

Mavacamten Efficacy in Mutation-specific Hypertrophic Cardiomyopathy: an In-silico Approach to Inform Precision Medicine

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Abstract

Hypertrophic cardiomyopathy (HCM) is a common genetic heart disease characterised by hyperdynamic contraction and slowed relaxation. It has been proposed that cellular hypercontractility can derive from mutations that destabilise the energy-conserving myosin super relaxed state, SRX. A new drug, Mavacamten, has been shown to re-stabilise myosin SRX. Here we develop a human-based in-silico model to investigate how disease and drug-induced SRX changes alter cardiac contractility. We do this to mechanistically investigate how Mavacamten restores function in a HCM causing mutation. Our simulations show that hypercontractility is accounted for by an increased availability of crossbridges due to a reduced abundance of myosin SRX, but cellular diastolic dysfunction is only recapitulated if there is a direct crossbridge contribution to thin filament activation. Our model replicates reduced cellular contractility with Mavacamten treatment, which also rescues the hypercontractile phenotype in HCM. Our model demonstrates that Mavacamten is effective in correcting HCM abnormalities caused by mutations that destabilise SRX. However, genotypes that cause HCM via other molecular pathways may be incompletely salvaged by Mavacamten.

1. Introduction

Hypertrophic cardiomyopathy (HCM) is a common genetic heart disease, which can cause arrhythmias and heart failure [1]. Pharmacological therapy plays a key role in the clinical management of HCM, as of yet there are no therapies that specifically target disease mechanisms [2]. Mavacamten is the first drug developed to target HCM pathophysiology, with demonstrated efficacy for obstructive HCM [2,3]. The exact mechanisms that govern Mavacamten's mode of action are incompletely understood, and a deeper understanding is needed to guide effective and safe patient-specific therapy.

Recently a new state of myosin that regulates contractile function by tuning myosin head availability was observed in myocardium [4]. This myosin state was termed the super relaxed state (SRX) where myosin heads are sequestered

away from the actin thin filament. Broadly myosin SRX exists in a balance with the disordered relaxed state (DRX) which defines myosins that are available to drive contraction. Certain HCM variants decrease myosin SRX thereby increasing DRX myosin heads, which are able to interact with actin and drive the clinical hypercontractile phenotype [5]. Mavacamten has been shown to re-stabilise myosin SRX and reverse the cellular hallmarks of HCM [6].

Here we extend a coupled human electromechanical cardiomyocyte model [7] to incorporate myosin SRX/DRX. We do this to investigate how HCM and Mavacamten-induced changes in SRX alter cellular contractility. We benchmark our model using human experimental data and conduct a simulation study to mechanistically interrogate how Mavacamten restores cellular function in the HCM-causing β -myosin heavy chain variant MYH7^{R403Q/+}.

2. Methods

2.1. Baseline model

We extended our coupled ToR-ORd+Land model [7] of human electrophysiology, excitation-contraction coupling, and active contraction (Figure 1A) to include an explicit dependence of crossbridge formation on the myosin DRX:SRX ratio (Figure 1B). Our model is based on two key assumptions: 1) changes in DRX:SRX ratio modulate the number of functionally accessible myosin heads for interaction with actin; 2) Mavacamten shifts the myosin DRX:SRX ratio towards SRX [6].

A new parameter R (Figure 1B) was introduced in the model to represent the ratio $(\text{DRX:SRX})/(\text{DRX:SRX})_{\text{control}}$. In our modified model, R modulates the transitions between the states that represent: (i) unblocked myosin binding sites on actin without crossbridges bound (U), and (ii) bound crossbridges in the pre-powerstroke state (W). This means that crossbridges now enter in the pre-powerstroke state W based on both the actin binding sites available for crossbridge formation due to calcium activation U, and myosin heads functionally available for interaction with actin, modelled through the new parameter

R as follows:

$$\frac{dW}{dt} = k_{uw} * f_1(R) * U - k_{wu} * f_2(R) * W - k_{ws} * W - \gamma_{wu} * W$$

We impose the constraint $f_2(1) = f_1(1) = 1$, so that $R = 1$ at control conditions where $(DRX:SRX) = (DRX:SRX)_{control}$. We then quantified how changes in R affected simulated isometric twitch tension in intact cardiomyocytes and steady-state tension-calcium relationships in skinned cardiomyocytes. Optimal $f_1(R)$ and $f_2(R)$ were chosen based on their ability to replicate changes in contractility observed in experimental data. In particular we aimed to replicate the ~25-30% decrease in maximal tension with 0.5 μ M Mavacamten observed in human myocardium [8], and the ~40% increase in contractility caused by a 17% increase in DRX due to the pathogenic MYH7^{R403Q/+} mutation observed in human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) [6].

