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Dose-Dependent Effects of the Myosin Activator Omecamtiv Mecarbil on Cross-Bridge Behavior and Force Generation in Failing Human Myocardium

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Abstract

Background—Omecamtiv mecarbil (OM) enhances systolic function *in vivo* by directly binding the myosin cross-bridges (XBs) in the sarcomere. However, the mechanistic details governing OM-induced modulation of XB behavior in failing human myocardium are unclear.

Methods and results—The effects of OM on steady-state and dynamic XB behavior were measured in chemically-skinned myocardial preparations isolated from human donor and heart failure (HF) left ventricle. HF myocardium exhibited impaired contractile function as evidenced by reduced maximal force, magnitude of XB recruitment (P_{df}), and a slowed rate of XB detachment (k_{rel}) at submaximal Ca^{2+} -activations. Ca^{2+} -sensitivity of force generation (pCa_{50}) was higher in HF myocardium when compared to donor myocardium, both prior to and following OM incubations. OM incubation (0.5 μ M and 1.0 μ M) enhanced force generation at sub-maximal Ca^{2+} -activations in a dose-dependent manner. Notably, OM-induced a slowing in k_{rel} with 1.0 μ M OM but not with 0.5 μ M OM in HF myocardium. Additionally, OM exerted other differential effects on XB behavior in HF myocardium as evidenced by a greater enhancement in P_{df} and slowing in the time-course of cooperative XB recruitment (T_{rec}), which collectively prolonged achievement of peak force development (T_{pk}), compared to donor myocardium.

Conclusions—Our findings demonstrate that OM augments force generation but also prolongs the time course of XB transitions to force-bearing states in remodeled HF myocardium which may extend the systolic ejection time (SET) *in vivo*. Optimal OM dosing is critical for eliciting

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enhanced systolic function without excessive prolongation of SET which may compromise diastolic filling.

Keywords

Omecamtiv mecarbil; contractile function; human heart failure

Progression of heart disease to end-stage heart failure (HF) is often the result of long-term maladaptive remodeling coupled with deteriorating cardiomyocyte function. Although, the fundamental pathophysiological mechanisms that lead to HF are not completely understood¹, a common feature of many forms of HF is decreased contractility leading to diminished force generation at the cardiomyocyte level and diminished systolic performance at the whole-heart level. Importantly, despite significant therapeutic advances, the clinical course of HF patients remains unacceptably poor and continues to pose major clinical challenges.^{2, 3} Current therapies aimed at increasing cardiac contractility have not improved long-term outcomes, and carry the potential for undesirable adverse side effects related to activation of signaling pathways that enhance cardiomyocyte intracellular Ca^{2+} concentrations.^{2, 4, 5} Thus, recent efforts^{6, 7} have focused on designing HF therapies that circumvent complex signaling pathways, and instead directly target the force-generating contractile machinery of the myofilaments.

In this regard, omecamtiv mecarbil (OM), a molecule that selectively binds the catalytic domain of myosin, has gained significant attention due to its potentially beneficial effect on systolic function in HF patients.^{5, 7, 8} Early clinical trials have demonstrated that OM augments systolic indices such as systolic ejection time, stroke volume, and ejection fraction⁵ without altering cardiomyocyte Ca^{2+} transients.⁹ The mechanisms underlying OM-induced force enhancements have been linked to an enhanced stability of the actin-bound myosin cross-bridges (XBs)¹⁰, slowed strain-induced XB detachment from actin^{11, 12}, and slowed shortening velocity¹³, which act to prolong the XB duty cycle¹⁴. Additionally, OM has been shown to accelerate phosphate release^{10, 15} but concomitantly delays the XB powerstroke and actin-activated ATP turnover¹⁵, thereby, maintaining XBs in a strongly-bound state. Thus, in theory, a prolonged XB duty cycle should reduce ATP utilization and improve contractile efficiency¹¹, thereby reducing workload in failing hearts.

Initial clinical trials have demonstrated that OM induces dose-dependent increases in stroke volume and fractional shortening in healthy volunteers⁵ and HF subjects without adverse side-effects.^{2, 7} Despite these early positive results, the functional impact of OM on dynamic XB behavior at the cellular level has not been established, and there are no studies that have systematically examined the impact of OM on myofilament contractile function in human failing myocardium. This information would be valuable because it has been shown that failing human myocardium displays pathological remodeling, altered contractile protein phosphorylation, and diminished force generating capacity^{16, 17} –factors that could potentially modify the impact of OM on XB behavior. Thus, in this study we tested whether the dose-dependent impact of OM on XB behavior differs in ventricular myocardium isolated from donor and failing hearts.

Methods

An expanded Methods section is available in the Supplemental material.

Ethical approval and procurement of human myocardial tissue samples

Normal, non-failing (donor) and failing left ventricular (LV) human cardiac tissue samples were collected and stored until used for experiments as per the approved guidelines of the University of Sydney.

Determination of sarcomeric protein expression and phosphorylation levels

Coomassie and Pro-Q Diamond phosphoprotein staining was performed to quantify myofilament protein expression and phosphorylation in the cardiac samples as described previously^{18, 19} (Figures 1A and B). Quantification of β -myosin heavy chain (β -MHC) expression (n=6 per group) (Figure 1C) was performed as described previously.²⁰ Gels were scanned using Image J software.¹⁸

Preparation of chemically-skinned multicellular myocardial preparations, Ca^{2+} solutions, and OM solutions

Detergent-skinned multicellular ventricular preparations (~100 μm in width and ~400 μm in length) were prepared as described earlier.¹⁸ The composition of various Ca^{2+} solutions²¹ used for experiments was calculated using a computer program²² and using established stability constants²³. OM (Selleckchem, Houston, TX, USA) and was initially dissolved in DMSO¹¹ and diluted in a relaxing solution to achieve final concentrations of 0.5 μM or 1 μM OM. Functional measurements (Figures 2, 3, 4, and 5) were performed at ~24°C following a 2-minute incubation with 0.5 μM or 1 μM OM.

