



Effects of omecamtiv mecarbil on calcium-transients and contractility in a translational canine myocyte model

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Abstract

Omecamtiv mecarbil (OM) is a selective cardiac myosin activator (myotrope), currently in Phase 3 clinical investigation as a novel treatment for heart failure with reduced ejection fraction. OM increases cardiac contractility by enhancing interaction between myosin and actin in a calcium-independent fashion. This study aims to characterize the mechanism of action by evaluating its simultaneous effect on myocyte contractility and calcium-transients (CTs) in healthy canine ventricular myocytes. Left ventricular myocytes were isolated from canines and loaded with Fura-2 AM. With an IonOptix system, contractility parameters including amplitude and duration of sarcomere shortening, contraction and relaxation velocity, and resting sarcomere length were measured. CT parameters including amplitude at systole and diastole, velocity at systole and diastole, and duration at 50% from peak were simultaneously measured. OM was tested at 0.03, 0.1, 0.3, 1, and 3 $\mu\text{mol}\text{L}^{-1}$ concentrations to simulate therapeutic human plasma exposure levels. OM and isoproterenol (ISO) demonstrated differential effects on CTs and myocyte contractility. OM increased contractility mainly by prolonging duration of contraction while ISO increased contractility mainly by augmenting the amplitude of contraction. ISO increased the amplitude and velocity of CT, shortened duration of CT concurrent with increasing myocyte contraction, while OM did not change the amplitude, velocity, and duration of CT up to 1 $\mu\text{mol}\text{L}^{-1}$. Decreases in relaxation velocity and increases in duration were present only at 3 $\mu\text{mol}\text{L}^{-1}$. In this translational myocyte model study, therapeutically relevant concentrations of OM increased contractility but did not alter intracellular CTs, a mechanism of action distinct from traditional calcitropes.

KEYWORDS

muscle cells, heart failure, myocardial contraction, sarcomeres, omecamtiv mecarbil

Abbreviations: Cont-Dur, duration of sarcomere shortening measured at 50% of Cont-P; Cont-P, amplitude of sarcomere length shortening; Cont-V, maximal contraction velocity measured at the contraction phase; CT, calcium transient; CTAd, calcium-transient amplitude at diastole normalized by CTAs; CTAs, calcium-transient amplitude at systole; CTD50, calcium-transient duration measured at 50% CTAs; CTVd, calcium-transient velocity measured at diastole; CTVs, calcium-transient velocity measured at systole; HF, heart failure; ISO, isoproterenol; OM, omecamtiv mecarbil; Rel-V, maximal relaxation velocity measured at the relaxation phase; RSL, resting sarcomere length normalized by Cont-P.

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1 | INTRODUCTION

Heart disease is the leading cause of death in the United States, and heart failure (HF) accounts for 34% of all cardiovascular mortality.¹ About 6.5 million adults in the United States suffer from HF.² Total cost for HF was \$30.7 billion in 2012 and projections suggest an increase to \$69.8 billion by 2030.³ One of the treatment options for HF with reduced ejection fraction is to directly improve myocardial contractility. Drugs that could directly increase cardiac contractility are called inotropes. However, the general terminology is not sufficient in describing the mechanism of action and communicating the progress in developing novel therapeutics. Recently, a proposal was published that classifies inotropes based upon their mechanisms.⁴ This recommendation aimed at facilitating communication regarding inotropes' mechanism of action and recognizing advancement in developing novel therapeutics for HF with reduced ejection fraction. Traditional inotropes are classified as calcitropes because they modulate intracellular Ca^{2+} levels. On the other hand, inotropes that increase cardiac contractility by interacting with molecular motor and scaffolding without affecting intracellular calcium are called myotropes. Another class of inotropes, mitotropes, improve cardiac performance by regulating glucose metabolism and energy production. Traditional calcitropic agents, such as dobutamine and milrinone, are effective in regulating hemodynamics in acute HF patients.⁵ They enhance contractility; however, they also increase intracellular Ca^{2+} , activate maladaptive Ca^{2+} -dependent signaling cascades, increase heart rate and myocardial oxygen consumption, and may induce lethal arrhythmias.^{6,7} Long-term treatment with this class of calcitropes has been shown to increase the morbidity and mortality of patients with chronic HF.⁸ Another group of calcitropes called calcium sensitizers, such as levosimendan and pimobendan, was developed to increase troponin calcium binding affinity without increasing the level of intracellular Ca^{2+} .^{9,10} However, calcium sensitizers are non-specific and inhibit phosphodiesterase (PDE-3), a mechanism leading to elevation of intracellular Ca^{2+} .^{9,11} Clinical data suggest short-term symptomatic improvement with calcium sensitizers but similar mortality rates as with traditional inotropes.¹² Therefore, there is an urgent medical need to develop better agents in managing HF with reduced ejection fraction.

