In silico Identification of the Key Ionic Currents Modulating Human Pluripotent Stem Cell-Derived Cardiomyocytes towards an Adult Phenotype

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Abstract

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) provide a promising resource for regenerative therapies. However, their immature phenotype and heterogeneities raise concern for arrhythmia when hiPSC-CMs are inserted into the native tissue. In silico models can improve understanding of the electrophysiological differences between hiPSC-CMs and adult cardiomyocytes and inform risk predictions.

Our aim is to conduct a sensitivity analysis to identify the main ionic currents determining differences in electrophysiological properties between the Paci2020 model (hiPSC-CMs) and the ToR-ORd model (human ventricular cardiomyocytes). Our simulations highlighted the fast Na^+ current as the key modulator of upstroke velocity, while the inward and rapid delayed rectifier K^+ currents mainly contributed to a decrease of diastolic potential and action potential duration, respectively.

In conclusion, we identified an increase of the conductance of these currents essential to modulate the biomarkers of the hiPSC-CM model towards the adult phenotype.

1. Introduction

Cardiovascular diseases are the leading cause of mortality worldwide. Since heart self-regeneration after infarction is limited, efficient alternative strategies to reduce mortality are needed. Therapies with human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have been explored for cardio-protection, i.e. to prevent remodelling shortly after infarction, as well as for cardio-restoration, i.e. to reverse remodelling and restore cardiac function in the later stages of cardiac injury[1]. Despite their promising regenerative capabilities, hiPSC-CMs are predominately immature in their properties, structure and function compared with adult cardiomyocytes (CMs)[2]. From an electrophysiological point of view, ventricular-like hiPSC-CMs have an action potential (AP), characterised by slow upstroke velocity, prolonged AP duration (APD), depolarised diastolic potential and spontaneous beating[3]. These properties and heterogeneities between hiPSC-CMs and adult CMs give concern for arrhythmia when introducing hiPSC-CMs into the native tissue.

Computer models (*in silico*) are recognised as a powerful tool for investigations into arrhythmia mechanisms, drug safety studies[4] and to support clinical decisions[5]. They provide a fast and effective alternative to experimental models and present the unique opportunity to test in simulations the effects of cellular changes on heart electrophysiology. Therefore, they constitute the perfect tool to characterise the electrophysiological properties of hiPSC-CMs and their differences with adult CMs, to predict potential arrhythmia caused by their interaction.

In this paper we present a sensitivity analysis of the latest biophysically-detailed hiPSC-CM Paci model[6] (Paci2020), to identify the contribution of the different ionic currents to the modulation of AP biomarkers towards an adult phenotype.

2. Methods

Simulations were performed using the Paci2020 model[6] of hiPSC-CMs and the ToR-ORd model[7] representing the adult ventricular phenotype. Both models were paced at 1Hz at 37°C until steady state and the last AP was analysed. We computed the values of four biomarkers: maximum diastolic potential (MDP), peak voltage (V_{peak}), maximum upstroke velocity (dV/dt_{max}) and APD at 90% of repolarisation (APD_{90}).

The sensitivity analysis of Paci2020 focused on the conductances of eleven ionic currents: fast and late Na^+ currents (I_{NaF}, I_{NaL}), transient outward K^+ current (I_{to}), rapid and slow delayed outward rectifier K^+ currents (I_{Kr}, I_{Ks}), inward rectifier K^+ current (I_{K1}), L-type Ca^{2+} cur-

rent (I_{CaL}), Na^+/Ca^{2+} exchanger (I_{NaCa}), Na^+/K^+ pump (I_{NaK}), SERCA pump (I_{up}) and funny current (I_f). All these currents are present in both Paci2020 and ToR-ORd, except I_f, which is not expressed in adult cardiomyoctyes. Current conductances (defined as G_x, for current I_x) were scaled by $\{0, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10\}$ of their baseline values. We included a large range of scaling factors to account for considerable discrepancies in current expression between hiPSC-CMs and adult CMs.