Stretch activation experiments to measure dynamic XB contractile parameters

The stretch activation protocol used here was described in detail previously.^{11, 18} Skinned myocardial preparations were exposed to pCa solutions that generated ~25% of maximal force prior to OM incubations (i.e., pCa 6.6 and pCa 6.3 in HF and donor myocardium, respectively). As done earlier²⁴, a lower pCa (i.e., pCa 6.6) was chosen for HF group to account for an increased myofilament Ca^{2+} sensitivity when compared to the donor group. The key features of cardiac muscle stretch activation responses are depicted in Figure 4A. A mechanical slack-restretch protocol was used to measure the rate of tension redevelopment, k_{tr} , using linear transformation of the half-time of force redevelopment, i.e., $k_{\text{tr}} = 0.693/t_{1/2}$, where $t_{1/2}$ is the time (in milliseconds) required to reach half maximal force of the k_{tr} trace.²⁴

Data Analysis

Data were analyzed with a linear mixed (LM) effect model²⁵ with an unstructured covariance matrix across the conditions and across the groups. The R package *lme4* was used to implement the LM analysis.^{26, 27} Asterisks in figures and tables denote statistical significance using a Tukey *post hoc* multiple comparisons test, and type-I error was controlled by adjusting P-values for multiple comparisons using a false discovery rate (FDR)

method. Values are reported as mean \pm SEM and the criterion for statistical significance was set at $P < 0.05$.

Results

Sarcomeric protein expression and phosphorylation levels

The basal phosphorylation levels of cardiac myosin binding protein-C (MyBP-C), troponin T (TnT) and troponin I (TnI) were reduced in HF samples compared to donor samples. Phosphorylation levels of MyBP-C, TnT, and TnI in HF were $\sim 65 \pm 11\%$ ($P=0.001$), $\sim 68 \pm 10\%$ ($P=0.04$), and $\sim 33 \pm 8\%$ ($P<0.005$), respectively compared to donor myocardium ($n=4$; Figures 1A and 1B). Phosphorylation levels of other sarcomeric proteins such as regulatory light chain were not significantly different between the groups. Donor and HF ventricular samples expressed 100% β -MHC isoform without evidence of α -MHC expression (Figure 1C). We have previously shown that OM incubation does not affect the phosphorylation status of sarcomeric proteins.¹¹

Effects of OM on Ca²⁺-activated force generation

Ca²⁺-activated force generation was measured in skinned ventricular myocardial preparations in a range of pCa that yielded forces below half-maximal force (i.e., in pCa's 6.6, 6.5, 6.4, and 6.3). Force measurements were collected at baseline or following a 2-minute incubation in 0.5 μ M or 1.0 μ M OM. The increase in OM-induced force enhancement gradually decreased with increasing levels of Ca²⁺ activations (Figure 2), as reported previously.¹¹ Force enhancements following 0.5 μ M OM incubation were: $\sim 28 \pm 5\%$, $25 \pm 3\%$, $22 \pm 3\%$, and $19 \pm 4\%$ in the donor group; and $\sim 24 \pm 7\%$, $19 \pm 3\%$, $13 \pm 2\%$, and $8 \pm 2\%$ in the HF group at pCa's 6.6, 6.5, 6.4, and 6.3, respectively. Force enhancements following 1.0 μ M OM incubation were more pronounced: $\sim 85 \pm 11\%$, $63 \pm 9\%$, $54 \pm 7\%$, and $28 \pm 5\%$ in the donor group; and $\sim 78 \pm 13\%$, $67 \pm 17\%$, $36 \pm 10\%$, and $27 \pm 6\%$ in the HF group at pCa's 6.6, 6.5, 6.4, and 6.3, respectively. Importantly, OM-induced force enhancement was more pronounced in HF myocardium than in donor myocardium when comparisons were made at equivalent baseline levels of force production (i.e., at pCa 6.6 in HF vs. pCa 6.3 in donor). Furthermore, OM incubation did not alter either the Ca²⁺-independent force (i.e., F_{\min} measured at pCa 9.0) and maximal Ca²⁺-activated force (i.e., F_{\max} measured at pCa 4.5) in both donor¹¹ and HF groups (Table 1).

Effects of OM on myofilament Ca²⁺ sensitivity (pCa₅₀) and cooperativity of force development (n_H)

Under basal conditions HF myocardium displayed increased pCa₅₀ i.e., an increased responsiveness of cardiac myofilaments to Ca²⁺ when compared to donor myocardium, as indicated by a significant left-ward shift in the force-pCa relationships (Figure 3A), a trend that was maintained even after OM incubation (Table 1). The increase in Ca²⁺-sensitivity post-OM incubations was mainly confined to forces $< 40\%$ of maximal force, and hence pCa₅₀ was unaltered in both groups (Table 1; Figures 3B and 3C). n_H values decreased post-OM incubations in both groups, indicating that OM decreased the overall cooperativity of force production (Table 1).

Effects of OM on k_{tr} —When studied at equivalent levels of steady-state force generation (i.e., at pCa 6.3 and 6.6, in donor and HF myocardium, respectively), no differences in k_{tr} were observed between donor and HF prior to OM incubation (Table 2). OM differentially affected k_{tr} in HF and the donor group: following 0.5 μ M and 1.0 μ M OM incubations: k_{tr} was slowed by ~33% and ~32%, respectively in the HF group but not in donor group, suggesting that OM slows XB transitions from weakly-bound to strongly-bound states, or from strongly-bound to weakly-bound states, or both, resulting in an overall slowing of XB cycling in HF myocardium.

Effects of OM on dynamic stretch activation parameters

Stretch activation parameters (Figure 4) were assessed following OM incubations (0.5 μ M or 1.0 μ M) at equivalent levels of force generation (i.e., at pCa 6.3 and 6.6, respectively in donor and HF myocardium). The impact of OM on the rate of XB detachment was assessed by measuring k_{rel} . Our findings show that donor myocardium displayed accelerated k_{rel} (~35%; $p=0.03$), and a greater magnitude of XB detachment (P2) as indicated by more negative P2 values compared to HF myocardium (Table 2). Interestingly, incubation with OM did not alter k_{rel} in donor myocardium, but k_{rel} was slowed following 1.0 μ M OM by ~30% in HF myocardium, indicating a differential effect of OM on k_{rel} in HF myocardium (Table 2).