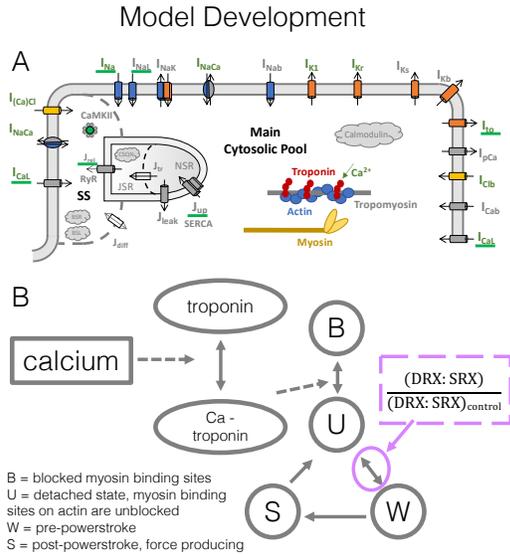


Figure 1. ToR-ORd+Land model of human cardiomyocyte electromechanical function with embedded description of myosin DRX:SRX. A: model schematic [7]. B: diagram of the active tension model. In pink the modifications introduced to simulate myosin DRX:SRX.

2.2. Control population

From our newly generated model incorporating myosin DRX:SRX ratios we constructed a control population of human cardiomyocytes. We first generated 2000 electromechanical models to account for cell heterogeneity due to variable expression of ion channels, pumps, and exchangers [9] as well as different levels of protein kinase A-mediated phosphorylation. The population was then

calibrated as in [9] with additional constraints on active tension duration at 50% and 95% decay.

2.3. MYH7^{R403Q/+} population

To investigate the pathogenic mechanisms of the HCM causing MYH7^{R403Q/+} variant we constructed an in-silico population of MYH7^{R403Q/+} cardiomyocytes by remodelling R to $R = 1.3$, as observed in experimental data [6]. The mechanical behaviours of the control and MYH7^{R403Q/+} populations were compared to control and MYH7^{R403Q/+} hiPSC-CMs data [6]. Modulating R alone did not fully capture the MYH7^{R403Q/+} phenotype of impaired cellular relaxation. Therefore, we incorporated a positive feedback mechanism from crossbridge cycling to thin filament activation to account for the significant prolongation of relaxation observed in-vitro. Specifically, calcium unbinding from troponin was slowed due to larger populations of active crossbridges. This has been shown experimentally where calcium unbinding from Troponin C was found to be slowed by increased crossbridge abundance [10], and that calcium unbinding is essential for appropriate and timely muscular relaxation [11].

2.4. In-silico trial of Mavacamten

We simulated the administration of 0.5 μ M Mavacamten on the MYH7^{R403Q/+} population, and evaluated the contractile response to the simulated drug intervention. Simulations results were then compared to experimental data [6].

The simulation of 0.5 μ M Mavacamten was achieved by further remodelling R to 0.79, after simulating the remodelling induced by the mutation.

2.5. Simulation design

All the simulations conducted in this study were run in MatLab (Mathworks Inc. Natwick, MA, USA) using the ordinary differential equation solver ode15s. A stimulus current of -53 μ A/ μ F with 1 ms duration was used and for each simulation steady-state was reached at 1 Hz pacing.

