

# Effects of omecamtiv mecarbil on failing human ventricular trabeculae and interaction with (-)-noradrenaline

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## Abstract

Omecamtiv mecarbil (OM) is a novel medicine for systolic heart failure, targeting myosin to enhance cardiomyocyte performance. To assist translation to clinical practice we investigated OM's effect on explanted human failing hearts, specifically; contractile dynamics, interaction with the  $\beta_1$ -adrenoceptor (AR) agonist (-)-noradrenaline and spontaneous contractions. Left and right ventricular trabeculae from 13 explanted failing hearts, and trabeculae from 58 right atrial appendages of non-failing hearts, were incubated with or without a single concentration of OM for 120 min. Time to peak force (TPF) and 50% relaxation ( $t_{50\%}$ ) were recorded. In other experiments, trabeculae were observed for spontaneous contractions and cumulative concentration-effect curves were established to (-)-noradrenaline at  $\beta_1$ -ARs in the absence or presence of OM. OM prolonged TPF and  $t_{50\%}$  in ventricular trabeculae (600 nM, 2  $\mu$ M,  $p < .001$ ). OM had no significant inotropic effect but reduced time dependent deterioration in contractile strength compared to control ( $p < .001$ ). OM did not affect the generation of spontaneous contractions. The potency of (-)-noradrenaline ( $pEC_{50}$  6.05  $\pm$  0.10), for inotropic effect, was unchanged in the presence of OM 600 nM or 2  $\mu$ M. Co-incubation with (-)-noradrenaline reduced TPF and  $t_{50\%}$ , reversing the negative diastolic effects of OM. OM, at both 600 nM and 2  $\mu$ M, preserved contractile force in left ventricular trabeculae, but imparted negative diastolic effects in trabeculae from human failing heart. (-)-Noradrenaline reversed the negative diastolic effects, co-administration may limit the titration of inotropes by reducing the threshold for ischemic side effects.

## KEYWORDS

arrhythmia, beta-adrenoceptor, contractility, diastole, heart failure, inotrope, noradrenaline, omecamtiv mecarbil, relaxation

**Abbreviations:** AR, adrenoceptor;  $Ca^{2+}$ , calcium; HF, heart failure; LV, left ventricle; OM, omecamtiv mecarbil; PPS, pre-power stroke; RAA, right atrial appendages; RV, right ventricle; SET, systolic ejection time;  $T_{50\%}$ , time to 50% relaxation; Tnl, troponin-I; TPCH, The Prince Charles Hospital; TPF, time to peak force; XB, cross bridge.

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## Summary

### What is already known

- Omecamtiv mecarbil (OM) increases systolic ejection time but imparts negative diastolic effects.
- In phase II trials, evidence of ischemia was revealed at supratherapeutic concentrations.

### What this study adds

- OM preserved contractile force and imparted negative diastolic effects on trabeculae from human failing hearts.
- Noradrenaline has competing diastolic effects, normalizing relaxation velocities in tissue exposed to OM.

### Clinical significance

- OM may preserve contractile force but reduce the threshold for ischemia.
- The negative diastolic effects of OM may limit the titration of noradrenaline.

## 1 | INTRODUCTION

Traditional inotropes enhance cardiac contractility by augmenting intracellular calcium ( $\text{Ca}^{2+}$ ) homeostasis by increasing **cyclic AMP** through activation of  **$\beta$ -adrenoceptors** (AR) or inhibition of **phosphodiesterase-3**.<sup>1</sup> However, clinical studies have failed to demonstrate long-term safety or efficacy of traditional inotropes; instead, they may increase the potential of sudden arrhythmic death due to their  $\text{Ca}^{2+}$  centric mechanism.<sup>2,3</sup> A novel therapeutic approach has redirected the focus to the sarcomere, specifically the actomyosin cross-bridge cycle, fundamental in determining the rate and magnitude of cardiac muscle force generation.

**Omecamtiv mecarbil** (OM) is a first-in-class selective small molecule activator of human cardiac  $\beta$ -myosin. OM influences the motor ATPase cycle by binding an allosteric pocket on myosin. This shifts the equilibrium towards the ADP.  $\text{P}_i$ -bound state, increasing the population of myosin in the pre-power stroke (PPS) state available for contraction, known as the 'duty ratio'.<sup>3-6</sup> Early models demonstrated increased stability of actin-bound myosin cross bridges (XB), and improved contractility with increased fractional shortening. Whilst this was independent of alterations in  $\text{Ca}^{2+}$  transients,<sup>4,7,8</sup> OM, by recruiting more XBs for contraction, provides a downstream  $\text{Ca}^{2+}$ -sensitizing mechanism for force production.<sup>9,10</sup> Challenging these positive findings has been the observation of a concentration dependent reduction in force development and relaxation velocities.<sup>8,10</sup> Two mechanisms have been proposed. OM may stabilize a proportion of myosin in a weak actin affinity state, forming non-force generating XBs, which impose a drag force on the functional myosin heads.<sup>8</sup> Alternatively, by stabilizing myosin it may prevent the rate of XB detachment by slowing ADP isomerization.<sup>9</sup> In canine and murine models of heart failure (HF), OM increased stroke volume, cardiac output, and left ventricular (LV) systolic ejection time (SET).<sup>11,12</sup> However, the increase in SET and

reduced relaxation velocities may inversely compromise diastolic filling and reduce coronary perfusion, resulting in myocardial ischemia.<sup>13</sup> In early dose finding trials, plasma concentrations exceeding 1200 ng/mL were associated with chest pain, troponin-I (TnI) rise and ECG findings consistent with ischemia.<sup>13-15</sup> Nonetheless, patients with refractory advanced HF may still benefit from this novel mechanism, which has provided equipoise for the phase III trial, GALACTIC-HF.<sup>16</sup>

As monotherapy, the concentration of OM can be controlled to minimize adverse effects. However, for HF patients who subsequently present in cardiogenic shock, the negative diastolic effects of OM are a concern for co-administration with traditional inotropes which increase heart rate and myocardial oxygen demand, further compromising diastole and reducing ischemic threshold. Such scenario highlights the need for information regarding drug-drug interactions of OM in the presence of  $\beta$ -AR agonist in human failing myocardium. Heart failure induces abnormal phosphorylation and negative remodeling from stress induced mechanisms which impact XB behavior and  $\beta$ -AR activity.<sup>17</sup> Therefore, scientific models utilizing human failing myocardium will best reflect and allow translation to clinical practice.