To summarise the results of the sensitivity analysis, we computed the relative sensitivities, as described in [8], using the biomarkers simulated for scaling factors 2 and 0.5, and compared with their baseline values. Relative sensitivities vary between -1 (strong negative correlation) and +1 (strong positive correlation).

3. Results

3.1. AP and ionic current comparison of baseline Paci2020 and ToR-ORd

Figure 1 shows the APs and the main ionic currents for the baseline Paci2020 and ToR-ORd models. In the top panel, we can see that compared to ToR-ORd, the AP in Paci2020 displays a slower upstroke (dV/dt_{max}, 118 vs 349 V/s, Paci2020 vs ToR-ORd), depolarised MDP (-74 vs -89 mV), longer AP (APD_{90}, 481 vs 273 ms) and higher V_{peak} (35 vs 33 mV). All current peaks were found lower in Paci2020 compared to ToR-ORd, except for I_{Ks} which has a similar amplitude (+3%, Paci2020 vs ToR-ORd). The currents whose peaks show the largest differences are shown in the remaining panels of Figure 1: I_{NaF} (-62%), I_{NaL} (-80%), I_{to} (-88%), I_{Kr} (-59%) and I_{up} (-95%). I_{CaL}, I_{NaCa}, I_{NaK} and I_{K1} peaks are also lower in Paci2020, but to a lesser extend (-35%, -26%, -32%, -10%, respectively).

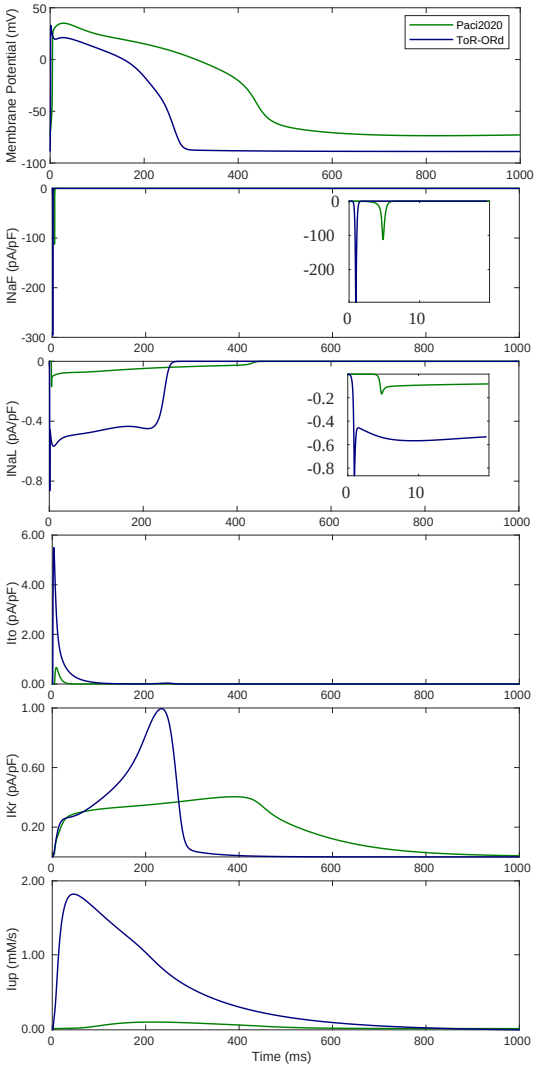

Figure 1. Simulated AP and selection of current traces for Paci2020 (green) and ToR-ORd (blue).

3.2. Sensitivity analysis

Figure 2 summarises the sensitivity analysis results of Paci2020, which was performed to identify the ionic currents key in modulating hiPSC-CM biomarkers. The values in Figure 2 correspond to the relative sensitivities, computed as described in the Methods section.

