Mutation-specific Hypertrophic Cardiomyopathy and Mavacamten: a Mechano-energetic In Silico Study

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Abstract

Mavacamten is the first drug with proven efficacy for hypertrophic cardiomyopathy (HCM), the most prevalent genetic cardiac disorder. Our goal is to investigate the pathophysiology of the R403Q HCM mutation and the capability of Mavacamten to ameliorate the impaired cellular mechano-energetics. We incorporated a reparametrized metabolic-sensitive contractile element (CE) into our model of human induced pluripotent stem cell derived cardiomyocyte (hiPSC-CMs) electromechanics relating the effects of MgATP, MgADP, inorganic phosphate, and ATP hydrolysis-derived proton to the tension development. Our results suggest that the prolonged contractile relaxation duration, observed in vitro, due to R403Q mutation (~33%) is captured by a variant of the metabolic-sensitive CE without assuming additional fluxes to the thin filaments. Further, our HCM model could correctly predict the unaltered ATPase activity and ~40% increase in fractional shortening (FS) in R403Q mode. In R403Q mode, our model simulates the improved FS, contractile relaxation, and ATPase rate due to 0.5 μM Mavacamten: 14.6%, 21%, and 19.3%, respectively, consistent with the experiments. This work is a step toward robust computational models of cardiac electro-mechano-energetic coupling for pharmacological investigations on sarcomeric cardiomyopathies.

1. Introduction

HCM is mostly caused by pathogenic mutations hosted by myosin binding protein C, coded by the MYBPS3 gene, and adult cardiac myosin isoforms, coded by MYH7, in the sarcomere [1]. As the crossbridge (XB) cycling and function of sarcomeres comprise a predominant portion of the cardiac energy consumption [2], the impairment due to the sarcomeric mutations requires metabolic-wise analyses in studying the function of diseased human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs).

Correspondingly, considering energetics in the drug effect analysis is also vital as the promising drugs introduced for HCM are designed to directly target XB cycling, such as the myosin ATPase activity inhibitor Mavacamten [3].

The mechanism of action of Macacamten and its effect on HCM mutations is under active research [1,4]. On the other hand, the computational studies on the effect of Mavacamten and pathophysiology of HCM, specifically R403Q, are not many. In a notable study, Margara et al. simulated the effect of 0.5 μM Mavacamten on the mechanical response of ToR-ORd+Land model [5] of human adult cardiomyocyte electromechanics [6]. Of note, to simulate the improved active tension impaired relaxation due to Mavacamten, they hypothesized an additional XB-to-thin-filament feedback [6]. This inspired us to further investigate Mavacamten mechanism of action, considering an *in silico* hiPSC-CM model with a metabolic-sensitive thermodynamically constrained contractile element (CE), built upon our previous model [7]. This extended model considers the dynamics of MgADP, inorganic phosphate (Pi), and ATP hydrolysis-derived proton in the XB cycling interactions enabling us to incorporate the level of energetics affected in R403Q HCM pathological condition and the administration of Mavacamten. The newly developed model, hiMCE, is benchmarked against the available experimental data on Mavacamten and R403Q HCM.

2. Methods

2.1. The metabolic-sensitive extension of the model

We reparametrized and integrated the metabolic-sensitive CE by Tran et al. [8] into the Paci2020 model of hiPSC-CMs electrophysiology [9] (Fig. 1) to simulate biomarkers of action potential (AP), Ca^{2+} transients (CaT), and active tension (AT) reported for hiPSC-CMs, as the CE was originally calibrated for guinea pig data. The CE consists of a non-permissive and permissive XB binding states, N and P, respectively. The latter is in equilibrium with two strongly-bound phases indicating myosin heads pre-rotation, XB_A, and the force generating state denoting bound myosin heads post-rotation XB_B.

2.2. In silico simulation in control and HCM conditions

For the control hiMCE model, we proceeded similarly as in [6], by introducing into the XB cycling the DRX:SRX ratio between the disordered relaxed state DRX, which defines the myosins available to drive the contraction, and the recently observed super relaxed SRX state, which tunes the myosin head availability.