The study sought to investigate how clinically relevant concentrations of OM would impact myocardial contractile function and the generation of spontaneous contractions. Second, it was hypothesized OM would increase the potency of **noradrenaline** via the  $\beta_1$ -AR through increased  $\text{Ca}^{2+}$  sensitivity and that noradrenaline would reverse the negative diastolic effects.

## 2 | MATERIALS AND METHODS

In this study, all tissue samples were collected during cardiac surgery at The Prince Charles Hospital (TPCH). Right atrial appendages

(RAA) were obtained from adults undergoing coronary artery bypass surgery, valve surgery or a combination of both. Right and left ventricular trabeculae were obtained from end-stage HF patients undergoing cardiac transplantation at TPCH. Patients provided written informed consent prior to surgery. Patient characteristics are outlined in Table 1. The study was approved by the Metro North Hospital and Health Services Human Ethics Committee according to the Declaration of Helsinki, approval references HREC/12/QPCH/275 and EC28114.

## 2.1 | Preparation of tissues for contractility and spontaneous contractions

Following excision, cardiac tissue was immediately immersed in ice-cold pre-oxygenated modified Krebs solution containing (mM); Na<sup>+</sup> 125, K<sup>+</sup> 5, Ca<sup>2+</sup> 2.25, Mg<sup>2+</sup> 0.5, Cl<sup>-</sup> 98.5, SO<sub>4</sub><sup>2-</sup> 0.5, HCO<sub>3</sub><sup>-</sup> 32, HPO<sub>4</sub><sup>2-</sup> 1, ethylenediaminetetraacetic acid 0.04, and equilibrated with 95% O<sub>2</sub>/5% CO<sub>2</sub> and transported to the laboratory (within ~5 min of surgical removal). Intact atrial and ventricular trabeculae (width 1.96 ± 0.76 mm; cross-sectional area 3.7 ± 2.1 mm<sup>2</sup>; 180/13 trabeculae/patients) were dissected and set up, often in pairs, in a 50-ml organ bath containing modified Krebs solution at 37°C.<sup>18</sup> Trabeculae were attached to Swema SG4-45 strain gauge or ADI MLT0240 force transducers and connected to a PowerLab Data Acquisition System. Atrial trabeculae were stimulated with square-wave pulses of 5 ms duration just above threshold voltage to contract at 1 Hz, while ventricular trabeculae contracted at 0.2 Hz. Force was recorded on a computer using LabChart 8 for Windows (ADInstruments, Bella Vista, Australia). A length-tension curve was constructed to determine the optimal length at which maximal contraction occurred ( $L_{max}$ ). Subsequently, atrial trabeculae were adjusted to 50% of the force observed at  $L_{max}$ , whilst ventricular trabeculae remained unchanged. The incubation medium was exchanged for 50 ml of modified Krebs supplemented with amino acids (mM): Na<sup>+</sup> 15, fumarate 5, pyruvate 5, L-glutamate 5, glucose 10 at 37°C.<sup>19</sup> Ventricular tissues were then stimulated at 1 Hz and contractile force allowed to reach a steady state prior to the commencement of experiments with OM.

## 2.2 | Experimental protocols

### 2.2.1 | Effect of OM on contractility in human atrium and ventricle

Initial contractility studies incubated atrial or ventricular trabeculae with or without a single concentration (60, 200, 600 nM, 1 or 2 μM) of OM. Contractile force, time to peak force (TPF) and time to 50% relaxation ( $t_{50\%}$ ) were recorded over 2 h.<sup>20,21</sup> In a separate experiment, to ensure the lack of β-AR support did not confound force of contraction over the 2 h period, the effect of OM, in human ventricle, was determined in the presence of (-)-noradrenaline. Following preparation, trabeculae were incubated, for 90 min, with

TABLE 1 Patient characteristics

	Transplant n = 13	Atrial n = 58
Gender, M/F	12/1	36/22
Age, years (mean ± SD)	48.3 ± 13.47	62 ± 10.47
Baseline BMI, kg/m <sup>2</sup> (mean ± SD)	28.53 ± 3.64	31.37 ± 6.4
Transplant	13 (100%)	
Coronary artery bypass grafting		39 (68%)
Coronary artery bypass grafting + aortic valve replacement		13 (22%)
Aortic valve replacement		2 (3%)
Mitral valve replacement		3 (5%)
Myomectomy		1 (2%)
Cause of cardiomyopathy		
Idiopathic dilated cardiomyopathy	5 (38%)	
Ischemic dilated cardiomyopathy	3 (23%)	18 (31%) <sup>a</sup>
Familial dilated cardiomyopathy	2 (15%)	
Giant cell myocarditis	1 (8%)	
Peri-partum Cardiomyopathy	1 (8%)	
Congenital heart disease	1 (8%)	
Baseline left ventricular ejection fraction, % (mean ±SD)	19.83 ± 13.62	56.05 ± 10.32
Previous malignant arrhythmia	6 (46%)	
Atrial fibrillation	2 (15%)	11 (19%)
Diabetes	1 (8%)	13 (22%)
Coronary artery disease	3 (23%)	52 (90%)
Ventricular assist device	6 (46%)	
Cardiovascular medications, n		
Angiotensin-converting enzyme inhibitors	5 (38%)	26 (45%)
Angiotensin <sub>1</sub> -receptor blocker	4 (31%)	15 (26%)
β-Blockers	7 (54%)	40 (69%)
Bisoprolol	6 (46%)	6 (10%)
Carvedilol	1 (8%)	
Metoprolol		26 (45%)
Nebivolol		1 (2%)
Sotalol		4 (7%)
Atenolol		3 (5%)
Mineralocorticoid receptor antagonist	7 (54%)	4 (7%)
Digoxin	2 (15%)	1 (2%)
Flecainide		1 (2%)
Amiodarone	6 (46%)	

(Continues)

TABLE 1 (Continued)

	Transplant <i>n</i> = 13	Atrial <i>n</i> = 58
Lignocaine infusion	1 (8%)	
Dobutamine infusion	1 (8%)	

<sup>a</sup>13 patients undergoing coronary artery bypass grafting had a reduced left ventricular ejection fraction secondary to ischemia.