The impact of OM on the rate and magnitude of XB recruitment was assessed by measuring k_{df} and P_{df} , respectively. k_{df} was slowed by ~28% and ~44%, respectively, in donor myocardium following 0.5 μ M and 1.0 μ M OM incubations. Whereas k_{df} was slowed by ~42% and ~57%, respectively, in HF myocardium following 0.5 μ M and 1.0 μ M OM incubations (Table 2). Prior to OM incubation, donor myocardium exhibited greater P_{df} (~73% higher vs. HF myocardium; $p=0.02$, Table 2), suggesting impairments in XB recruitment in HF myocardium. OM incubations did not affect the new steady-state force attained following the imposed stretch (i.e., P3) in either group (Table 2). P_{df} was enhanced by ~74% and ~65% ($p=0.02$) following 0.5 μ M and 1.0 μ M OM incubations in HF myocardium, respectively, such that the overall number of XBs recruited into the force-bearing state was substantially enhanced, whereas no OM-induced enhancement in P_{df} was observed in donor myocardium (Table 2) –indicating a differential effect of OM on P_{df} in HF myocardium.

To gain further insights into the mechanisms by which OM differentially affects XB behavior in donor and HF myocardium, we measured T_{rec} , the time lag between k_{rel} and k_{df} (please see supplement for a detailed explanation). T_{rec} can be interpreted as the time required for the transition between XB detachment and the commencement of XB attachment to actin due to the recruitment of XBs into the force-bearing state. T_{rec} was not statistically different between HF and donor myocardium (Table 2; Figure 5A) under basal conditions. Importantly, following OM incubation HF myocardium displayed ~47% and ~62% increases in T_{rec} (Table 3; Figure 5A) at 0.5 μ M and 1.0 μ M OM, respectively, but no effects of OM on T_{rec} were observed in donor myocardium. Thus, T_{rec} in HF myocardium was ~109% and ~149% longer than in donor myocardium following 0.5 μ M and 1.0 μ M OM incubations ($p<0.005$; Table 3, Figure 5A), suggesting a differential effect of OM on T_{rec} in HF myocardium.

No differences in T_{pk} (i.e., time to achieve peak force development) were observed between donor and HF myocardium under basal conditions, but T_{pk} was ~32% and ~38% longer ($p < 0.005$) (Figures 4A and 5B) in HF myocardium vs. donor myocardium following 0.5 μ M and 1.0 μ M OM incubations, respectively (Table 3). This indicates a greater delay in achievement of peak force generation in HF myocardium and thereby a differential effect of OM on T_{pk} in HF myocardium.

Discussion

Omecamtiv mecarbil (OM) is currently in clinical trials as a potential therapy for systolic heart failure (HF)^{6, 7} due to its ability to augment acto-myosin XB interactions and force generation without the undesirable secondary effects of other inotropic agents. However, despite early evidence of the utility of OM in HF patients, the detailed mechanisms underlying OM-induced effects on XB behavior at the cellular level in HF myocardium have not been established. Here we show that OM enhances force generation in HF myocardium by shifting the XB equilibrium towards a force-bearing state, however, the time course of XB recruitment is prolonged such that achievement of peak force generation is delayed when compared to donor myocardium.

HF myocardium exhibits reduced magnitude of XB recruitment

Poor systolic function is a common feature of HF patients, and the contractile deficits can often be traced to the contractile machinery's inability to elicit sufficient force-generating interactions between myosin and actin. Indeed, previous studies have reported contractile abnormalities in HF myocardium¹⁷, as evidenced by reduced maximal force generation and enhanced myofilament Ca^{2+} sensitivity (i.e., pCa_{50}). However, the mechanisms underlying the changes in dynamic regulation of XB behavior in HF myocardium are unknown. Consistent with previous reports¹⁷, we observed reduced steady-state maximal force generation and increased pCa_{50} in HF myocardium when compared to donor myocardium (Table 1; Figure 3A). However, unlike previous studies^{11, 25}, OM incubation did not enhance the pCa_{50} (Table 1). The cause of this discrepancy is unclear, but may be related to a basal temperature-dependent sensitization of thin filaments which acts to decrease the impact of OM on pCa_{50} (15°C²⁵ and 22°C¹¹ in earlier studies vs. 24°C in the present study). Results from stretch activation experiments revealed a slowed rate of XB detachment (i.e., k_{rel} , Table 2) in HF myocardium, which acts to prolong XB duty ratio and increase force generation at low Ca^{2+} -activations. Furthermore, despite a lack of difference in basal rates of XB recruitment between HF and donor myocardium (i.e., k_{tr} and k_{df} , Table 2), HF myocardium displayed impairments in the magnitude of XB recruitment, (i.e., P_{df} , Table 2), thereby contributing to a reduced force generating capacity. These findings suggest that factors inherent to HF myocardium, such as contractile protein dephosphorylation, contribute to impaired force generation. Collectively, prolonged XB on time due to slowed XB detachment and a reduced magnitude of XB recruitment are likely contributors to slowed rates of diastolic relaxation and impaired systolic function displayed by HF patients *in vivo*.

It is unclear why HF myocardial samples generate lower maximal forces, display slower rates of XB detachment, and recruit less XBs into force-bearing states, but these effects

could be related to diminished phosphorylation of key contractile proteins such as MyBP-C, TnI, and TnT (Figure 1), which have been shown to be reduced in HF¹⁷. In particular, we have previously shown that increased MyBP-C phosphorylation by protein kinase A (PKA) accelerates the rates and enhances the magnitudes of XB detachment and recruitment^{24, 28}, whereas MyBP-C dephosphorylation slows the rates and reduces the magnitudes of XB detachment and recruitment^{18, 24}. In addition, PKA-mediated TnI phosphorylation has been shown to accelerate XB detachment and decrease myofilament Ca²⁺ sensitivity²⁹. Thus, reduced MyBP-C and TnI phosphorylation in HF myocardium would be expected to slow the rate of XB detachment (Table 2; Figure 5), and decrease the number of XBs entering the cycling pool during myofilament activation. Furthermore, our finding that TnT phosphorylation is reduced in HF myocardium (Figure 1) suggests that TnT may also be a component of β -adrenergic signaling^{21, 30}, a pathway known to be downregulated during HF³¹, although the specific impact of PKA-mediated TnT phosphorylation is yet to be elucidated. Moreover, it is likely that although total TnT phosphorylation is reduced, PKC-mediated TnT phosphorylation may be upregulated in HF³², contributing to slowed XB detachment in HF myocardium. In contrast to reduced MyBP-C, TnI, and TnT phosphorylation, we did not observe changes in RLC phosphorylation which is consistent with our data demonstrating that baseline k_{df} is similar in HF and donor groups (Table 2), because increased RLC phosphorylation would be expected to accelerate k_{df} ³³.