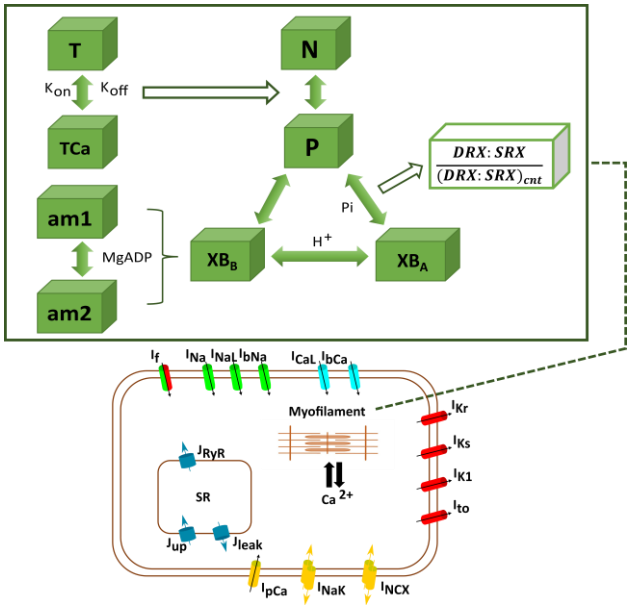

Figure 1. The model of hiPSC-CM electromechanics with the metabolic-sensitive contractile element. The metabolites and myosin DRX:SRX ratios have been embedded in the crossbridge cycling. DRX:SRX represents the disturbed relaxed state (DRX) over the super relaxed state (SRX) ratio. T: troponin, TCa: Ca^{2+} bound troponin. N: non-permissive state where XB formation is prevented. P: permissive state where XB formation is permitted. XB_A: pre-rotation strongly-bound state. XB_B: post-rotation state of strongly bound state. The strongly-bound substates in rapid equilibrium that equally contribute to the tension generation are am1 and am2 and we assumed MgADP binds to am1 [8].

The DRX:SRX ratio represented by F1 and F2 (with default values of 1) modulates the XB in our model as:

$$\frac{d}{dt}XB_{preR} = ap1 \times F1 \times P_{XB} - am1 \times F2 \times XB_{preR} - ap2 \times XB_{preR} + am2 \times XB_{postR} \quad (1)$$

where ap1 and ap2 affect the XB detachment, and am1 and am2 (two strongly-bound substates) affect Pi- and MgATP-related metabolic regulations in the XB cycling, respectively [8].

In HCM hiMCE model, in addition to modifying F1 and F2 values, we altered the values of metabolites according to the increase in Pi and ADP by-products, reported for R403Q-induced HCM [10]. Thus, maintaining the consistency in the conservation of phosphate and creatin reaction [8], we changed the energetic parameters to obtain the HCM variant of the model as given in Table 1.

Table 1. The values of parameters in control and the HCM variant of the model. Pi_ref is the inorganic phosphate (Pi) reference value and ap2 influences the rapid XB detachment. F1 and F2 are constants affecting pre-rotational states in XB cycling, as in [6].

Parameter	Control hiMCE	HCM hiMCE
Pi_ref (mM)	2	18.9
MgADP (mM)	0.036	0.072
ap2 coef.	1	0.315
F1	1	1.3
F2	1	1.3

2.3. In silico simulation of Mavacamten

In [6], Mavacamten modulates the transitions between XB_A and P states by altering the parameters representing DRX:SRX. We also altered other XB parameters to accurately simulate the effect of 0.5 µM Mavacamten on Ca^{2+} signalling [11], the ATPase rate [3], and Pi [12] (Table 2), obtaining the HCM-MAVA hiMCE model.

Table 2. Altered parameters of the contractile element to simulate the effect of 0.5 µM Mavacamten on the R403Q variant of the model. K_{on} is the constant rate affecting Ca^{2+} binding to troponin, n_{perm} the hill coefficient affecting nearest neighbor cooperativity in nonpermissive to permissive transitions, C1 to C5 the constants influencing XB cycling rates transitions including Pi- and MgADP-dependent regulations.

Parameter	Control hiMCE	HCM-MAVA hiMCE
K_{on} (mM^{-1} s^{-1})	62.5×10^3	65.5×10^3
n_{perm}	11.55	7.95
C1	1	0.26
C2	1	0.4
C3	1	5.4
C4	1	0.4
C5	1	2.39
ap1 coef.	1	1.45
ap3 coef.	1	0.28
F1	1	0.1
F2	1	0.1

Of note, C1 modulates P to XB_A transition, C2 affects Pi-dependent XB_A to P transition, C3 influences XB_A to

XB_B transition, C4 modulates proton-dependent XB_B to XB_A transition, and C5 affects MgATP-dependent transition from XB_B to P states.

3. Results

3.1. Validation of the metabolic-sensitive model

As Table 3 and Figure 2 show, the new hiMCE model successfully simulates key AP, CaT and AT biomarkers within their experimental ranges [7].

Table 3. Simulated *vs* in vitro biomarkers (non-paced condition). APA: AP amplitude, MDP: max diastolic potential, dV/dt max: max upstroke velocity, APD_{90}: AP duration at 90% of repolarization, CaT Dur: duration of Ca^{2+} transient, $tRise_{10,\ peak}$: rise time from 10% of max threshold to peak CaT, AT: active tension, RT_{50}: time from peak contraction to 50% of relaxation, %FS: fractional sarcomere shortening.