50 nM ICI 118,551 to block  $\beta_2$ -ARs and 5  $\mu$ M phenoxybenzamine to irreversibly block  $\alpha$ -ARs and neuronal/extraneuronal uptake of (-)-noradrenaline.<sup>19</sup> Trabeculae were then washed with 50 ml of Krebs solution, and ICI 118,551, amino acids and glucose re-added. 1  $\mu$ M of (-)-noradrenaline, a concentration which increases contractile force by ~50% of the maximal effect of (-)-noradrenaline at the  $\beta_1$ -AR, was added. Contractile force was allowed to reach a steady state before OM was added and trabeculae observed for 2 h.

### 2.2.2 | Spontaneous contraction

To determine whether spontaneous contractions could be induced by OM, ventricular trabeculae were set up as described above. Following a 2-h incubation with OM, the electrical stimulators were turned off and tissue observed for spontaneous contractions.

### 2.2.3 | (-)-Noradrenaline concentration-effect curves

Ventricular trabeculae were incubated with or without 600 nM or 2  $\mu$ M OM, concentrations derived from atrial studies, in the presence of 50 nM ICI 118,551 and 5  $\mu$ M phenoxybenzamine.<sup>19</sup> After 90 min, trabeculae were washed, and ICI 118,551 and OM re-added. After another 30 min, a cumulative concentration-effect curve was established to (-)-noradrenaline. This was achieved by sequential administration of  $\frac{1}{2}$  log unit increments in concentration until a maximal (-)-noradrenaline effect was observed, followed by a single concentration (200  $\mu$ M) of (-)-isoprenaline to obtain a maximal effect through stimulation of both  $\beta_1$ -AR and  $\beta_2$ -AR.<sup>18,22</sup>

### 2.2.4 | Statistics and calculations

Group size numbers are indicated as numbers of (trabeculae/patients). Values for *n* refer to the number of patients (not replicates) for statistical analysis. Group sizes were always  $\geq 5$  and studies were designed to generate groups of equal size and tissues randomly assigned to experimental groups. However, due to the variable volume of right atrial tissue and number of available trabeculae in explanted hearts, group sizes were not always the same. Experiments were always conducted with a control. With ventricular experiments, trabeculae from both the right ventricle (RV) and LV were obtained for each condition. Statistical analysis was performed first

by combining data from both ventricles (for each patient there was a left and right trabeculum). Second, pre-defined subgroup analysis was performed on each separate ventricle. Investigators A.D, L.C, and P.M were unblinded for practical reasons and performed all facets of the experiment and analysis. Changes in peak contractile force,  $\Delta$ TPF and  $\Delta t_{50\%}$  were calculated using baseline (prior to incubation with OM) as the reference. The force, TPF and  $t_{50\%}$  was determined by a custom in-house developed MATLAB R2018b script. Labchart 8 recording data files were first converted to MATLAB using Jim Hokanson ADInstruments (LabChart) SDK. The script then applied a 40 Hz lowpass filter to channel data to reduce noise. To determine the effect of OM on contractility, an individual contraction every 2 min was identified and the force, TPF and  $t_{50\%}$  was calculated by a developed contraction property algorithm function. Concentration effect curve experiments identified the maximal contraction for each concentration. All data, including outliers, were exported into Microsoft Excel for further statistical analysis and presentation. The script was validated against manually determined calculations. For agonists,  $pEC_{50}$  (-log concentration causing 50% of maximal response) and maximal responses were expressed as a percentage of maximal effect through stimulation of  $\beta_1$ -AR with (-)-noradrenaline or  $\beta_1$ -AR and  $\beta_2$ -AR with 200  $\mu$ M (-)-isoprenaline. Data are expressed as mean  $\pm$  standard error. Differences between experimental data were compared and tested for significance ( $p \leq .05$ ) with one-way ANOVA and, if significant and variance in inhomogeneity, followed by Tukey-Kramer multiple comparisons analysis. Fisher's exact probability test was used to test differences in the number of spontaneous contractions between groups. Differences in the rates of change over time between conditions were assessed by fitting linear mixed effects models with fixed effects for condition, time and their interaction and random intercepts and slopes for heart ID. Separate models were fitted for each side and for the combined data adjusted for side. Statistical analyses were performed using GraphPad Prism<sup>®</sup> Version 8.2.1 and Stata (Version 15). The manuscript complies with the British Journal of Pharmacology's recommendations and requirements of experimental design and analysis.<sup>23</sup>

### 2.2.5 | Drugs

Omecamtiv mercarbil (CK-1827452, #A11206) was purchased from Sapphire Bioscience (Redfern, Australia), (-)-noradrenaline, (-)-isoprenaline, ICI 118,551 were from Sigma Aldrich (Castle Hill, Australia). Phenoxybenzamine was a gift from GlaxoSmithKline (Stevenage, UK).

### 2.2.6 | Nomenclature statement

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to

PHARMACOLOGY,<sup>24</sup> and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.<sup>25</sup>

### 3 | RESULTS

#### 3.1 | Contractile effects of omecantiv mecarbil on right atrial trabeculae

Atrial experiments were performed to determine the concentrations of OM to be used on ventricular trabeculae. At all concentrations, OM produced no significant inotropic effect. Instead, all trabeculae displayed a time dependent reduction in force (fade). The progression of which was not significantly reduced by OM at 120 min ( $p = .63$ ; Figure 1A). At the conclusion of the experiment, the addition of (-)-isoprenaline (200  $\mu$ M) caused an increase in contractile force (Table S1). OM caused a concentration-dependent prolongation of TPF and  $t_{50\%}$  in right atrial trabeculae. At 120 min, 600 nM, 1 and 2  $\mu$ M OM produced significant increases from baseline for both TPF (600 nM:  $p = .01$ , 1  $\mu$ M:  $p = .047$ , 2  $\mu$ M:  $p < .001$ ; Figure 1B) and  $t_{50\%}$  (600 nM:  $p < .001$ , 1  $\mu$ M:  $p = .0003$ , 2  $\mu$ M:  $p < .001$ ; Figure 1C). Based on these results and phase II trials,<sup>13,14,26</sup> 600 nM and 2  $\mu$ M were chosen to further evaluate therapeutic and supratherapeutic concentrations of OM respectively, against a control group (no OM present), on failing ventricular myocardium.