In basic research and drug development, canines have been widely used as a large animal model because canine hearts and human hearts share many similarities in terms of their structure, protein expression, electrophysiology, and hemodynamics. It is well accepted among cardiovascular researchers that canine hearts represent human cardiac physiology and pathophysiology much better than rodent hearts.¹³ Isolated cardiac myocytes provide a higher throughput platform requiring fewer animals. For image-based studies, isolated myocytes are well suited for experiments aimed at visualizing Ca^{2+} homeostasis and cellular contractility.¹⁴⁻¹⁷ Thus, isolated cardiomyocytes can be utilized to provide valuable insight into cellular Ca^{2+} homeostasis and contractility by measuring both endpoints simultaneously. In addition, the direct effects on the myocytes can

be easily evaluated due to the lack of influence by heart rate, coronary flow, and neuro-hormonal factors. Previous studies have shown that the isolated myocyte assay could successfully detect both positive and negative inotropes.^{16,18,19} Furthermore, a recent publication showed that isolated canine myocytes responded to supratherapeutic omecamtiv mecarbil (OM) treatment with increased duration of contraction without change in the calcium transient (CT), suggesting that isolated canine ventricular myocyte is a suitable model for evaluating cardiac contractility and CT.^{20,21}

Omecamtiv mecarbi is a first-in-class selective cardiac myosin activator that prolongs myocardial systole, which is decreased in HF patients with reduced ejection fraction and further reduced by traditional inotropes.²² As compared with calcium sensitizers, OM directly enhances myocyte contractility without affecting Ca^{2+} homeostasis. In both animal models and clinical studies, OM prolongs systolic ejection time yet reduces heart rate and increases stroke volume and cardiac output, but does not change myocardial oxygen consumption.²²⁻²⁶ For example, OM infusion in normal and HF dogs produced pronounced enhancement of cardiac function measured by echocardiography.²² Previous studies performed in isolated myocytes have limited OM testing concentrations; one tested only 0.2 $\mu\text{mol/L}$ concentration in rat myocytes,²² the other tested only 1 and 10 $\mu\text{mol/L}$ supratherapeutic concentrations in canine myocytes.²¹ The present study leverages an enhanced understanding of the therapeutic concentrations of OM to characterize the mechanism of action of OM at therapeutically relevant concentrations. In the COSMIC-HF trial, the maximal free plasma concentration of OM was 0.5 $\mu\text{mol/L}$.²⁵ The present study simultaneously measured the effects of OM on sarcomere length shortening and intracellular CTs in isolated canine ventricular myocytes at 0.03, 0.1, 0.3, 1, and 3 $\mu\text{mol/L}$. In addition, to differentiate OM from calcitropes, the effects of a β -adrenergic agonist, isoproterenol (ISO), on cardiac contractility and CTs were also measured under the same experimental conditions for comparison.

2 | MATERIALS AND METHODS

2.1 | Animals

Adult male beagle dogs (12- to 15-month old; Marshall BioResources, New York, NY, USA) were single-housed at an American Association for Accreditation of Laboratory Animal Care (AAALAC)-accredited facility and acclimated for at least 7 days. Veterinary care was provided in accordance with the Guide for the Care and Use of Laboratory Animals, 8th Edition (NRC, 2011). All in vivo experiments were conducted in compliance with Amgen Institutional Animal Care and Use Committee (IACUC) and United States Department of Agriculture (USDA) regulations. After removal from anesthetized dogs, hearts were transported in cardioplegic solution for myocyte isolation. A total of three hearts were used for the studies reported in this manuscript. Myocytes from heart #1 were tested with positive control compound isoproterenol. Myocytes from heart #2 were

tested with OM at a range of 0.03-3 $\mu\text{mol/L}$. Myocytes from heart #3 were used for a repeating OM study (at a range of 0.3-3 $\mu\text{mol/L}$) with a washout process.

2.2 | Solutions

The standard Tyrode solution for myocyte isolation and IonOptix recording comprises (mmol/L): 135 NaCl, 5.4 KCl, 1 CaCl_2 , 1 MgCl_2 , 0.33 NaH_2PO_4 , 10 HEPES, 10 glucose, and is adjusted to pH 7.4 with NaOH. CaCl_2 is substituted with equimolar NaCl in the calcium-free Tyrode solution. The cardioplegic solution for heart transportation contained (mM): 27 KCl, 20 NaHCO_3 , 255 glucose, 63 mannitol, and heparin 1000 units/L. OM, obtained from Amgen's sample bank, was first dissolved in dimethyl sulfoxide (DMSO) at 1000-fold of the highest testing concentration (3 μM), then diluted to testing concentrations of 0.03, 0.1, 0.3, 1, and 3 $\mu\text{mol/L}$. DMSO was kept at 0.1% at baseline and at all testing concentrations. ISO (Sigma-15627, Sigma-Aldrich, St. Louis, Missouri, USA) was first dissolved in ddH_2O , then diluted to the testing concentrations of 1, 3, 10, 30, and 100 nmol/L.

2.3 | Myocyte isolation

As previously described,^{18,27} isolated heart was cannulated via left anterior descending coronary artery and mounted on a Langendorff perfusion apparatus. At 37°C, the heart was perfused with Ca^{2+} -free Tyrode solution for 10 minutes, followed by 1 mg/mL collagenase type II (Worthington Biochemical Corporation-LS004176) plus 0.5 mg/mL bovine serum albumin (BSA, Fraction V, Sigma) and 5 $\mu\text{mol/L}$ Ca^{2+} for 20 minutes. After washing with 0.2 mmol/L Ca^{2+} -Tyrode for 10 minutes, a ~ 1.5 cm \times 1.5 cm transmural left ventricular wall was minced into smaller pieces, swirled, and filtered through a 100 μm Teflon mesh. The dissociated myocytes were harvested and washed three times in 0.2 mmol/L Ca^{2+} -Tyrode solution containing 5 mg/mL BSA, then transferred to 1 mmol/L Ca^{2+} -Tyrode and recovered for 1 hour at room temperature before storage. The myocytes were stored at 4°C in 1 mmol/L Ca^{2+} -Tyrode and 30 $\mu\text{mol/L}$ N-benzyl-p-toluene sulfonamide (BTS, TCI America-B3082). On the next day, myocytes were equilibrated to room temperature for at least 1 hour, then washed three times with Tyrode solution before contractility and CT measurement. All experiments were performed within 24 hours of isolation during which the isolated myocytes maintained optimal morphology and contractility.²⁸ They were quiescent, rod-shaped with clear striations, and exhibited consistent contraction/relaxation cycles under pacing.