3. Results

3.1. Cellular active tension generation is modulated by myosin availability

Figure 2 shows that our phenomenological model of SRX abundance appropriately describes changes in twitch and steady-state tension generation due to crossbridge availability. It generated a ~40% increase in twitch tension

amplitude for $R = 1.3$ (Figure 2A) and a $\sim 30\%$ decrease in steady-state maximal tension for $R = 0.79$ (Figure 2B), meeting our calibration targets. For $R = 0.79$ the chosen formulation predicted a shift in calcium sensitivity of force production of ~ 0.05 units from control, compared to experiments reporting a shift of ~ 0.1 units in human myocardium at $0.5 \mu\text{M}$ Mavacamten [8]. For $R = 0.49$ simulations predicted a decrease in maximal tension of $\sim 70\%$, consistent to animal experiments [3] at $1 \mu\text{M}$ Mavacamten.

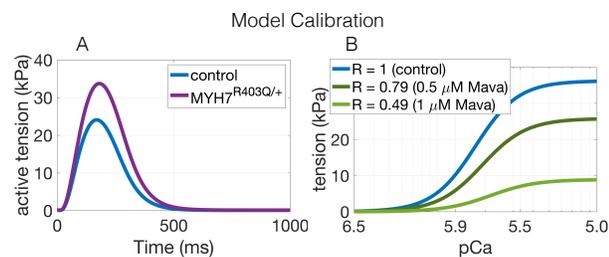


Figure 2. Active tension generation is modulated by myosin availability. A: active tension increase under the $\text{MYH7}^{\text{R403Q/+}}$ mutation. B: maximal steady-state tension and calcium sensitivity reduction under simulated action of Mavacamten.

3.2. Mavacamten corrects hypercontractility and abnormal relaxation in simulated $\text{MYH7}^{\text{R403Q/+}}$ cardiomyocytes

Next, we conducted a simulation study to investigate the mechanisms that cause HCM in the $\text{MYH7}^{\text{R403Q/+}}$ variant. For this, we first constructed a control population and applied a remodelling of $R = 1.3$ on the 348 control models that satisfied the calibration criteria. Figure 3A illustrates the changes in contractility (tension amplitude, TaAmpl) and relaxation (time to 90% decay, T90off) that follows from $R = 1.3$ in our simulated population of human cardiomyocytes (light purple). We observed a median increase in contractility (active tension peak) of 43% and a median increase in relaxation time of 3% under $\text{MYH7}^{\text{R403Q/+}}$ compared to control. Tension abnormalities were corrected by applying $R = 0.79$ as representative of $0.5 \mu\text{M}$ Mavacamten (Figure 3A, in green). This correlates well with the experimental dose-dependent responses of sarcomere shortening and relaxation time obtained in hiPSC-CMs (Figure 3B, [6]).

The model without thin filament feedback had fidelity in simulating mutation-induced percentage change in contractility when compared to experimental data. However, the model did not well describe the $\sim 35\%$ prolongation of relaxation observed in-vitro in hiPSC-CMs. We therefore hypothesised that the prolongation of relaxation observed experimentally in $\text{MYH7}^{\text{R403Q/+}}$ could be explained by a positive feedback mechanism from

crossbridge cycling to thin filament activation. Accordingly, a larger number of crossbridges favour thin filament activation and slows the release of calcium from troponin. When we incorporated this thin filament feedback into our model, we found significantly prolonged tension relaxation in our simulations in agreement with the $\sim 35\%$ change in relaxation time observed in experiments (Figure 3A, dark purple dashed line). Simulation of Mavacamten in this system lowers the amount of crossbridge cycling and the myosin-based contribution to thin filament activation restoring cardiac relaxation.

Mavacamten corrects hypercontractility and abnormal relaxation in simulated and experimental pathogenic $\text{MYH7}^{\text{R403Q/+}}$