#### 3.2 | Effect of omecantiv mecarbil on contractile dynamics in failing ventricular trabeculae

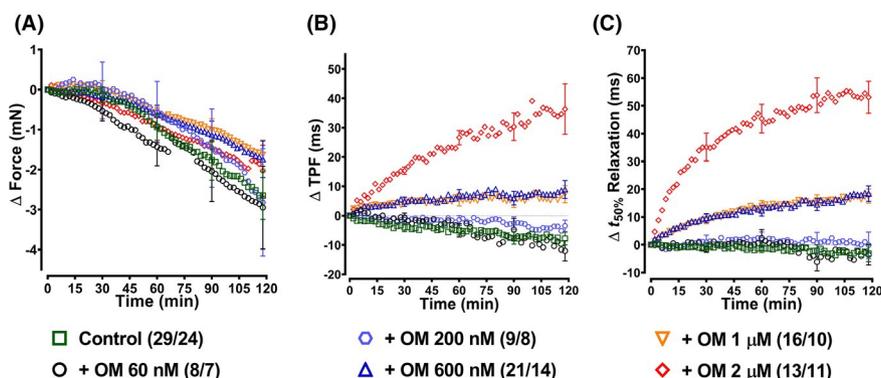
To obtain the most detailed appreciation of how OM affects failing ventricles, experiments were performed on both right and left ventricular myocardium and ended with (-)-isoprenaline providing maximal  $\beta$ -AR ( $\beta_1$ -AR +  $\beta_2$ -AR) activation. At 120 min, 600 nM and 2  $\mu$ M OM prolonged TPF (600 nM:  $p < .001$ , 2  $\mu$ M:  $p < .001$ ; Figure 2A) and

$t_{50\%}$  (600 nM:  $p < .001$ , 2  $\mu$ M:  $p < .001$ ; Figure 2B) in failing human trabeculae. In pre-specified sub-group analysis of left and right trabeculae, results remained significant.

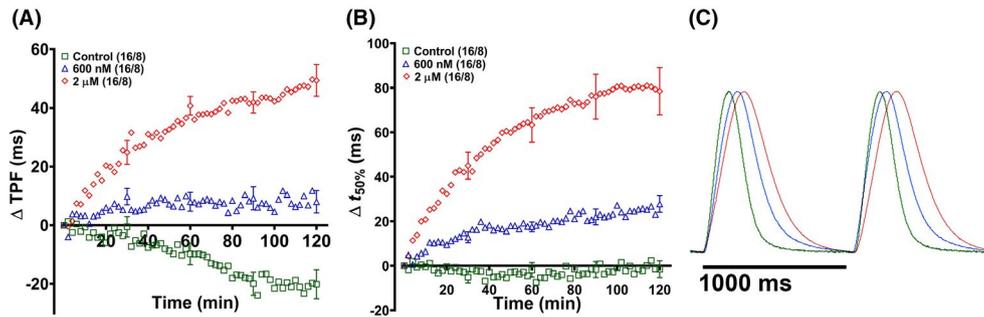
OM did not produce a concentration-dependent increase in contractile force in human failing ventricle. Instead, ventricular trabeculae displayed time dependent reduction in contractile force (Figure 3). Based on the linear mixed models, rates of change in force differed significantly between groups ( $p < .001$ ) (Table 2). There was a significant reduction in average force from baseline to 120 min in all control groups ( $p < .05$ ). However, both therapeutic and supratherapeutic OM concentrations preserved the contractile strength of LV trabeculae and the combined group (LV +RV), with no significant change over 120 min. RV trabeculae exhibited deterioration in force from baseline to 120 min which was of borderline significance (OM 600 nM:  $p = .055$ , OM 2  $\mu$ M  $p = .057$ ). Compared to the control groups, the mean force of trabeculae from the LV and combined group was significantly higher at 120 min for both OM conditions ( $p < .001$ ). Only RV trabeculae exposed to OM 2  $\mu$ M had a significantly greater mean force at 120 min compared to control ( $p < .001$ ). Mean force was significantly lower in the right ventricle compared to the left. At completion of the experiment, the addition of (-)-isoprenaline (200  $\mu$ M) caused an increase in contractile force (Tables S2–S4).

#### 3.3 | Effect of omecantiv mecarbil on contractile force in the presence of (-)-noradrenaline

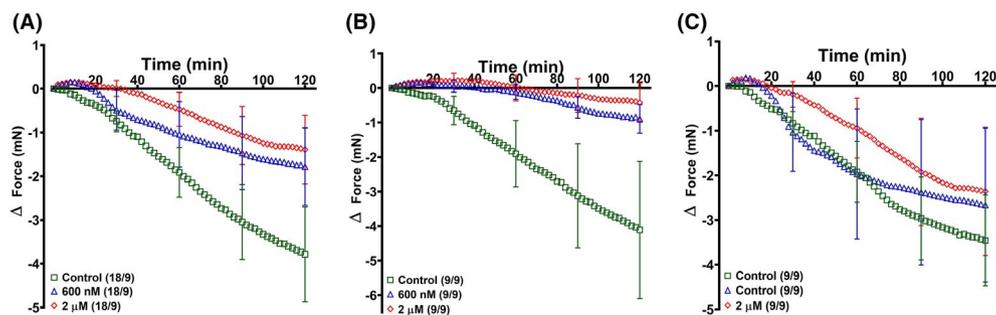
To reduce time-dependent fade, the effects of OM in the presence of selective  $\beta_1$ -AR stimulation by (-)-noradrenaline were investigated. This strategy also mimics the clinical condition of sympathetic nervous system activation to support the heart. At baseline, after a new steady state was attained with 1  $\mu$ M (-)-noradrenaline, there were no significant differences in peak contractile forces between groups ( $p = .66$ ; Figure 4). Trabeculae incubated with OM maintained contractile force, with no significant difference when compared to baseline after