Additionally, it is also possible that HF myocardium harbors an increased number of super-relaxed (SRX) myosin heads, characterized by substantially slower ATP turnover rates, which have been linked to reduced MyBP-C phosphorylation³⁴. These SRX heads are proposed to be folded over and interacting with the thick filament backbone rather than interacting with myosin binding sites on actin, thus potentially decreasing thin filament activation and force generation in HF myocardium, although this possibility requires further investigation.

OM enhances the magnitude of XB recruitment in HF myocardium

Earlier studies have shown that OM enhances myofilament force generation by altering the kinetic properties of the β -MHC motor.^{11, 13, 35} As shown earlier¹¹, we observed that OM-induced force enhancements were primarily confined to sub-maximal Ca²⁺-activations and that force enhancements gradually decline with increasing Ca²⁺ levels (Figure 2). As reported earlier¹¹, OM treatment also reduced the cooperativity of force generation, n_H (Table 1) which may be related to the fact that at low [Ca²⁺], thin filament activation relies more on XB-mediated cooperative XB recruitment as most of the regulatory units (RU) are in the *off* state.³⁶ However, as intracellular [Ca²⁺] rises most of the RUs switch to the *on* state, effectively reducing the dependence on XB-mediated cooperative XB recruitment, which offsets the impact of OM and decreases the overall steepness of n_H ³⁷.

In addition to enhanced force generation, OM slowed the rate of XB recruitment (k_{df}) (Table 2) which can be linked to OM's ability to shift XB equilibrium toward a force-bearing state through enhanced XB-mediated cooperative XB-recruitment to neighboring RUs, a slow and time consuming process. At higher doses (1.0 μ M), OM also slowed the rate of XB detachment (k_{rel}) in HF myocardium (Table 2). Thus, at higher concentrations, OM-induced

force increases maybe, in part, attributed to a slowing in k_{rel} which concomitantly increases the time that myosin XBs remain bound to actin (increased XB duty ratio)^{11, 25}, an effect which has been proposed to be due to allosteric changes in myosin heads³⁸ and delayed actin-induced myosin light chain domain rotation.¹⁵ Furthermore, OM also traps a population of XBs in a weak actin affinity state²⁵, and those myosin heads may not be readily available for recruitment, or they may transition towards the force-bearing state very slowly – a mechanism that contributes to a prolonged time course of cooperative XB recruitment and slowing of force generation.

Importantly, when tested at equivalent submaximal activation levels, OM elicited differential effects on XB behavior in HF myocardium. Notably, the basal time course of XB recruitment to a force-generating state, T_{rec} , was not different between donor and HF myocardium, however, following OM incubation T_{rec} was significantly slower in HF myocardium (Table 3). A greater OM-induced slowing of T_{rec} in HF myocardium would be predicted to slow the overall rate of XB cycling from a weakly-bound to a strongly-bound state and vice versa (k_{tr}).³⁹ Indeed, OM-induced slowing in k_{tr} is observed in HF myocardium but not in donor myocardium (Table 2). An indirect effect of slowed XB cycling in HF myocardium is a further prolongation of the XB duty ratio, which acts to prolong the open state of tropomyosin, thus permitting more XB recruitment and binding to additional open actin sites. In support of this notion, we observed that the magnitude of XB recruitment (P_{df}) is enhanced to a greater extent in HF myocardium compared to donor myocardium following OM incubation (Table 2).

Our observation that OM slows XB kinetics may seem to differ from results of enzymology studies which showed that OM accelerated the rate of phosphate release^{10, 15}, which would be expected to accelerate the rate of force generation. Interestingly, although not tested at multiple doses of OM, Rohde et al¹⁵ also showed that OM slows the force-generating myosin powerstroke step by delaying the rotation of myosin light chain domain and effectively uncouples the powerstroke from actin-activated phosphate release.¹⁵ This mechanism may resolve the seemingly contradictory findings that OM accelerates phosphate release yet slows XB kinetics, as we show here. An additional consideration in analyzing results of OM experiments, is that some studies have employed systems that lack an intact myofilament lattice^{10, 13, 15}, in contrast to skinned myocardium utilized here. Notably, ADP release is a strain-sensitive mechanism⁴⁰, a property that would be difficult to capture using unregulated systems. Nevertheless, studies using regulated systems (i.e., *in vitro* motility assays or skinned myocardium) reported slowed rates of shortening velocity and XB detachment^{11, 13, 14}, suggestive of a slowed ADP release (although this was not directly measured in these studies). Collectively, these findings suggest that OM acts to slow the rate of XB transitions to force-bearing states and prolongs XB duty cycle, which together act to promote XB-mediated cooperative XB recruitment and slow the rate of force generation, as observed in our studies.

Another key finding is that despite a significant OM-induced enhancement in the magnitude of XB recruitment, HF myocardium displayed a delay in achievement of peak force development, T_{pk} , when compared to donor myocardium, in part, due to a prolongation of T_{rec} in HF myocardium (Table 3; Figure 5). Collectively, these findings suggest that the

beneficial effects of OM manifest more slowly in the HF myocardium. This differential response to OM may be due to inherent deficits in force generating capacity in HF myocardium such as reduced numbers of readily available XBs for recruitment, impaired XB cycling due to contractile protein dephosphorylation, or both.

