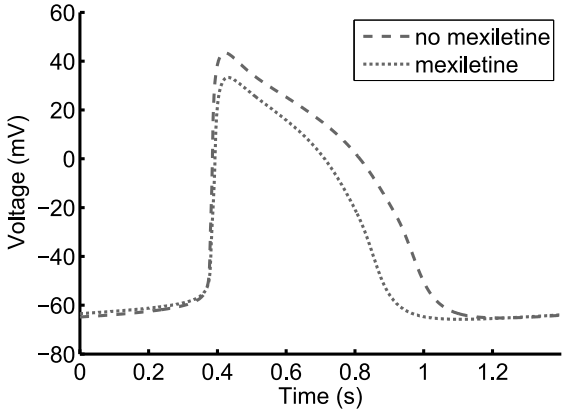

Figure 7. Simulated effects of mexiletine on patient APs. The main effect of this drug consists in a reduced APD.

4. Discussion and conclusions

In this paper, we propose a preliminary model of LQT3 syndrome in hiPSC-CMs, based on the recent experimental data by Ma *et al.* [2] from control and patient hiPSC-CMs. We aim to provide an *in silico* tool suitable to investigate the role of I_{Na} in LQT3 and in this specific cell type. Our simulations showed that both the control and the patient models reproduce all the AP features that significantly differ in control and patient hiPSC-CMs, i.e. APD at several percentages of repolarization and Rate. This is achieved by replacing only the control I_{Na} by the mutated one. Our models have limitations reproducing quantitatively some AP features, but they succeeded in simulating qualitatively the APA in patient cells while the MDP remained unchanged as in experiments. To respond to the current debate regarding the real value of hiSPC-CMs as models of adult cardiac cells, e.g. as *in vitro* arrhythmia models [9], we run additional simulations with the ORd model, offering a proof-of-concept comparison of the effects of the increment of I_{NaL} in hiPSC-CMs and adult cardiomyocytes. The APD prolongation resulted similar in the 2 models, e.g. APD_{50} (33.4% *vs* 33.6%) or APD_{90} (32.3% *vs* 30.2%). Our findings support the idea that hiPSC-CMs can be considered representative, or at least comparable, models for the specific LQT3 syndrome.

Acknowledgements

Michelangelo Paci was financially supported by Tekes, Finland (decision: 40346/11) and by the Finnish Cultural Foundation (Central Fund), Finland.

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