2.4 | Myocyte contractility and CT measurement

Before CT measurement, the isolated myocytes were loaded with 5 $\mu\text{mol/L}$ Fura-2 AM (Molecular Probes-F1221) for 30 minutes at

room temperature, then washed three times with Tyrode solution (followed by a wait time of 0.5-1 hour) to de-esterify the dye.

Sarcomere length and intracellular CTs were recorded using a video-based edge-detecting IonOptix system. Briefly, myocytes were loaded into the recording chamber mounted on the stage of an inverted microscope (Motic AE31, New York Microscope Co) and perfused with Tyrode solution, which was kept in a $\sim 37^\circ\text{C}$ water bath (VWR, model 1202) and perfused via a micro pump (ISMATEC, 827B) to the recording chamber at a rate of ~ 6 mL/min. Myocytes were paced via a MyoPacer (IonOptix LLC, Westwood, MA, USA) at 1 Hz (60 beats/min) frequency and illuminated with an LED alternating dual wavelength (340 nm/380 nm). Pacing induced intracellular CT fluorescence change (detected at 510 nm with a Hamamatsu H7360 photomultiplier tube), and sarcomere length shortening was simultaneously recorded. Data were sampled at a rate of 240 Hz. Background fluorescence was recorded and manually subtracted from the total fluorescence. If a myocyte fulfilled the selection criteria (good morphology, well-followed pacing, no spontaneous beating, < 10% decrease in contractility during the 10-minute pre-baseline recording, etc),¹⁸ baseline measurements were collected for approximately 10-20 seconds. Afterwards, myocytes were exposed to ascending testing concentrations, with each concentration applied for 2 minutes before data collection. Sarcomere shortenings and CT signals (F340, F380, and ratio) were acquired for 10-20 seconds at each concentration.

2.5 | Data analysis

Data analysis was performed using IonWizard software. Sarcomere shortening was measured from averaged sarcomere waveforms (Figure 1). The following parameters were analyzed for contractility:

- Cont-P: Amplitude of sarcomere length shortening
- RSL: Resting sarcomere length normalized by Cont-P
- Cont-V: Maximal contraction velocity measured at the contraction phase
- Rel-V: Maximal relaxation velocity measured at the relaxation phase
- Cont-Dur: Duration of sarcomere shortening measured at 50% of Cont-P

CT parameters were analyzed from the calcium-fluorescence ratios (F340/F380) collected at baseline and at each testing concentration (Figure 1). The following parameters were measured from the averaged ratio waveform:

- CTAs: CT amplitude at systole
- CTAd: CT amplitude at diastole normalized by CTAs
- CTVs: CT velocity measured at systole
- CTVD: CT velocity measured at diastole
- CTD50: CT duration measured at 50% CTAs

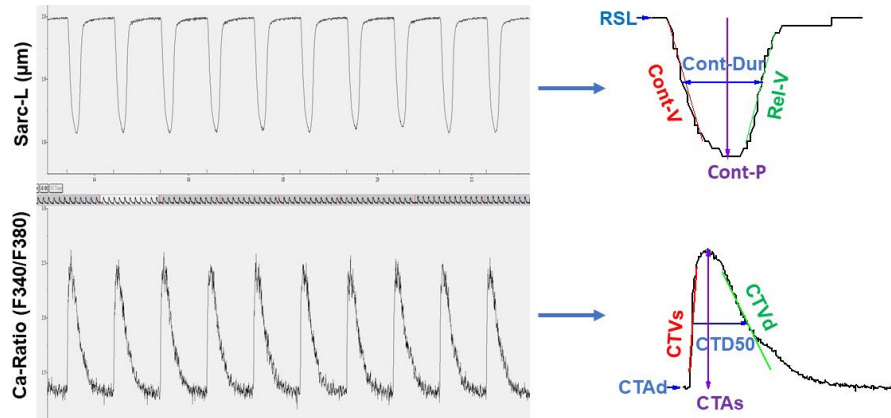


FIGURE 1 Illustration of measured contractility and calcium-transient parameters. Cont-Dur, duration of sarcomere shortening measured at 50% of Cont-P; Cont-P, amplitude of sarcomere length shortening; Cont-V, maximal contraction velocity measured at the contraction phase; CTAd, calcium-transient amplitude at diastole normalized by CTAs; CTAs, calcium-transient amplitude at systole; CTVd, calcium-transient velocity measured at diastole; CTVs, calcium-transient velocity measured at systole; CTD50, calcium-transient duration measured at 50% CTAs; Rel-V, maximal relaxation velocity measured at the relaxation phase; RSL, resting sarcomere length normalized by Cont-P

Based on our validation and previously published results,¹⁸ drug-related effects for contractility and CT are defined as a $\geq 10\%$ change in the measured parameters, and significance was assessed with a one-way analysis of variance (ANOVA) followed by a post hoc multiple comparison using the raw data (unnormalized). If the criteria were met to indicate a significant change in these parameters, an asterisk (*) is marked on the representing data point in the corresponding figures. Qualified researchers may request data from Amgen clinical studies. Complete details are available at the following: <http://www.amgen.com/datasharing>.