Potential *in vivo* consequences of OM-induced effects on XB behavior

OM differentially impacts HF myocardium, in that there is a greater slowing of cooperative XB recruitment, T_{rec} , and a greater prolongation of the time to reach peak force development, T_{pk} , –a cumulative impact is a substantially enhanced XB recruitment, P_{df} in HF myocardium. The differential dose-response to OM may be related to inherent differences in basal contractile function in donor and HF myocardium and has significant implications for *in vivo* contractile function in HF patients. Specifically, during the initial phase of the isovolumic contraction, the Ca^{2+} levels surrounding the myofilaments is very low.⁴¹ Because the effects of OM are more prominent at low Ca^{2+} levels, OM treatment enhances XB recruitment and amplifies the number of XBs ultimately participating in force generation during this initial phase of isovolumic contraction thereby augmenting ventricular pressure development later in systole.¹⁰

Specifically, our data show that a higher OM dose (1.0 μ M), which may be greater than the effective doses administered to HF patients in clinical trials to date^{6, 7}, results in robust enhancements in force generation, but also significantly slows XB detachment (k_{rel}) in HF myocardium. Slowed k_{rel} likely plays a major role in determining the total systolic ejection time (SET) and the commencement of ventricular relaxation and diastolic filling.⁴² In particular, in the late systolic ejection phase, during which a substantial amount of blood is pumped into the circulation, the Ca^{2+} transient wanes off⁴³ and XBs begin to rapidly detach due to shortening-induced cooperative deactivation of the thin filaments.⁴¹ Thus, slowed k_{rel} at higher OM doses may act to prolong XB-mediated thin filament activation which is a net effect of all bound XBs, thereby prolonging the SET –potentially delaying ventricular pressure decay and reducing diastolic filling.⁴² In contrast, incubations of skinned myocardium with 0.5 μ M OM produced relatively smaller enhancements in force generation, but did not slow k_{rel} , suggesting that it is possible to optimize OM dose to elicit beneficial effects on systolic force generation without the potential adverse impact of excessive prolongation of SET that can infringe on the diastolic filling phase.

Study limitations

It is important to note that our *in vitro* experiments cannot fully account for a slew of complexities that are inherent to clinical HF studies such as alterations in neuro-hormonal signaling, Ca^{2+} -cycling, and post-translational modifications in non-contractile proteins that typically occur in a remodeled HF myocardium. Furthermore, our experiments cannot specifically account for various comorbidities such as diabetes, hypertension, and peripheral arterial disease, or supplementary pharmacological interventions such as diuretics, ACE inhibitors, β -blockers, etc., that may be administered to HF patients in addition to OM.⁶ All the above factors may influence the *in vivo* impact of OM to some degree, and cannot be fully recapitulated *in vitro*. Thus, caution should be exercised when extrapolating our data to clinical outcomes.

Conclusions

Our novel findings demonstrate that OM promotes XB formation but prolongs the time course of cooperative XB recruitment leading to delayed peak force development in HF myocardium when compared to donor myocardium. At higher doses of OM, slower rates of force development are also accompanied by slower rates of XB cycling, which may underlie the prolongation of systolic ejection time *in vivo*. Of clinical significance, our results demonstrate that correct OM dosing may be critical to achieve a balance of increased systolic performance without diminished diastolic filling *in vivo*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

R.M., J.L., and J.E.S contributed to the conception and design of the experiments. R.M., J.L K.S.G., C.Y.D., C.G.D.R., A.L., S.L., and J.E.S participated in performing the experiments, data acquisition, data analysis, data interpretation, drafting, and revising the manuscript. S.V. participated in data analysis, data interpretation, drafting, and revising the manuscript. All authors approved the final version of the manuscript.

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Clinical Perspective

What is New

- We investigated if omecamtiv mecarbil (OM) affects actomyosin cross-bridge (XB) behavior similarly in healthy and pathologically remodeled heart failure (HF) myocardium.
- Dose-dependent impact of OM on XB function was examined in detergent-skinned myocardial preparations made from donor and HF heart samples.
- OM significantly enhanced the magnitude of XB recruitment to a force-bearing state in HF myocardium, effectively reducing the basal gap in force generation compared to donor myocardium.
- However, dose-dependent OM-induced force enhancement in HF myocardium was also accompanied by slowed peak force development and XB cycling compared to donor myocardium, particularly at higher OM doses.

What are the Clinical Implications

- Slowed XB kinetics at the cellular level may underlie the observed prolongation of systolic ejection time (SET) at the whole-heart level following OM treatment in healthy volunteers and HF patients.
- Our results highlight the importance of administering optimal OM doses to HF patients to balance increases in force generation and stroke volume while avoiding excessive prolongation of SET which can delay diastolic filling.