**FIGURE 1** Effects of OM on the contractile dynamics of atrial trabeculae. Five different concentrations of OM were tested and compared to control (absence of OM). Time dependent reduction in contractile force (fade) was observed in all trabeculae (A). OM did not increase contractile force, nor reduce fade ( $p = .$ ). OM produced concentration-dependent increases in TPF (B) and  $t_{50\%}$  (C). At 120 min, there was a significant increase from the control group with 600 nM, 1  $\mu$ M and 2  $\mu$ M for both TPF and  $t_{50\%}$ . Statistical analysis by one-way ANOVA test followed by Tukey's multiple comparisons test



**FIGURE 2** Effects of OM on the contractile dynamics of human failing ventricular trabeculae. Concentration-dependent increase in TPF (A) and  $t_{50\%}$  (B) in human failing ventricle. At 120 min, OM produced a significant increase in TPF (600 nM:  $p < .001$ , 2  $\mu\text{M}$ :  $p < .001$ ) and  $t_{50\%}$  (600 nM:  $p < .001$ , 2  $\mu\text{M}$ :  $p < .001$ ). (C) Representative examples of two consecutive contractions from left ventricular trabeculae exposed to different concentrations of OM stimulated at 1 Hz (Green Control, Blue 600 nM, Red 2  $\mu\text{M}$ ). Statistical analysis by one-way ANOVA test followed by Tukey's multiple comparisons test



**FIGURE 3** Effect of OM on preserving contractile force in ventricular trabeculae. (A) All trabeculae produced a time-dependent reduction in contractile strength. Sub-group analysis revealed the left (B) ventricular ( $p < .001$ ), but not right (C) ventricular trabeculae in the OM groups exhibited preservation of contractile force. Statistical analysis by linear mixed effects modelling followed by Bonferroni's multiple comparison adjustment

120 min (600 nM:  $p = .18$ , 2  $\mu\text{M}$ :  $p = .75$ ). In comparison, trabeculae not exposed to OM (control group) displayed a significant force reduction after 120 min ( $p = .005$ ). Comparing the force achieved at 120 min there was a significant difference between the control group and OM 2  $\mu\text{M}$  ( $p < .001$ ) and control versus OM 600 nM ( $p = .02$ ).

### 3.4 | Effect of omecantiv mecarbil on spontaneous contractions

Following 2-h exposure to OM, there was no significant difference in the amount of observed spontaneous contractions between groups ( $p = .88$ : Control 5/21 (trabeculae exhibiting spontaneous contraction/no spontaneous contractions), OM 600 nM 5/20, OM 2  $\mu\text{M}$  5/26) (Figure 5).

### 3.5 | Effect of selective $\beta_1$ -adrenoceptor stimulation by (-)-noradrenaline in the presence of omecantiv mecarbil on ventricular trabeculae

(-)-Noradrenaline caused a concentration dependent positive inotropic response in failing human ventricular trabeculae (Figure 6).

The potency of (-)-noradrenaline ( $p\text{EC}_{50}$  6.05  $\pm$  0.10) was unchanged in the presence of OM 2  $\mu\text{M}$  ( $p\text{EC}_{50}$  6.12  $\pm$  0.12,  $p = .67$ ) or 600 nM ( $p\text{EC}_{50}$  6.18  $\pm$  0.11,  $p = .90$ ) (Figure 6A), with no observed difference in left ( $p = .55$ ; Figure 6B) and right ( $p = .65$ ; Figure 6C) ventricular trabeculae.

(-)-Noradrenaline caused concentration-dependent reductions in TPF and  $t_{50\%}$  (Figure 7). Under basal conditions, there was no significant difference between groups for TPF and  $t_{50\%}$ . Following incubation with OM 600 nM and 2  $\mu\text{M}$  for 120 min, there was a significant increase in TPF and  $t_{50\%}$ . (-)-Noradrenaline concentration effect curves were then constructed. Trabeculae with no exposure to OM, achieved significant improvements in mean TPF and  $t_{50\%}$  with (-)-noradrenaline when compared to their respective baseline values (TPF:  $-36.3 \pm 7.3$  ms;  $p < .001$ ,  $t_{50\%}$ :  $-49.2 \pm 5.0$  ms;  $p < .001$ ). Significant improvements, although less dramatic, were also observed for 600 nM (TPF:  $-23.1 \pm 8.7$  ms;  $p = .01$ ,  $t_{50\%}$ :  $-30.7 \pm 5.3$  ms;  $p < .001$ ). However, despite maximal  $\beta_1$ -AR effect, TPF and  $t_{50\%}$  for trabeculae exposed to 2  $\mu\text{M}$  OM did not achieve mean baseline values (TPF:  $5.3 \pm 7.7$  ms;  $p = .49$ ,  $t_{50\%}$ :  $3.6 \pm 6.8$  ms;  $p = .60$ ). These results were consistent when analysing ventricles separately (Figure S1). There was no significant difference in the magnitude of change in TPF and  $t_{50\%}$ , produced by (-)-noradrenaline in the absence or presence of OM (TPF:  $p = .87$ ,  $t_{50\%}$ :  $p = .22$ ) (Tables S5–S6).

TABLE 2 Estimates of mean differences for contrasts indicated derived from linear mixed-effects model

Contrast <sup>a</sup>	Category	Combined		Left		Right	
		Coefficient (95% CI)	p-value	Coefficient (95% CI)	p-value	Coefficient (95% CI)	p-value
Group at baseline	Control	Reference		Reference		Reference	
	600	-0.09 (-0.62 to 0.44)	1	-0.02 (-0.58 to 0.54)	1	-0.16 (-0.96 to 0.64)	1
	2000	0.16 (-0.37 to 0.69)	1	-0.03 (-0.59 to 0.53)	1	0.34 (-0.45 to 1.14)	1
Group at 120 min	Control	Reference		Reference		Reference	
	600	2.03 (1.67 to 2.38)	<.001	3.36 (2.80 to 3.93)	<0.001	0.69 (-0.11 to 1.48)	.17
	2000	2.55 (2.19 to 2.90)	<.001	3.89 (3.33 to 4.45)	<0.001	1.21 (0.41 to 2.00)	<.001
Average change from baseline to 120 min	Control	-4.20 (-6.63 to -1.76)	<.001	-4.50 (-7.12 to -1.88)	<0.001	-3.89 (-6.96 to -0.82)	.003
	600	-2.08 (-4.52 to 0.36)	.18	-1.12 (-3.73 to 1.50)	1	-3.04 (-6.12 to 0.03)	.055
	2000	-1.81 (-4.24 to 0.63)	.45	-0.58 (-3.30 to 2.03)	1	-3.03 (-6.10 to 0.04)	.057
Average difference between sides	Left	Reference					
	Right	-0.8 (-0.95 to -0.65)	<.001				