3 | RESULTS

3.1 | Effects of OM and ISO on Cont-P and CTAs

In isolated canine ventricular myocytes, the classical inotrope ISO increased Cont-P and CTAs in a concentration-dependent fashion (Figure 2A, B). In addition to the increases in Cont-P and CTAs, ISO also induced a faster rising phase and a second waveform with a lesser amplitude at high testing concentrations, representing its “arrhythmic-like” activity (Figure 2B). The average Ca-ratio waveforms almost mirrored the Sarc-L waveforms, reflecting the concentration-dependent effects of ISO on intracellular CT amplitude (Figure 2B).

In contrast, while OM showed a similar concentration-dependent increase in Cont-P, no change was seen in CTAs (Figure 2C, D). Higher concentrations of OM caused longer Sarc-L time but the same CT time, except for a slightly extended duration at $3 \mu\text{mol/L}$ (Figure 2D). In multiple myocytes, the average changes in Cont-P and CTAs compared to baseline are shown in Figure 3 (N = 11 for ISO, N = 10 for OM). The largest increase of about 207% in Cont-P was induced by ISO at 30 nmol/L . This contractility

change was accompanied by increased CTAs, which also plateaued at $\text{ISO} = 30 \text{ nmol/L}$ with a 37% increase. Similarly, a concentration-dependent increase in Cont-P was observed with OM, with the largest increase of 41% at $3 \mu\text{mol/L}$. However, OM did not change CTAs up to $3 \mu\text{mol/L}$. In addition, the magnitude of increase in Cont-P was much smaller with OM than with ISO.

3.2 | Effects of OM and ISO on RSL and CTAd

The average effects of ISO and OM on RSL and CTAd in multiple myocytes were compared (Figure 4) (N = 11 for ISO, N = 10 for OM). ISO decreased RSL by 38% at 100 nmol/L , indicating sarcomeres could not fully relax in the presence of ISO. Meanwhile, ISO caused a concentration-dependent increase in CTAd, suggesting that intracellular Ca^{2+} level remained higher than normal during diastole. OM caused a concentration-dependent decrease in RSL. The largest decrease occurred at $3 \mu\text{mol/L}$ with approximately 59% reduction. In contrast to ISO, OM had no effect on CTAd at any of the testing concentrations.

3.3 | Effects of OM and ISO on Cont-V and CTVs

OM and ISO affected the velocity of contraction phase differently as measured by the maximal velocity at contraction (rising) phase of sarcomere length shortening (Cont-V) and CT at systole (CTVs) (Figure 5) (N = 11 for ISO, N = 10 for OM). ISO increased both Cont-V and CTVs in a concentration-dependent manner. OM had a more complex biphasic effect on Cont-V with the peak enhancement at $0.3 \mu\text{mol/L}$. At $3 \mu\text{mol/L}$, OM reduced Cont-V by 35%. OM had no effects on CTVs at any of the other testing concentrations.