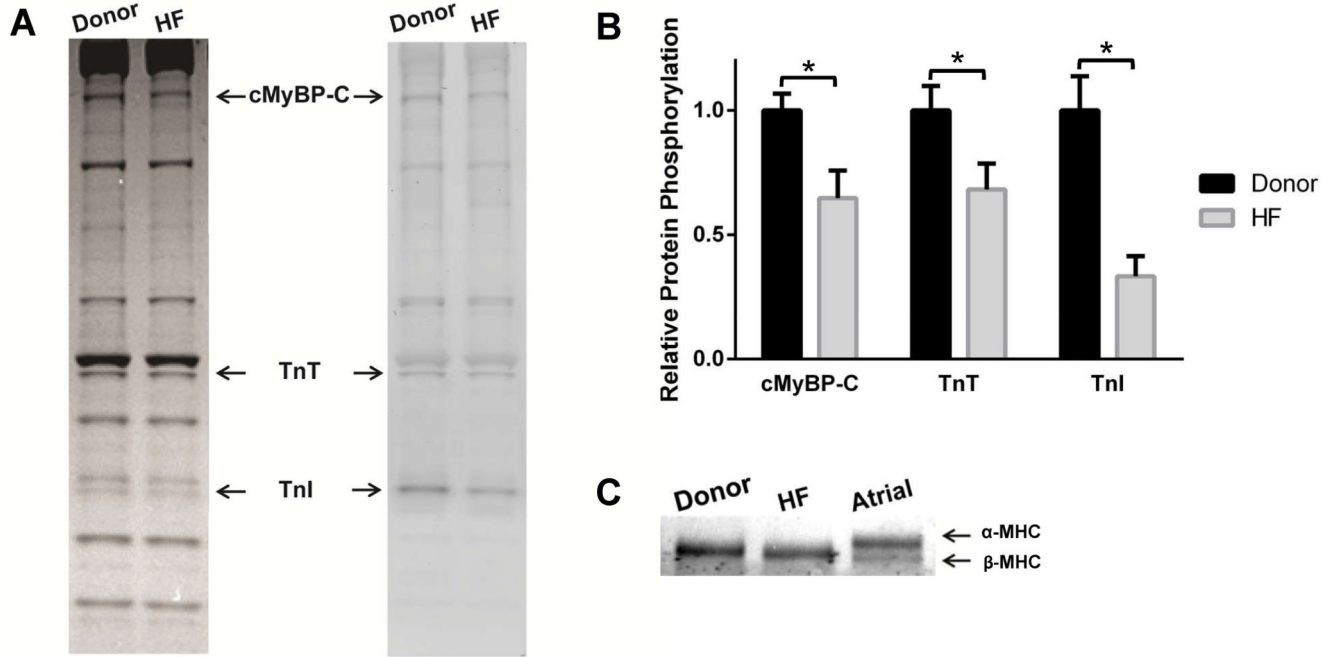


Figure 1. Phosphorylation of sarcomeric proteins and MHC expression in donor and HF myocardium

(A) Representative Coomassie stained (left) SDS gel and Pro-Q Diamond-stained (right) showing the expression and phosphorylation status of myofilament proteins in donor and HF samples. Cardiac samples isolated from 4 donor hearts and 4 failing hearts were used to analyze contractile protein expression and phosphorylation levels. (B) Quantification of phosphorylation of MyBP-C, TnT, and TnI in donor and HF samples. (C) Representative 5% Tris-HCl gel showing MHC isoform expression in donor and HF myocardium. Values are expressed as mean \pm S.E.M. * P < 0.05.

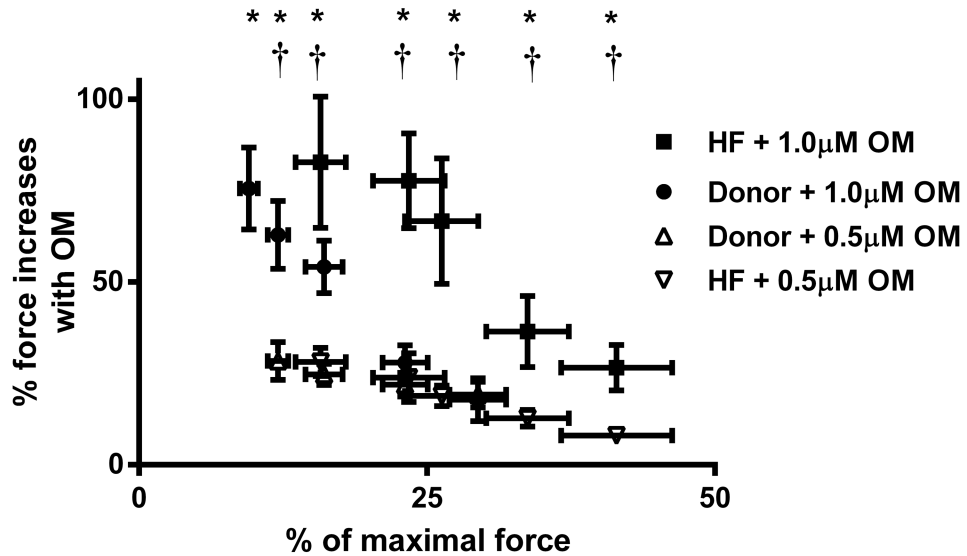


Figure 2. Effect of OM on force enhancements at variable $[Ca^{2+}]$ activations in donor and HF myocardium

Baseline forces produced by the skinned myocardial preparations were first measured in Ca^{2+} solutions producing a range of submaximal forces. Forces were measured at baseline or following a 2-minute incubation with 0.5µM or 1.0µM OM. The net increase in force generation from the untreated baseline (pre-OM) following OM incubation was calculated and is expressed as % increase in force from baseline. Force enhancements were pronounced at submaximal Ca^{2+} activation but declined as the level of Ca^{2+} was increased. 12 skinned myocardial preparations (3 fibers each from 4 hearts) were used for both groups. *, significant force enhancement from baseline level in donor and HF myocardium following 1.0µM OM incubation; †, significant force enhancement from baseline level in donor and HF myocardium following 0.5µM OM incubation. Asterisks indicate $P < 0.05$.