There were no significant differences between groups at baseline. At 120 min, there was a significant difference in mean contractile force for both concentrations of OM (600 nM and 2  $\mu$ M) in ventricular trabeculae from the LV and combined (left and right) groups when compared to control ( $p < .001$ ). In *right* ventricular trabeculae, only 2  $\mu$ M produced a significant difference ( $p < .001$ ). The control groups of left, right and combined ventricular trabeculae all displayed a significant reduction in contractile force from baseline to 120 min ( $p < .05$ ). Left and combined ventricular trabeculae groups displayed evidence of preservation in force with no significant reduction ( $p > .05$ ). Force in right ventricular trabeculae declined from baseline to 120 min, which was of borderline significance, in the presence of OM (600 nM:  $p = .055$ , 2  $\mu$ M:  $p = .057$ ). Mean force was significantly lower in the right ventricle compared to the left.

<sup>a</sup>Contrasts derived from linear mixed models fitted with random intercept and slope for heart ID and fixed effects for group, time and their interaction (time\*group) (all models) and side (combined) (1647 measurements per side on 9 hearts);  $p$ -values adjusted for multiple comparisons.

## 4 | DISCUSSION

The utilization of explanted failing hearts to investigate effects of clinically relevant concentrations of OM,<sup>13,14</sup> should facilitate translation to clinical practice and may aid clinicians with an understanding of its interaction with traditional inotropes. Both therapeutic and suprathreshold OM concentrations exerted concentration dependent increases in TPF and  $t_{50\%}$ , and preserved contractile strength, preventing a significant decline in force overtime compared to control. OM did not affect the potency of (-)-noradrenaline but the positive lusitropic effects of (-)-noradrenaline reversed the delay in TPF and  $t_{50\%}$  imparted by OM.

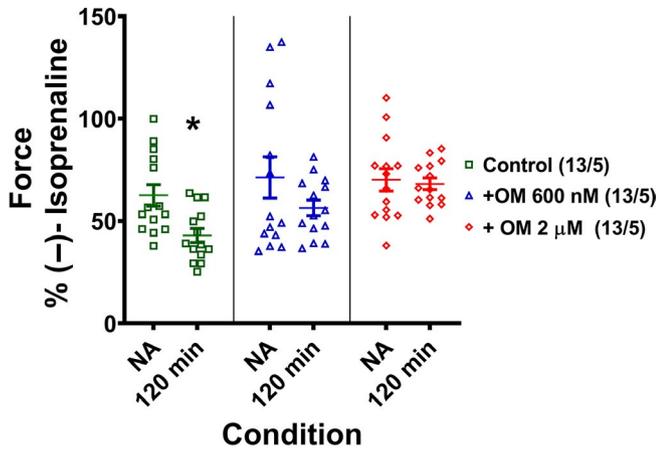
### 4.1 | Effect on ventricular contractile dynamics

In human failing ventricle, OM increases TPF and  $t_{50\%}$  in a concentration-dependent manner (Figure 2). Clinical models explained the enhanced cardiac performance achieved by OM was through prolongation of the SET, which TPF and  $t_{50\%}$  contribute.<sup>12,13</sup> OM increases the fraction and strength of actin-bound myosin XBs. However, the cost is an increased internal load, creating drag and reducing filament velocity, delaying TPF.<sup>6,7</sup> The sliding velocity was not directly measured in this study, but previous groups have reported a 15- to 20-fold reduction with porcine and human cardiac myosin.<sup>7,8</sup> In addition, OM impairs diastolic relaxation by reducing XB detachment increasing the time XBs remain bound to actin.<sup>27</sup> It is important to confirm the original findings in healthy human myocardium,<sup>4,9,10</sup>

as cardiomyopathies can affect components of the actomyosin XB cycle and reduce the duty ratio.<sup>28</sup> Furthermore, recent stretch activation experiments in permeabilized human failing myocardium displayed reduced XB recruitment and force generating capacity compared to normal myocardium.<sup>27</sup> These studies were performed at Lmax, imparting stretch representing preload found in normal physiology. Despite these differences, human failing myocardium, under Lmax, continued to produce concentration-dependent increases in TPF and  $t_{50\%}$  to OM.

### 4.2 | Effect on peak contractile strength

Unlike earlier studies, no significant inotropic response by OM was detected.<sup>4,8,9</sup> Instead OM preserved contractile strength, reducing the progressive reduction in force (fade) over time. Trabeculae exposed to OM displayed a significantly higher mean force at 120 min compared to control. This was mainly driven by results in the LV trabeculae (Figure 3 and Table 2). There are several factors which might explain the differing results observed here and in prior studies.<sup>4,8,27</sup> First, humans predominantly express the  $\beta$ -myosin heavy chain (MHC) compared to the  $\alpha$ -MHC isoform in rodents.<sup>4</sup>  $\beta$ -MHC is a slower myosin motor with a longer XB duty ratio which may explain the disparity in force enhancement.<sup>9,29</sup> Second, early human myocyte studies used healthy hearts<sup>8,15</sup> and permeabilized or skinned preparations.<sup>8,9,27</sup> Failing myocytes have alterations in phosphorylation status of key contractile proteins which could account for reduced maximal forces and XB detachment.<sup>30-32</sup> The structurally intact trabeculae prepared in these experiments