The MDP is very stable to changes in current conductances and mainly modulated by G_{K1} and G_{Kr} (10% and 3% MDP decrease for two-fold increase of G_{K1} and G_{Kr}). dV/dt_{max} is mainly modulated by G_{NaF}. A two-fold increase in G_{NaF} caused a considerable increase in dV/dt_{max} (+78%) and V_{peak} (+42%). This is expected, since I_{NaF} is the main depolarising current in the upstroke phase. On the contrary, an increase of G_{CaL} caused a decrease of dV/dt_{max}. Our investigation concluded this is a consequence of reduced I_{Na} availability, caused by the longer APD, which in turn is caused by the increased I_{CaL}. Indeed, we observed a lower value for the I_{NaF} recovery gate at the start of the AP, when G_{CaL} is increased.

V_{peak} is increased when augmenting G_{NaF}, G_{CaL}, and G_{NaK}. On the contrary, higher G_{to}, G_{Kr}, G_{K1}, or G_{NaCa} reduce V_{peak}, since they are all outward currents in the initial AP phase. The APD is mainly shortened when increasing the outward K^+ currents, especially G_{Kr} and G_{K1} (APD_{90} -45% and -30% for a two-fold increase in G_{Kr} and G_{K1}, respectively). I_{Ks} does not significantly shorten the APD, since its magnitude is very small.

In summary, the AP biomarkers of the hiPSC-CM Paci2020 model are most sensitive to changes in G_{NaF}, G_{Kr}, G_{K1}, G_{CaL} and G_{NaK}. This is reflected in the changes observed in AP morphology when varying these conductances (Figure 3). The results of the sensitivity

	MDP	dV/dt_{max}	V_{peak}	APD_{90}
G_{NaF}		1.00	0.59	
G_{NaL}				
G_{to}			-0.15	
G_{Kr}	0.41	0.43	-0.27	-1.00
G_{Ks}				
G_{K1}	1.00	0.64	-0.16	-0.38
G_{CaL}	-0.10	-0.10	0.68	0.63
G_{NaCa}	0.13		-0.28	0.21
G_{NaK}		0.52	1.00	0.66
G_{up}				
G_f	-0.15	-0.21	-0.19	-0.13

Figure 2. Relative sensitivities computed from the biomarker changes observed in Paci2020 when scaling current conductances between 0.5 and 2. Darker shades of green/red correspond to larger positive/negative relative sensitivity. Values below +/-0.1 are not shown.

analysis for I_{CaL} and I_{K1} agree with the sensitivity analysis of one of the previous Paci models[9]. Moreover, our simulation provided additional insights into the role of I_{NaK}, I_{NaL} and I_{up} in hiPSC-CMs.

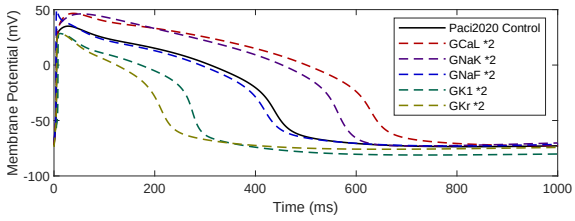

Figure 3. Changes in hiPSC-CM AP when increasing G_{CaL}, G_{NaK}, G_{NaF}, G_{K1} and G_{Kr}: baseline (black solid line), G_{CaL}*2 (dashed red line), G_{NaK}*2 (dashed purple line), G_{NaF}*2 (dashed blue line), G_{K1}*2 (dashed green line) and G_{Kr}*2 (dashed yellow line).

3.3. I_{NaF}, I_{K1} and I_{Kr} as main drivers of hiPSC-CM versus adult cardiomyocyte differences

Based on the simulation results presented in Sections 3.1 and 3.2, we investigated if by scaling the current conductances in Paci2020 to match the current peaks of ToR-ORd we can modulate the hiPSC-CMs biomarkers towards the adult phenotype.

Results are summarised in Figure 4, showing the ratio between Paci2020 biomarkers and peak currents and their corresponding values for ToR-ORd. For each of the currents included in Figure 1 (I_{NaF}, I_{NaL}, I_{to}, I_{Kr} and I_{up}), we chose the scaling factor that brings the current peak closer to the one of ToR-ORd. We also included the results obtained with a two-fold increase in I_{K1} conductance, due to its strong effect on MDP, and by removing I_f, which is not present in ToR-ORd.