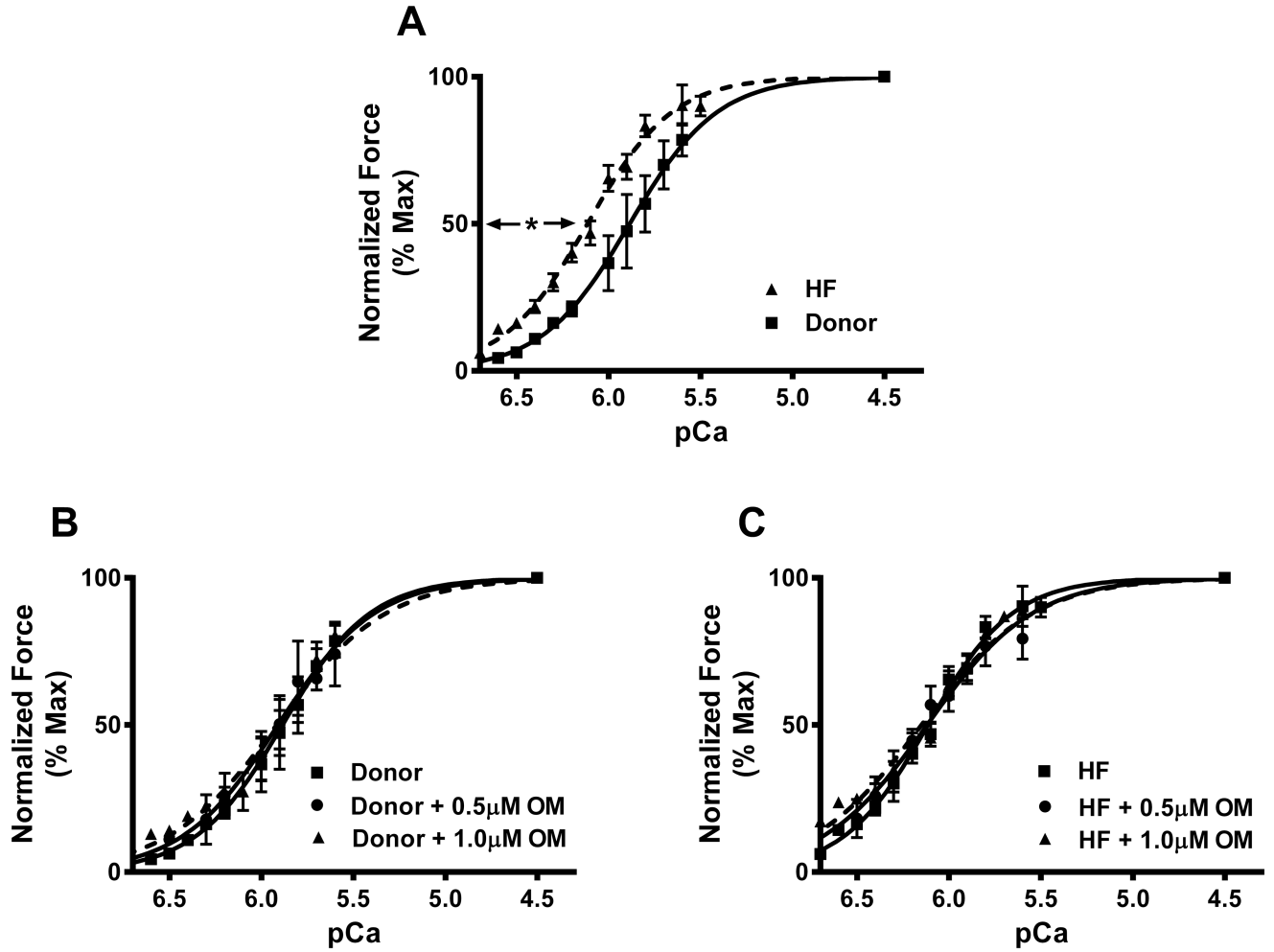


Figure 3. Effect of OM on pCa₅₀ in donor and HF myocardium

Skinned myocardial preparations were exposed to a 2-minute incubation with 0.5 μM or 1.0 μM OM. (A) Effect of OM on the force-pCa relationships in donor and HF myocardium. (B) Effect of OM on the force-pCa relationships in donor myocardium. (C) Effect of OM on the force-pCa relationships in HF myocardium. 12 skinned myocardial preparations (3 fibers each from 4 hearts) were used for both groups. *, pCa₅₀ is significantly higher in HF when compared to the donor myocardium. Asterisks indicate P < 0.05.

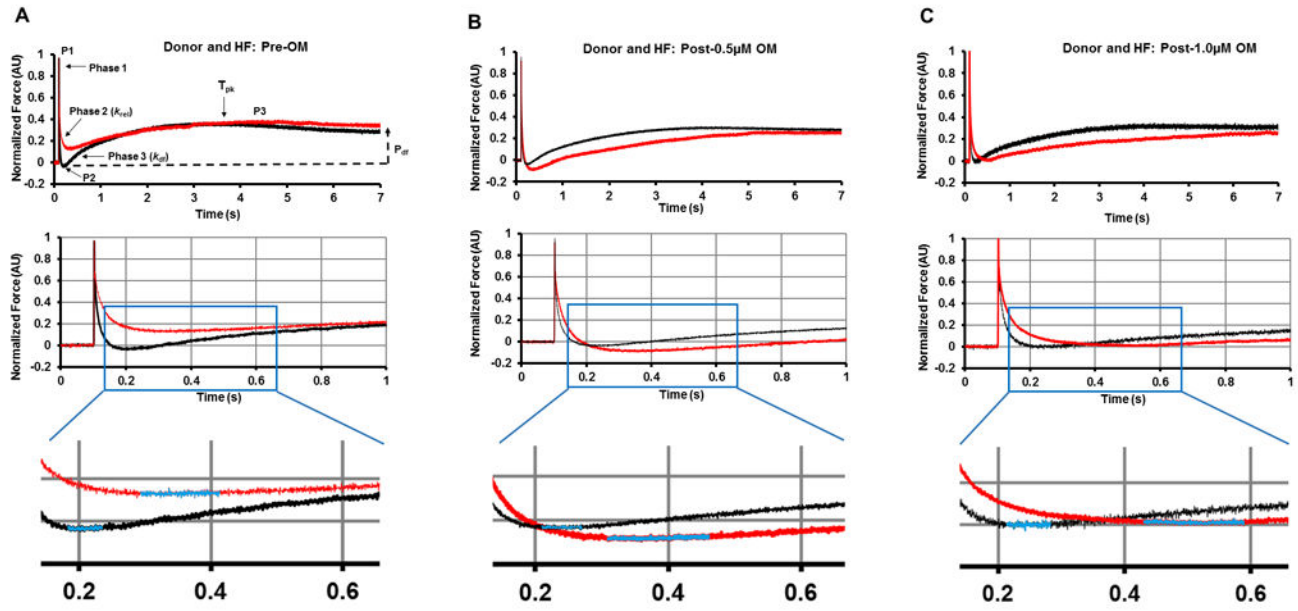


Figure 4. Representative stretch activation responses in donor and HF myocardium prior to and following OM incubations

Representative force responses are shown following a sudden 2% stretch in muscle length (ML) in isometrically-contracting donor (black traces), and HF (red traces) myocardial preparations prior to (A) and following incubation with 0.5 μ M OM (B) or 1.0 μ M OM (C). Expanded views are shown below panels A to C to demonstrate that k_{rel} is slower in HF myocardium compared to donor myocardium both prior to and following OM incubation. Insets below the expanded views depict the T_{rec} region of the trace. The highlighted parts of the stretch activation traces (blue colored regions) in the insets show that the time required for XB recruitment, T_{rec} , is prolonged post-OM incubations in the HF myocardium. Panel A highlights the important phases of the force transients and various stretch activation parameters that are measured from force responses to 2% stretch in ML (explained in methods section).