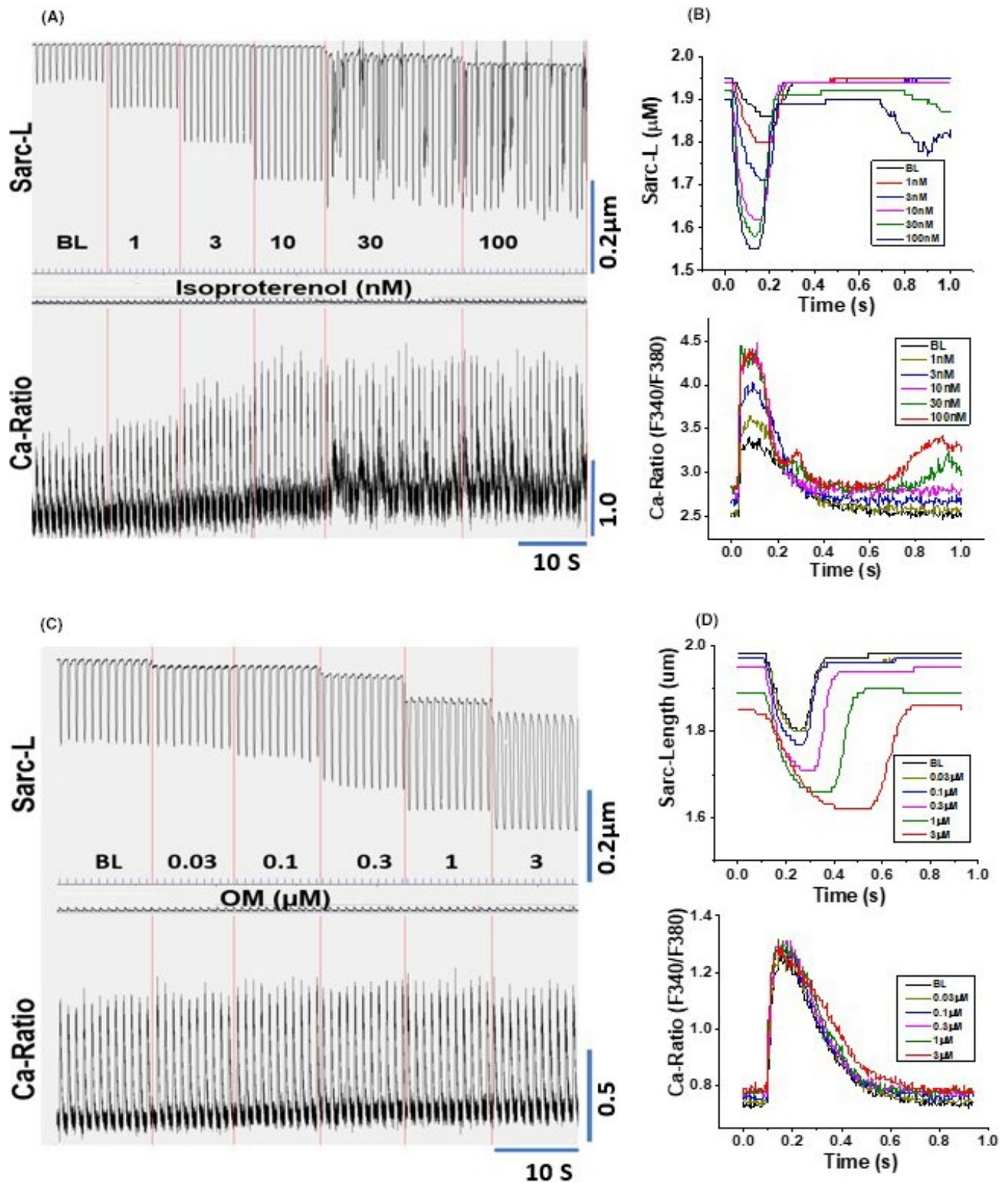


FIGURE 2 Effects of ISO and OM on sarcomere length shortening and calcium-transients in two single ventricular myocytes. (A) Raw data traces of sarcomere length shortening (upper trace, Sarc-L) and calcium-transient (lower trace, Ca-Ratio) from one representative myocyte in response to ISO. (B) Average traces of Sarc-L and Ca-Ratio from the same myocyte on the left at BL and at each testing concentration of ISO. (C) Raw data traces of Sarc-L and Ca-Ratio from one representative myocyte in response to OM. (D) Average traces of Sarc-L and Ca-Ratio from the same myocyte on the left at BL and at each testing concentration of OM. BL, baseline; ISO, isoproterenol; OM, omecamtiv mecarbil

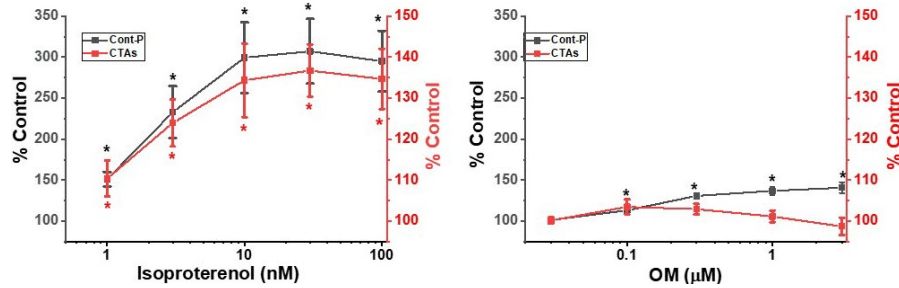


FIGURE 3 Average changes of systolic sarcomere length shortening (Cont-P) and calcium-transients (CTAs) in multiple myocytes induced by ISO (N = 11 from heart #1) and OM (N = 10 from heart #2). Y-axis represents mean \pm SEM of normalized percentage change of Cont-P (left, black) and CTAs (right, red). X-axis represents testing concentrations. *indicates significant ($P < .05$) difference from baseline. Cont-P, amplitude of sarcomere length shortening; CTAs, calcium-transient amplitude at systole; ISO, isoproterenol; OM, omecamtiv mecarbil; SEM, standard error of the mean

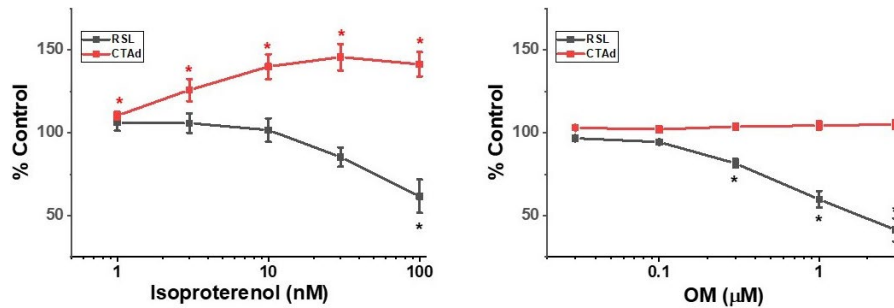


FIGURE 4 Average changes of resting sarcomere length (RSL) and diastolic calcium level (CTAd) in multiple myocytes induced by ISO (N = 11 from heart #1) and OM (N = 10 from heart #2). Y-axis represents mean \pm SEM of normalized percentage change of RSL and CTAd. X-axis represents testing concentrations. *indicates significant ($P < .05$) difference from baseline. ISO, isoproterenol; OM, omecamtiv mecarbil; SEM, standard error of the mean