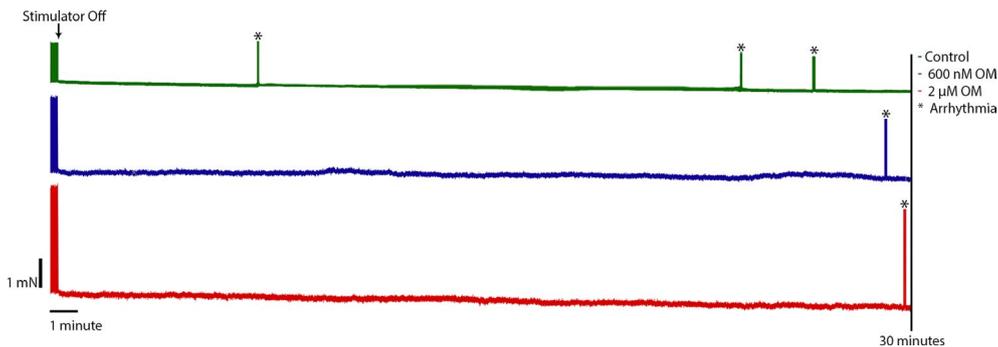


**FIGURE 4** Effect of OM on force of contraction following incubation with 1  $\mu\text{M}$  (-)-noradrenaline (NA). Following 1  $\mu\text{M}$  (-)-noradrenaline (NA), there was no significant difference in contractile force between groups at baseline (see NA bar) ( $p = .66$ ). At 120 min, contractile force was maintained in trabeculae incubated with OM (600 nM and 2  $\mu\text{M}$ ), whilst the control group displayed a significant reduction in force ( $*p = .005$ ). There was a significant difference in the contractile force achieved at 120 min between control versus 2  $\mu\text{M}$  OM ( $p < .001$ ) control versus 600 nM OM ( $p = .02$ ). Statistical analysis by one-way ANOVA test followed by Tukey's multiple comparisons test was performed

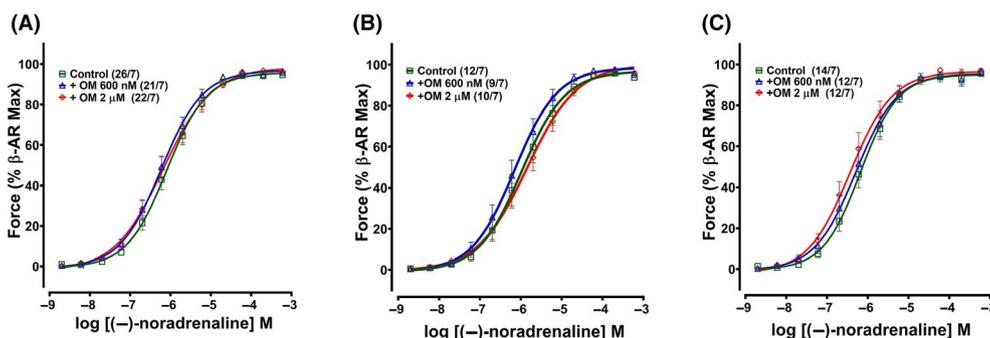
may affect the diffusion of OM molecules across the membrane to equilibrate around the sarcomere lattice. Compared to skinned preparations, this could alter OM's interaction and 'blunt' any induced increase in force. Third, OM's effect on force enhancement is affected by  $[\text{Ca}^{2+}]$ ,<sup>10,15</sup> with increased force at submaximal concentrations (pCa 6.2) and then diminishing effects as the  $[\text{Ca}^{2+}]$  increased.<sup>9</sup> In this study, a fixed 2.25 mM concentration of  $\text{Ca}^{2+}$  was used in all experiments, reflecting the normal physiological state, but may have contributed to the lack of inotropic response. Last, any possible inotropic effect is balanced against a time dependent fading baseline. The incubation solution used in the initial protocols lacked a  $\beta$ -AR agonist which would be present in vivo as part of neurohormonal signalling. To investigate whether this contributed to fade, trabeculae were co-incubated with 1  $\mu\text{M}$  (-)-noradrenaline. At 120 min, contractile force was not significantly reduced in trabeculae exposed to OM (Figure 4). This requires further investigation but from a purely inotropic perspective, OM and (-)-noradrenaline may work synergistically.

### 4.3 | Interaction with (-)-noradrenaline via $\beta_1$ -adrenoceptor

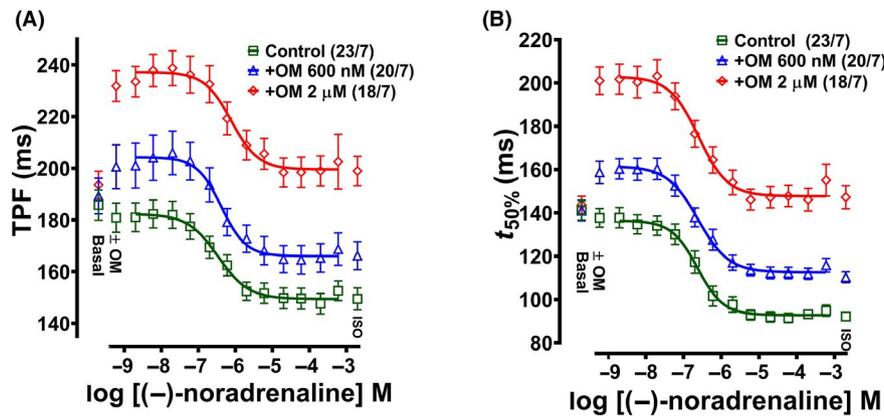
This present study extends our current knowledge by investigating the impact of OM in the presence of (-)-noradrenaline and selective



**FIGURE 5** Arrhythmia protocol. Following incubation with OM at 60 bpm (1 Hz), stimulators were turned off, and trabeculae observed for spontaneous contractions (\*). There was no increase in spontaneous contractions ( $p > .05$ ), statistical analysis using Kruskal-Wallis test



**FIGURE 6** Conservation of the inotropic effects of (-)-noradrenaline in the presence of OM. OM (600 nM or 2  $\mu\text{M}$ ) does not affect the potency or maximal inotropic effect of (-)-noradrenaline. This finding was reproduced with all trabeculae (A) and when analysing left (B) and right (C) ventricular trabeculae separately ( $p > .05$ ). An ordinary one-way ANOVA test followed by Tukey's multiple comparisons test was performed