Biomarker comparison between the baseline models highlighted that the simulated AP in Paci2020 has a depolarised MDP (-17%), a lower dV/dt_{max} (-66%), a slightly higher V_{peak} (+7%), and a significantly prolonged APD_{90} (+76%), compared to the AP obtained with ToR-ORd. This mostly agrees with experimental results[3].

A three-fold increase in G_{NaF} in Paci2020 shifts dV/dt_{max} closer to the value obtained with the ToR-ORd model, reducing the difference to –30%, Paci2020 vs ToR-ORd. It also slightly decreases the APD_{90} (-13%). However, V_{peak} moves further away from ToR-ORd (+76%). Increased G_{to} and G_{Kr} strongly reduce V_{peak}, but also shorten the APD_{90}, bringing it closer to the ToR-ORd value (+49 and -3%, respectively). G_{NaL} and G_{up} increases bring the I_{NaL} and I_{up} current peaks closer to ToR-ORd, but do not significantly affect any biomarker, as expected from the results in Figure 2. Doubling I_{K1} contributes to depolarise the MDP in Paci2020, decreasing the difference to the MDP in ToR-ORd from –17% to –9%. It also decreases V_{peak} to 28.5 mV and moves APD_{90} closer to ToR-ORd (from +76% to +24%). Scaling G_f to 0 had almost no effect on the biomarkers, but we verified that this inhibits spontaneous beating in unpaced conditions. In addition to the changes in biomarkers, Figure 4 also shows the effect on the scaled current peaks and differences to ToR-ORd, which were significantly reduced: I_{NaF} (from –62% to –19%), I_{NaL} (from –80% to –18%), I_{to} (from -88% to –17%), I_{Kr} (from –59% to –23%) and I_{up} (from –95% to +4%).

Based on these results, we identified the increase of the following currents as essential in modulating Paci2020 towards the ToR-ORd phenotype: I_{NaF}, for its effect on dV/dt_{max}, I_{K1}, for its effect on MDP, and I_{Kr}, for its effect on APD_{90}.

	$I_{peak,Baseline}$	$I_{peak,Scaled}$	MDP	dV/dt_{max}	V_{peak}	APD_{90}
Baseline			0.83	0.34	1.07	1.76
G_{NaF} *3	0.38	0.81	0.81	0.70	1.76	1.54
G_{NaL} *5	0.20	0.92	0.82	0.33	1.22	2.06
G_{to} *8	0.12	0.83	0.85	0.34	0.68	1.49
G_{Kr} *2	0.41	0.77	0.85	0.34	0.85	0.97
G_{up} *6	0.05	1.04	0.84	0.35	1.16	1.86
G_{K1} *2	0.90	1.79	0.91	0.36	0.86	1.24
G_f *0	N/A	N/A	0.86	0.40	1.17	1.97

Figure 4. Ratio between Paci2020 and ToR-ORd ionic current peaks and AP biomarkers for selected current conductance scalings. Shades of blue indicate how close the values are to an adult phenotype (ratio equal to 1). The first 2 columns show the current peak ratios with the baseline values on the left, while the last 4 columns show the AP biomarker ratios, with the baseline values at the top.

4. Discussion

In this study, we compared AP biomarkers and currents in the hiPSC-CM Paci2020 model with the ones of the human adult CM ToR-ORd model. Next, we performed a sensitivity analysis of Paci2020 to identify the main ionic currents determining its modulation from hiPSC-CM to adult CM, considering ToR-ORd as benchmark.

We showed that I_{NaF}, I_{NaL}, I_{to}, I_{Kr} and I_{up} current peaks are significantly lower in Paci2020 than in ToR-ORd. In partial agreement with this, lower expression of I_{Na}, I_{to} and I_{Kr} has been reported experimentally [10,11].