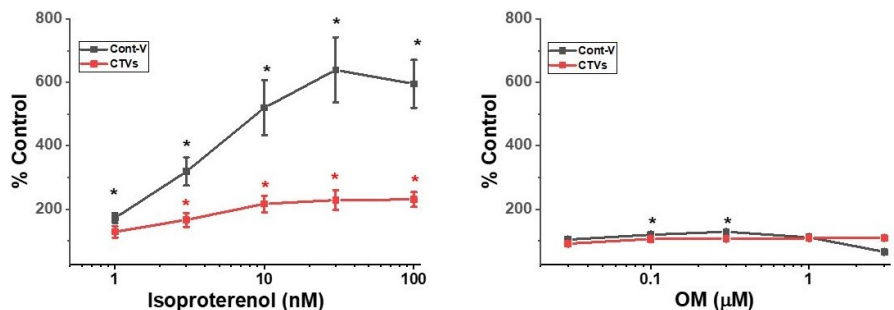


FIGURE 5 Average changes of sarcomere contraction velocity (Cont-V) and systolic calcium-transient velocity (CTVs) in multiple myocytes induced by ISO (N = 11 from heart #1) and OM (N = 10 from heart #2). Y-axis represents mean \pm SEM of normalized percentage change of Cont-V and CTVs. X-axis represents testing concentrations. *indicates significant ($P < .05$) difference from baseline. ISO, isoproterenol; OM, omecamtiv mecarbil; SEM, standard error of the mean

3.4 | Effects of OM and ISO on Rel-V and CTvd

OM and ISO affected the velocity of the relaxation phase differently, as measured by the maximal velocity at relaxation (declining) phase of sarcomere length shortening (Rel-V) and CT at diastole (CTvd) (Figure 6) (N = 11 for ISO, N = 10 for OM). ISO caused a concentration-dependent increase in both Rel-V and CTvd. OM had a biphasic effect on Rel-V with an increase at ≤ 1 $\mu\text{mol/L}$ and a decrease at 3 $\mu\text{mol/L}$. OM had no effects on CTvd at ≤ 1 $\mu\text{mol/L}$. However, a

significant 26% reduction was induced by OM at 3 $\mu\text{mol/L}$, indicating a slower declining phase of CTs.

3.5 | Effects of OM and ISO on Cont-Dur and CTD50

The average effects of ISO and OM on the duration of contraction (Cont-Dur) and CT at 50% from the peak (CTD50) in multiple

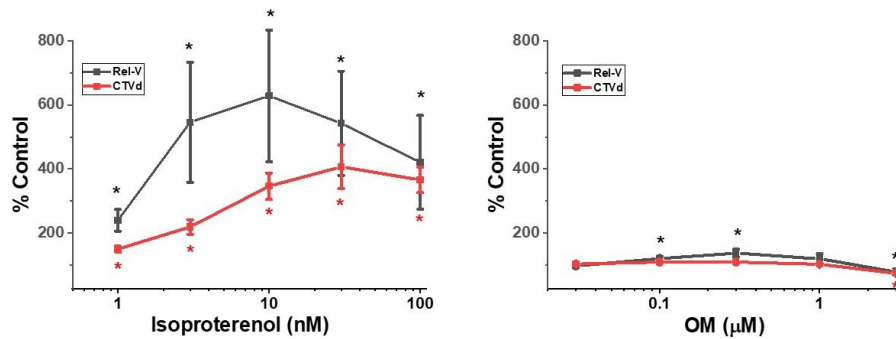


FIGURE 6 Average changes of sarcomere relaxation velocity (Rel-V) and diastolic calcium-transient velocity (CTVd) in multiple myocytes induced by ISO (N = 11 from heart #1) and OM (N = 10 from heart #2). Y-axis represents mean \pm SEM of normalized percentage change of Rel-V and CTVd. X-axis represents testing concentrations. * indicates significant ($P < .05$) difference from baseline. ISO, isoproterenol; OM, omecamtiv mecarbil; SEM, standard error of the mean

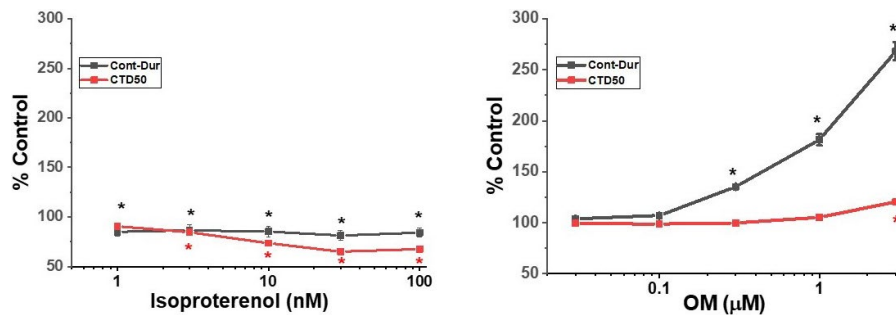


FIGURE 7 Average changes of sarcomere contraction duration (Cont-Dur) and calcium-transient duration (CTD50) in multiple myocytes induced by ISO (N = 10 from heart #1) and OM (N = 10 from heart #2). Y-axis represents mean \pm SEM of normalized percentage of Cont-Dur and CTD50. X-axis represents testing concentrations. * indicates significant ($P < .05$) difference from baseline. Cont-Dur, duration of sarcomere shortening measured at 50% of Cont-P; CTD50, calcium-transient duration measured at 50% CTAs; ISO, isoproterenol; OM, omecamtiv mecarbil; SEM, standard error of the mean

myocytes were compared (Figure 7) (N = 11 for ISO, N = 10 for OM). ISO caused a concentration-dependent decrease in both Cont-Dur and CTD50, indicating that ISO decreased the duration of sarcomere length shortening and CTs. Contrary to ISO, OM caused a concentration-dependent increase in Cont-Dur. However, OM did not change CTD50 at $\leq 1 \mu\text{mol/L}$. At $3 \mu\text{mol/L}$, OM induced a 20% significant prolongation of CTD50, consistent with the reduction of CTVd.