Biomarker	hiMCE	Exp. range
APA (mV)	103	104±6
MDP (mV)	-75.0	-75.6±6.6
dV/dt max (V/s)	24	27.8±26.3
APD_{90} (ms)	403	415±119
CaT Dur. (ms)	693	805±188
Cat $tRise_{10,50}$ (ms)	102	82.9±50.5
AT magnitude (kPa)	0.055	0.055±0.009
RT_{50} (ms)	158	158±12.1
%FS	3.23	3.27±0.37

3.2. R403Q HCM and Mavacamten; the mechano-energetics

Based on the model reparameterizations for Mavacamten and HCM (Tables 1&2), we simulated the electro-mechano-energetic results (Fig. 3). Interestingly, the level of energetics included in the CE responds to the HCM pathological variant capturing 33% prolonged contractile relaxation (Fig. 3C) in accord with hiPSC-CM experimental data [1]. Further, the insignificant changes in the CaT and ATPase rate due to R403Q HCM (Fig. 3B,D) and ~40% elevated fractional sarcomere shortening (Fig. 3F) were captured consistent with the hiPSC-CM experimental reports [1,13,14]. Moreover, the simulated reduction in the ATPase rate, 19.3%, due to Mavacamten lies within the experimental range [17.9, 28.5]% reported for bovine [4] (Fig. 3D). Of note, the metabolic-sensitive model CaT was not affected by Mavacamten, in agreement with animal experimental data [3]. The amended prolonged tension relaxation (21%, Fig. 3C) and fractional cell shortening (14.6%, Fig. 3F) quantitatively follow the experiments on 0.5 µM Mavacamten effect on R403Q HCM hiPSC-CMs [1].

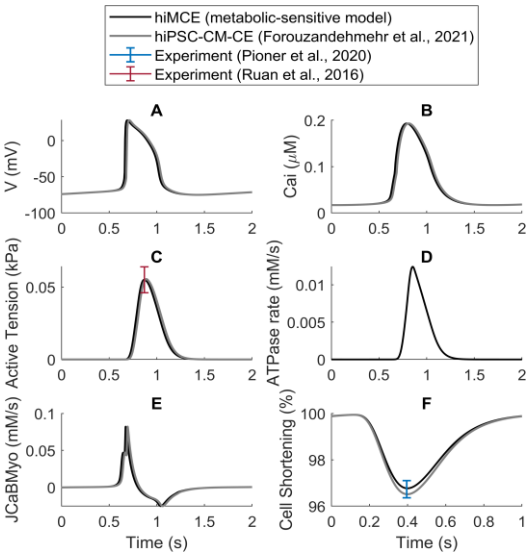

Figure 2. Key outcomes of the hiMCE, its predecessor hiPSC-CM-CE, and the experimental ranges. A: action potentials, B: Ca^{2+} transients, C: active tensions, D: ATPase activity, E: Ca^{2+} flux toward myofilament, and F: fractional cell shortening.

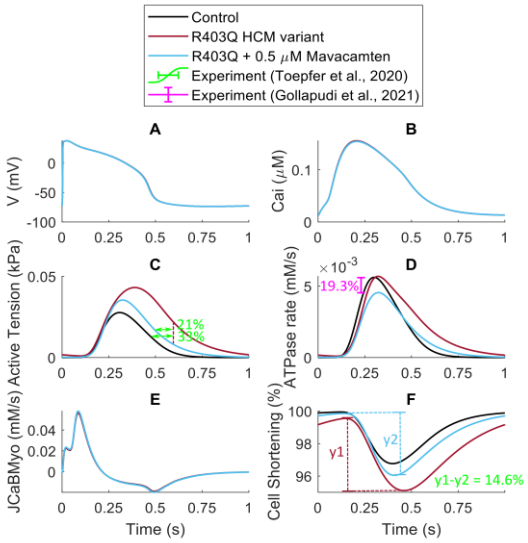

Figure 3. Key electro-mechano-energetic simulations by hiMCE in control and HCM conditions (with and without Mavacamten). A: action potential, B: Ca^{2+} transients, C: active tensions, D: ATPase activities, E: Ca^{2+} flux toward myofilament, F: fractional cell shortenings.