Studies hypothesised that the main cause for the more depolarised MDP in hiPSC-CMs is a significant underexpression of I_{K1}[12]. Our *in silico* comparison was not able to confirm this and instead found comparable current peaks in Paci2020 and ToR-ORd. However, a recent review by Paci et al. highlights that I_{K1} in hiPSC-CMS is not necessarily lower than in human adult CMs[13].

Our investigation concluded that increased I_{NaF} was the main driver to increase dV/dt_{max} towards the ToR-ORd phenotype. We also showed that increased I_{K1} and I_{Kr} contribute to a lower MDP and shorter APD_{90}, respectively. This agrees with [12] where it was hypothesised that increasing I_{K1} could improve MDP and reduce pro-arrhythmia of hiPSC-CMs.

Based on these results, future work may investigate the combinations of different scaling factors to modulate Paci2020 from hiPSC-CM to an adult phenotype, through automated optimisation methods.

5. Conclusion

In this study, we performed a sensitivity analysis of an *in silico* model of hiPSC-CM and highlighted the contribution of the single ionic currents to AP biomarker changes. We concluded that increased I_{NaF}, I_{K1} and I_{Kr} are essential to modulate the hiPSC-CM model towards an adult phenotype.

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References

[1] Terzic A, Behfar A. Regenerative heart failure therapy headed for optimization. Eur Heart J May 2014; 35(19):1231–1234.

[2] Machiraju P, Greenway SC. Current methods for the maturation of induced pluripotent stem cell-derived cardiomyocytes. World J Stem Cells Jan. 2019;11(1):33–43.

[3] Ma J, Guo L, Fiene SJ, et al. High purity human-induced pluripotent stem cell-derived cardiomyocytes: electrophysiological properties of action potentials and ionic currents. Am J Physiol Heart Circ Physiol Nov. 2011;301(5):H2006–H2017.

[4] Passini E, Britton OJ, Lu HR, et al. Human in silico drug trials demonstrate higher accuracy than animal models in predicting clinical pro-arrhythmic cardiotoxicity. Front Physiol Sep. 2017;8:668.

[5] Morrison TM, Pathmanathan P, Adwan M, et al. Advancing regulatory science with computational modeling for medical devices at the FDA's office of science and engineering laboratories. Front Med Sep. 2018;5:241.

[6] Paci M, Passini E, Klimas A, et al. All-optical electrophysiology refines populations of in silico human iPSC-CMs for drug evaluation. Biophys J May 2020;118(10):2596–2611.

[7] Tomek J, Bueno-Orovio A, Passini E, et al. Development, calibration, and validation of a novel human ventricular myocyte model in health, disease, and drug block. Elife Dec. 2019;8:e48890.

[8] Romero L, Pueyo E, Fink M, et al. Impact of ionic current variability on human ventricular cellular electrophysiology. Am J Physiol Heart Circ Oct. 2009;297(4):H1436–H1445.

[9] Paci M, Hyttinen J, Rodriguez B, et al. Human induced pluripotent stem cell-derived versus adult cardiomyocytes: an in silico electrophysiological study on effects of ionic current block. Br J Pharmacol Aug. 2015;172(21):5147–5160.

[10] Garg P, Garg V, Shrestha R, et al. Human induced pluripotent stem cell-derived cardiomyocytes as models for cardiac channelopathies: A primer for non-electrophysiologists. Circ Res Jul. 2018;123(2):224–243.

[11] Blinova K, Stohlman J, Vicente J, et al. Comprehensive translational assessment of human-induced pluripotent stem cell derived cardiomyocytes for evaluating drug-inuced arrhythmias. Toxicol Sci Jan. 2016;155(1):234–247.

[12] Goversen B, van der Heyden MAG, van Veen TAB, et al. The immature electrophysiological phenotype of iPSC-CMs still hampers in vitro drug screening: Special focus on IK1. Pharmacol Ther Mar. 2018;183:127–136.

[13] Paci M, Penttinen K, Pekkanen-Mattila M, et al. Arrhythmia mechanisms in human induced pluripotent stem cell–derived cardiomyocytes. J Cardiovasc Pharmacol Mar. 2021;77(3):300–316.

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