3.6 | Reversibility of the effects of OM

To confirm that prolongation of CT at $3 \mu\text{mol/L}$ OM was not due to either deterioration of myocyte function or variation of an individual dog, myocytes isolated from another dog were tested at 0.3, 1, and $3 \mu\text{mol/L}$ followed by washout (Figure 8). The recovery of CT and sarcomere length shortening after 6 minutes of washout demonstrated that the effects were OM-induced and partially reversible. Among the seven tested myocytes, an average of 31% reduction in CTVd and 12% prolongation in CTD50 at $3 \mu\text{mol/L}$ OM were detected. These effects were partially reversible upon washout for 6 minutes.

4 | DISCUSSION

In the present study, we performed a direct comparison of a classical calcitrope, ISO, with a first-in-class myotrope, OM, by measuring intracellular CT and sarcomere shortening simultaneously in isolated canine ventricular myocytes. To the best of our knowledge, this is the first study to characterize the concentration-dependent effects of OM in its therapeutic and supratherapeutic exposure levels in primary myocytes. Both contractility and CTs were analyzed for their amplitude, duration, and velocity to understand the differences between calcitropes and myotropes. ISO and OM enhanced myocyte contractility by different mechanisms. ISO mainly increased the amplitude and velocity of contraction with relatively small effects on duration. OM mainly increased the duration of contraction with relatively small effects on amplitude and velocity of contraction. Consistent with its calcitropic mechanism, ISO also increased the amplitude and velocity of CT in a concentration-dependent manner concurrent with changes in contractility. On the other hand, OM did not change any parameter of CT up to $1 \mu\text{mol/L}$ despite profound increase in contractility measured simultaneously, confirming its myotropic mechanism.

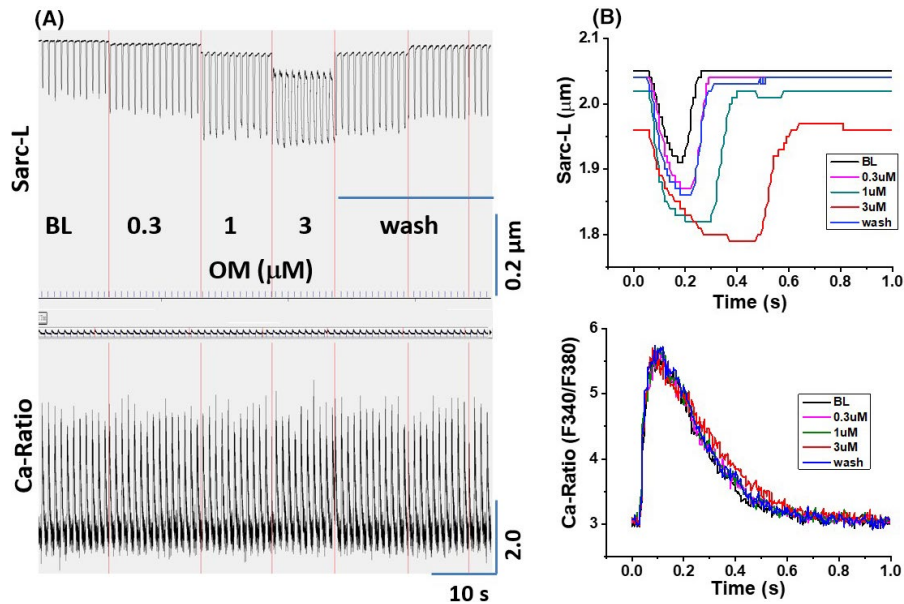


FIGURE 8 Effects of OM on Cont-P and CTAs in a ventricular myocyte (from heart #3) with washout process. (A) Raw data traces of sarcomere length shortening (upper trace, Sarc-L) and calcium-transient (lower trace, Ca-Ratio) from one representative myocyte in response to OM and washout. (B) Average traces of Sarc-L and Ca-Ratio from the same myocyte on the left at BL and at each testing concentration of OM and washout. BL, baseline; Cont-P, amplitude of sarcomere length shortening; CTAs, calcium-transient amplitude at systole; OM, omecantiv mecarbil

4.1 | Comparison with previous publications

OM has been tested in isolated rat ventricular myocytes at a single concentration of 0.2 μmol/L.²² OM at 0.2 μmol/L significantly increased the fractional shortening (percent cell length change) of adult rat cardiac myocytes, but did not have any effect on the CT as measured by the ratiometric fluorescent calcium indicator Fura-2. This observation in rat ventricular myocytes is similar to our findings in isolated canine myocytes, by measuring sarcomere length shortening and CT in a more expanded concentration range (0.03-3 μmol/L), indicating that activation of cardiac myosin by OM has similar functional effects and mechanisms in rats and dogs. A recent study in isolated mongrel ventricular myocytes, only supratherapeutic concentrations of OM, 1 and 10 μmol/L, were investigated for unloaded cell shortening, action potential morphology, and changes in CTs.²¹ In this report, OM increased cell shortening without altering intracellular Ca²⁺ handling, consistent with the myotropic mechanism of OM.