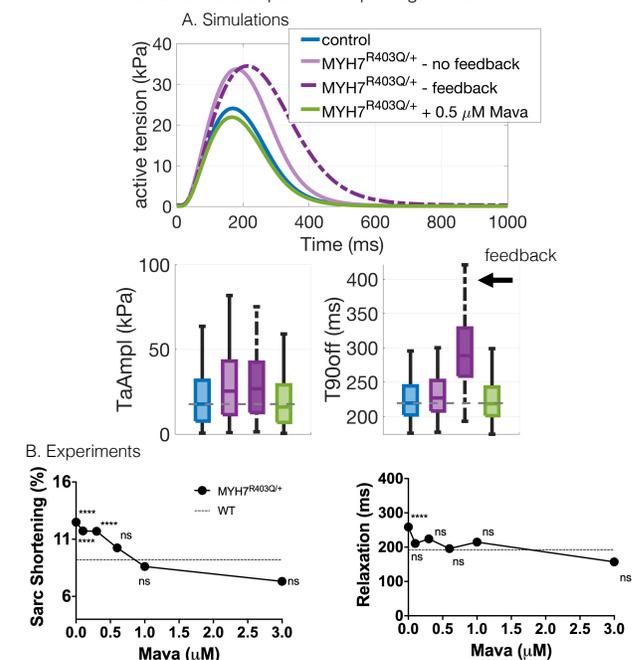


Figure 3. Explanation of disease phenotype through modelling and simulation. A: simulated active tension changes under the $\text{MYH7}^{\text{R403Q/+}}$ mutation and Mavacamten. The feedback from crossbridge cycling to thin filament activation allows to recover the mutation-induced prolongation in relaxation. B: experimental sarcomere shortening and relaxation time dose-dependent responses of hiPSC-CMs to Mavacamten [6].

4. Discussion

We conducted a modelling and simulation study to mechanistically unravel how Mavacamten restores tension generation in a β -myosin heavy chain mutation that causes HCM. Our results contribute to a better mechanistic understanding of Mavacamten efficacy in a specific HCM subgroup, which is needed to guide effective and safe patient-specific therapy. To investigate how HCM and Mavacamten alter cellular contractility, we extended a

human cardiomyocyte electromechanical model to include representation of myosin sequestration and release from the energy-conserving state SRX.

Our simulations show that hypercontractility is a consequence of increased crossbridge cycling due to reduced myosin SRX abundance. We also show that this mechanism in isolation cannot explain the significantly impaired relaxation observed experimentally. For this reason, we modelled a crossbridge-based contribution to thin filament activation that favours a prolonged calcium binding to troponin, thereby slowing cellular relaxation.

We also show that our phenomenological model of myosin sequestration is able to replicate the reduction in maximal active tension and reduction in calcium sensitivity of force production observed with 0.5 and 1 μ M Mavacamten treatment. When Mavacamten exposure was simulated in human adult cardiomyocytes with the MYH7^{R403Q/+} variant, our simulations showed that Mavacamten rescued the hypercontractile phenotype and impaired relaxation by reducing the proportion of crossbridges that can interact with actin. This suggests that Mavacamten can be very effective in correcting HCM abnormalities caused by mutations that destabilise SRX, but careful consideration should be taken in extrapolating these results to different HCM genotypes that could act through different pathways. According to our simulations, the modulation of contractility by reduction of myosin head availability provides a robust mechanism to reduce hyperdynamic contraction, a common pathogenic mechanism in HCM. This supports the effectiveness of Mavacamten in clinical trials for obstructive HCM [2]. However, specific genotypes may remain untreated if the observed hypercontractile phenotype does not occur through SRX destabilization [12]. We suggest that future mechanistic investigations, like the ones conducted here, on mutation-specific disease pathways will be key to advance our understanding of HCM and to inform effective patient-specific pharmacological interventions.

Acknowledgments

This work was funded by the European Union's Horizon 2020 research and innovation programme (Personalised In-Silico Cardiology project, grant agreement 764738; CompBioMed 1 and 2 Centre of Excellence in Computational Biomedicine, grant agreements 675451 and 823712; TransQST project, Innovative Medicines Initiative 2 Joint Undertaking, grant agreement 116030), a Wellcome Trust Fellowship in Basic Biomedical Sciences to B.R. (214290/Z/18/Z), a British Heart Foundation (BHF) Intermediate Basic Science Fellowship to A.B. (FS/17/22/32644), an NC3Rs Infrastructure for Impact Award (NC/P001076/1), the Oxford BHF Centre of Research Excellence (RE/13/1/30181), a Sir Henry Wellcome fellowship to

C.N.T. (206466/Z/17/Z), and a University of Oxford BHF CRE Intermediate Transition Fellowship to C.N.T. (RE/18/3/34214). We would like to acknowledge the use of the University of Oxford Advanced Research Computing (ARC) facility in carrying out this work.

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