**FIGURE 7** Opposing diastolic effects of noradrenaline and OM in both left and right ventricular trabeculae. Concentration-dependent effects of (-)-noradrenaline on TPF (A) and  $t_{50\%}$  (B) in left and right ventricular trabeculae previously exposed to OM. In both figures, basal represents the TPF and  $t_{50\%}$  of trabeculae before exposure to any compound, there was no significant difference between groups (TPF:  $p = .65$ ,  $t_{50\%}$ :  $p = .91$ ).  $\pm$  OM represents the TPF and  $t_{50\%}$  after 120 min of exposure to  $\pm$ OM. ISO reflects maximal  $\beta$ -AR ( $\beta_1$ -AR +  $\beta_2$ -AR) activity. (-)-Noradrenaline had powerful lusitropic effects on the control group (Green), which were not exposed to OM, significantly reducing TPF and  $t_{50\%}$  below baseline. Trabeculae exposed to 2  $\mu$ M and 600 nM OM had an increase in TPF and  $t_{50\%}$  prior to the addition of (-)-noradrenaline. Despite maximal  $\beta$ -AR effect, the TPF and  $t_{50\%}$  remained greater than basal for 2  $\mu$ M OM, whilst 600 nM also exhibited a significant reduction

$\beta_1$ -AR stimulation. OM did not affect the potency or positive inotropic effects of (-)-noradrenaline (Figure 6). By virtue of the proposed downstream  $\text{Ca}^{2+}$  sensitizing effects from the recruitment of XBs, it was expected the potency ( $pEC_{50}$ ) of (-)-noradrenaline would increase.<sup>8,33</sup> This could have proved clinically favorable due to the adverse arrhythmic and metabolic effects with higher concentrations of traditional inotropes. Importantly, (-)-noradrenaline, via  $\beta_1$ -AR, counteracted the negative diastolic effects of OM. However, in trabeculae co-incubated with OM, (-)-noradrenaline, at best, only restored diastolic parameters to baseline values at maximal  $\beta_1$ -AR agonism (Figure 7). In human clinical studies, supratherapeutic levels of OM produced excessive prolongation of systole, compromising diastolic coronary blood flow, inducing chest pain and myocardial ischemia.<sup>13,26</sup> Diastolic dysfunction is an independent risk factor for refractory cardiogenic shock and mortality.<sup>34,35</sup> Stimulation of  $\beta_1$ -AR by (-)-noradrenaline has powerful lusitropic effects.<sup>36,37</sup> Coordinated by cAMP-dependent protein kinase, phosphorylation of phospholamban and TnI accelerates the recycling of cytosolic  $\text{Ca}^{2+}$  into the sarcoplasmic reticulum and reduces the affinity of  $\text{Ca}^{2+}$  for troponin C.<sup>20,31,37</sup> Together, these two mechanisms improve diastolic performance. It is a concern that in trabeculae exposed to OM, diastolic indices only improved to their baseline values at maximal  $\beta_1$ -AR activation. In clinical practice, submaximal (-)-noradrenaline concentrations are infused due to potential adverse pro-arrhythmic and metabolic effects. For the foreseeable future, traditional inotropes will remain standard medical therapy for management of cardiogenic shock. In the presence of OM, inotropes may not overcome the negative diastolic effects. Their titration may be impaired as the threshold for ischemia will be reduced, a function of increased metabolic demand (imparted by inotropes) and reduced diastolic perfusion. A final cautionary note and limitation to this study is that ventricular trabeculae

were stimulated at 60 bpm. Models have revealed that with increasing concentrations of OM, cardiac contraction and diastolic performance is further impaired at increased pacing rates.<sup>38,39</sup> Unfortunately, patients in cardiogenic shock are generally tachycardic, which inotropes compound.

#### 4.4 | Effect on spontaneous contractions

There was no increase in spontaneous contractions in the presence of OM. Fatal ventricular arrhythmias in HF patients result from triggered activity termed delayed afterdepolarization and result of pathological diastolic  $\text{Ca}^{2+}$  release.<sup>30,40,41</sup> The results (Figure 5) support a mechanism independent of  $\text{Ca}^{2+}$ .

#### 4.5 | Limitations

The contracting human atrial and ventricular trabeculae preparation is extremely valuable for understanding the effects and mechanisms of medicines directly relevant for clinical interpretation.<sup>18,20,42</sup> Superfused working human trabeculae will likely have a hypoxic core due to their size which may confound observed results, especially contractile strength. However, Pecha et al found only a very weak correlation between force generation and cross-sectional area.<sup>43</sup> Our studies used trabeculae with a mean width  $1.96 \pm 0.76$  mm and cross-sectional area of  $3.7 \pm 2.1$  mm<sup>2</sup>, therefore minimizing hypoxia. Second, Munro et al observed a high degree of variability in force generation, when normalized to cross-sectional area.<sup>44</sup> Instead, force generation was strongly related to myocyte content which is highly variable between failing hearts. In the failing heart, trabeculae have similar structural changes in comparison

with the ventricular free wall but have more variable myocyte content contributing to variable contractile force. Unfortunately, ventricular tissue from the donated hearts is currently not made available for research. This would have strengthened our study to compare results with non-failing human ventricle trabeculae. While the concentrations of OM were chosen to reflect clinical and suprathreshold doses, the pharmacodynamics present in whole systems is extremely different to in vitro. Consequently, one must use these results as guidance when extrapolating the results to the clinical setting.

## 5 | CONCLUSION

This study affirms known negative diastolic effects of OM, but on human failing ventricle. Whilst no significant inotropic effect was observed, OM preserved the contractile force over time. The findings provide new insights in drug-drug interactions of OM with traditional inotropes; the competing effects on diastolic performance and potential ischemic effects. Collectively, this will aid clinicians who administer OM in the treatment of patients with advanced heart failure.

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## DISCLOSURE

None declared.

## AUTHORS CONTRIBUTION

All listed authors have made substantial contributions to conception and design of the project, as well as critical revision of the manuscript. The final manuscript has been approved by all authors and agree to the integrity and accuracy of the work presented. Dr Alexander Dashwood, Professor Peter Molenaar and Elizabeth Cheesman were present for all experiments conducted. Karen Hay provided additional statistical analysis and critical appraisal of data interpretation.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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