In our study reported here, OM increased sarcomere shortening, that is, myocyte contractility in a concentration-dependent manner by primarily prolonging duration of contraction; these observations are consistent with what have been reported in isolated rat and dog myocytes,^{21,22} as well as in vivo dog studies.²² In normal and HF dogs, infusion of OM improved left ventricular systolic function primarily by increasing systolic ejection time.²²

Amplitude, velocity, and duration of CTs recorded simultaneously in the same myocyte were not affected up to 1 μmol/L, which is a supratherapeutic concentration. However, at 3 μmol/L, the velocity

of CT during relaxation decreased (Figure 6) and the duration of diastole increased (Figure 7). These changes were truly induced by OM treatment because the velocity and duration of CT during diastole recovered upon washout of OM (Figure 8). The mechanism for these effects is speculated to be off-target effects based upon their concentration dependences. The on-target effects of OM, activation of myosin and promotion of contractility, were statistically significant at 0.1 μmol/L (Figure 3, right panel). On the other hand, the effects on CT did not reach statistical significance until 3 μmol/L, a 30-fold higher concentration compared with that for the on-target effects, strongly suggesting that OM affected CT at 3 μmol/L via an off-target mechanism.

The published study in isolated canine myocytes reported by Horvath et al, 2017 only reported the amplitude of CT without analyzing the kinetics.²¹ At 10 μmol/L, there were no visually discernible effects on CTs. Unfortunately, 3 μmol/L was not tested in Horvath et al (2017); therefore, it is not possible to make a direct comparison with the current report.

4.2 | Translation into clinical findings

The clinical relevance of the data described in this paper can be interpreted by comparing the testing OM concentrations in the current study to the free OM therapeutic plasma exposure detected in human clinical trials. The maximal OM plasma concentration was 200-318 ng/mL in a randomized, placebo-controlled phase 2 trial,²⁵ which corresponds to a 0.5-0.8 μmol/L total plasma concentration. Given the plasma protein binding of OM (82% in humans),²⁹ the effective free plasma concentration is estimated to

be 0.09-0.14 $\mu\text{mol/L}$. Therefore, the 0.1 $\mu\text{mol/L}$ concentration in the current study is in-line with the effective free plasma levels achieved in humans. In the phase 2 clinical trial,²⁵ OM increased systolic ejection time duration and stroke volume and reduced left ventricular diameters and volumes, which could be interpreted by the increase of sarcomere shortening of isolated canine myocytes at 0.1-0.3 $\mu\text{mol/L}$ OM.

The lack of any changes in CT measured simultaneously with sarcomere shortening up to 1 $\mu\text{mol/L}$ in isolated canine ventricular myocytes provided direct and strong evidence that OM increased cardiac contractility primarily without modulating intracellular calcium homeostasis at therapeutic exposure levels. Since abnormality of Ca^{2+} homeostasis plays a key role in the pathogenesis of common cardiovascular disorders, including cardiac arrhythmias,³⁰ OM would carry less pro-arrhythmic risk than traditional calcitropes. This is tremendously beneficial for managing HF patients because disturbances of intracellular Ca^{2+} have been associated with atrial fibrillation and ventricular arrhythmias in failing hearts.

4.3 | Limitations

A common limitation of isolated cardiomyocyte models is the lack of complex multicellular environment and structural elements of the intact heart. There are several limitations to our present study. First, in contrast with in vivo animal studies, isolated myocytes are “unloaded,” which would influence the characteristics of contraction and relaxation. Second, we cannot exclude experimental bias in choosing suitable cells for functional studies, that is, only cells that demonstrate regular and forceful contractions are preferred. A high-throughput program (non-manual) that can objectively select a large population of myocytes for contractility measurement is needed to reduce cell selection bias. Third, each compound was tested in multiple myocytes harvested from a single dog; therefore, our results are based on the small number of dog hearts used for myocyte isolation. Fourth, the tissue in this study came from the left ventricle wall perfused by left anterior descending coronary artery, which includes epicardial, midcardial, and endocardial myocytes. In addition to well-documented differences in their electrophysiological properties,³¹ it has been recognized that unloaded cell shortening, CTs, and inward L-type Ca^{2+} current $I(\text{Ca}_L)$ characteristics show distinct regional differences when they were examined in epicardial, endocardial, and midmyocardial cells isolated from the canine left ventricle.³² For example, the time to peak and latency to onset of contraction were the shortest in epicardial cells, intermediate in midmyocardial cells, and the longest in endocardial cells.³² Therefore, depending on the region of the myocytes selected for recording, a drug's effect could be different due to the differences in electrophysiology, CT, excitation-contraction (EC) coupling, and contraction exhibited at myocytes isolated from epicardium, midcardium, and endocardium.

5 | CONCLUSIONS

Simultaneous recording of cardiac contractility and intracellular CT was performed in primary isolated normal canine ventricular myocytes to evaluate side-by-side a traditional calcitrope, ISO, with the first-in-class myotrope, OM. This assessment provides direct evidence that OM increases cardiac contractility at clinically relevant concentrations without modulating intracellular Ca^{2+} homeostasis, a mechanism distinct from traditional calcitropes, which may offer better risk to benefit ratio for managing HF with reduced ejection fraction.

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CONFLICT OF INTEREST

All authors are employees and stakeholders of Amgen Inc.

AUTHORS' CONTRIBUTIONS

B. Gao conceived of the study design and analysis. Y. Qu participated in the drafting of the manuscript. All authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this manuscript, take responsibility for the integrity of the work, and have given approval for its publication.

DATA AVAILABILITY STATEMENT

Requests may be submitted for data owned by Amgen from any of its completed clinical trials and observational studies relating to approved uses of products approved in both the US and Europe (or approved in one of the regions if Amgen has determined the data will not be submitted for regulatory review in the other region).

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