4. Discussion

The most significant portion of the consumed energy by heart is associated with sarcomere contractions [2]. Thus, metabolic investigations on the pathogenic

mutations affecting the function of cardiac contractile units become highly important. Therefore, the drug induced modelling here took a step further to also comply with the metabolic experimental findings reported for the effect of Mavacamten. Specifically, the prolonged relaxation simulated in R403Q HCM was previously suggested to depend on additional feedback to actins [6]. Here, that same change in function was achieved by focusing on the calibration of Pi, MgADP, altered DRX:SRX ratio, and an altered XB rapid detachment (ap2 in Table 1). Furthermore, the novel inclusion of C1 to C5 in Mavacamten calibration of the CE was suggested based on an unbalanced actomyosin signaling influencing the force-generating XB states in R403Q HCM etiology [15]. Markedly, this infers the effect of Mavacamten might not be only limited to the disturbed DRX:SRX ratio considered at play between permissive and pre-force generating bound XB states. Explicitly, Mavacamten might trigger a new interfilament balance modulating metabolic and force-generating terms that specifically affect the rapid XB detachment between the strongly bound pre-rotation and post-rotation states. Correspondingly, following experimental findings [3,11,12], we introduced Mavacamten calibration influencing the Pi-sensitive transition between the permissive XB state and strongly bound XB in the pre-rotation state, Ca^{2+} sensitivity of XB cycling, the MgADP-dependent dynamic at play between the permissive P state and the force generating XB_B state.

The limitations of this study include approximating the cooperative spatial protein dynamics with ODEs using a mean-field method [8] and the strong assumption that Mavacamten would restore the pathological values of metabolite to their baseline levels (although consistent with the drug mechanism of action).

In conclusion, this work demonstrated that the missing computational link in the accurate recapitulation of mutation-specific HCM, here R403Q, could be the energetic mechanisms involved in the XB cycling. This, together with the novel drug-induced calibration, are not only insightful in further understanding of mutation-specific HCM pathology, but also a step toward developing cardiac models of electro-mechano-energetic coupling for advanced drug trials.

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References

[1] C.N. Toepfer et al., Myosin sequestration regulates sarcomere function, cardiomyocyte energetics, and metabolism, informing the pathogenesis of hypertrophic cardiomyopathy, Circulation. 141 (2020).

[2] J.S. Ingwall, ATP and the heart, Springer US, Boston, MA, 2002.

[3] E.M. Green et al., A small-molecule inhibitor of sarcomere contractility suppresses hypertrophic cardiomyopathy in mice, Science (80-.). 351 (2016) 617–621.

[4] S.K. Gollapudi et al., Synthetic thick filaments: a new avenue for better understanding the myosin super-relaxed state in healthy, diseased, and mavacamten-treated cardiac systems, J. Biol. Chem. 296 (2021) 100114.

[5] F. Margara et al., In-silico human electro-mechanical ventricular modelling and simulation for drug-induced pro-arrhythmia and inotropic risk assessment, Prog. Biophys. Mol. Biol. 159 (2021) 58–74.

[6] F. Margara et al., Mavacamten efficacy in mutation-specific hypertrophic cardiomyopathy: an in silico approach to inform precision medicine, in: 2021 Comput. Cardiol., 2021: pp. 1–4.

[7] M. Forouzandehmehr et al., A mathematical model of hiPSC cardiomyocytes electromechanics, Physiol. Rep. 9 (2021).

[8] K. Tran et al., A Metabolite-sensitive, thermodynamically constrained model of cardiac cross-bridge cycling: implications for force development during ischemia, Biophys. J. 98 (2010).

[9] M. Paci et al., All-optical electrophysiology refines populations of in silico human iPSC-CMs for drug evaluation, Biophys. J. (2020).

[10] V. Sequeira et al., Energetic drain driving hypertrophic cardiomyopathy, FEBS Lett. 593 (2019) 1616–1626.

[11] P.O. Awinda et al., Effects of mavacamten on Ca 2+ sensitivity of contraction as sarcomere length varied in human myocardium, Br. J. Pharmacol. 177 (2020) 5609–5621.

[12] K. Alsulami et al., Small molecules acting on myofilaments as treatments for heart and skeletal muscle diseases, Int. J. Mol. Sci. 21 (2020) 9599.

[13] L.R. Sewanan et al., Extracellular matrix from hypertrophic myocardium provokes impaired twitch dynamics in healthy cardiomyocytes, JACC Basic to Transl. Sci. 4 (2019) 495–505.

[14] S.S. Sarkar et al., The hypertrophic cardiomyopathy mutations R403Q and R663H increase the number of myosin heads available to interact with actin, Sci. Adv. 6 (2020).

[15] S. Nag et al., Contractility parameters of human β-cardiac myosin with the hypertrophic cardiomyopathy mutation R403Q show loss of motor function, Sci. Adv. 1 (2015).

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