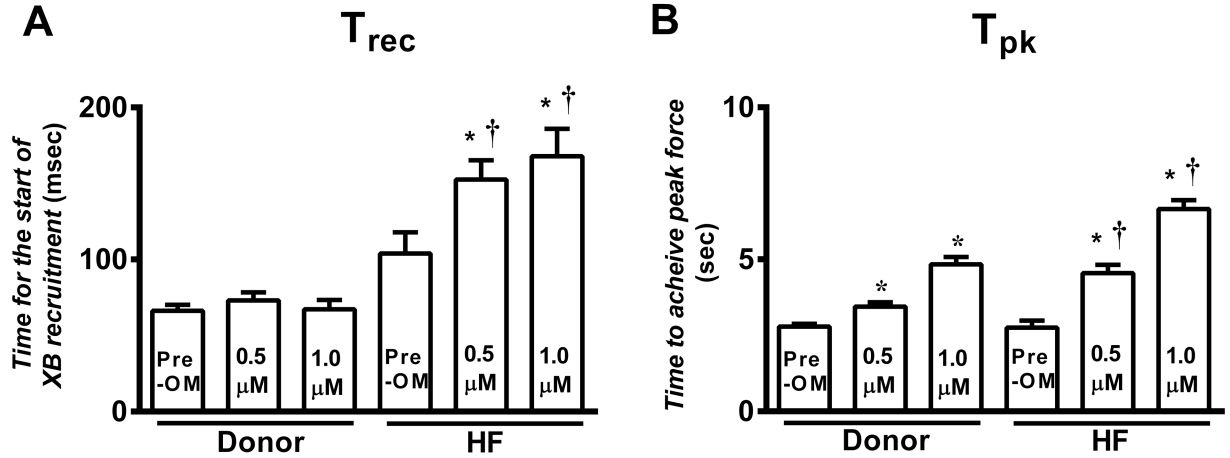


Figure 5. Effect of OM on the time required for XB recruitment (T_{rec}) and time required to achieve the peak of force development (T_{pk}) in donor and HF myocardium

The effect of OM was measured by incubating myocardial preparations in 0.5 μ M or 1.0 μ M OM. (A) T_{rec} prior to and following OM incubation in donor and HF myocardium. (B) T_{pk} prior to and following OM incubation in donor and HF myocardium. The supplement contains a description of how these parameters are measured. Values are expressed as mean \pm S.E.M. 12 skinned myocardial preparations (3 fibers each from 4 hearts) were used for both groups. *, different vs. the corresponding pre-OM group; †, different vs. the corresponding donor group. Asterisks indicate $P < 0.05$.

Table 1
Steady-state parameters measured prior to and following OM incubation in donor and HF myocardium

Group	pCa ₅₀	n _H	F _{max} (mN/mm ²)	F _{min} (mN/mm ²)
Donor				
Pre-OM	5.89±0.04	2.23±0.11	16.86±2.20	1.65±0.24
0.5μM OM	5.93±0.03	1.52±0.10 *	16.32±1.58	1.62±0.14
1.0μM OM	5.90±0.03	1.58±0.14 *	19.46±2.11	1.98±0.29
HF				
Pre-OM	6.09±0.04 †	1.95±0.14	8.96±0.99 †	0.87±0.07 †
0.5μM OM	6.11±0.02 †	1.39±0.11 *	10.11±0.92 †	1.08±0.14
1.0μM OM	6.12±0.04 †	1.43±0.10 *	9.37±0.90 †	1.06±0.10 †

pCa₅₀: myofilament Ca²⁺ sensitivity; n_H: cooperativity of force production; F_{max}: Ca²⁺-activated maximal force measured at pCa 4.5; F_{min}: Ca²⁺-independent force measured at pCa 9.0. Values are expressed as mean ± S.E.M. 12 skinned myocardial preparations (3 fibers each from 4 hearts) were used for both groups.

* different vs. the corresponding Pre-OM group;

† different vs. the corresponding donor group. Asterisks indicate P < 0.05.

Table 2
Dynamic stretch-activation parameters measured prior to and following OM incubation in donor and HF myocardium

Group	k_{tr} (s ⁻¹)	k_{rel} (s ⁻¹)	k_{df} (s ⁻¹)	Donor			P _{df}
				P1	P2	P3	
Pre-OM	1.23±0.08	88.49±5.70	1.44±0.11	0.569±0.017	-0.019±0.012	0.209±0.008	0.228±0.013
0.5µM OM	1.03±0.05	84.60±8.13	1.04±0.06*	0.725±0.048*	0.019±0.027	0.242±0.023	0.223±0.026
1.0µM OM	1.20±0.10	70.51±5.54	0.81±0.06*	0.633±0.020	-0.043±0.007	0.205±0.010	0.248±0.011
HF							
Pre-OM	1.18±0.11	65.59±6.75 [‡]	1.25±0.12	0.648±0.051	0.125±0.022 [‡]	0.258±0.020	0.133±0.024 [‡]
0.5µM OM	0.79±0.07*	64.55±3.98 [‡]	0.73±0.05 [‡]	0.705±0.027	0.009±0.019*	0.239±0.014	0.230±0.022*
1.0µM OM	0.80±0.06 [‡]	45.96±4.62 [‡]	0.54±0.05*	0.693±0.051	-0.014±0.019*	0.205±0.018	0.219±0.024*

k_{tr} : rate of tension redevelopment; k_{rel} : rate of XB detachment; k_{df} : rate of XB recruitment; P1: XB stiffness; P2: magnitude of XB detachment; P3: the new steady-state force attained following a 2% stretch in muscle length; P_{df}: magnitude of XB recruitment. As done in all our previous studies 11, 21, 24, 30, stretch activation amplitudes were normalized to pre-stretch steady-state Ca^{2+} -activated force, P0, which refers to the activation level (~25% of maximal Ca^{2+} -activated force), and is equivalent in the donor and HF groups (see methods for details). Values are expressed as mean ± S.E.M. 12 skinned myocardial preparations (3 fibers each from 4 hearts) were used for both groups.

* different vs. the corresponding Pre-OM group;

[‡] different vs. the corresponding donor group. Asterisks indicate $P < 0.05$.

Table 3
Time required for XB recruitment (T_{rec}) and time required to reach the peak of force development (T_{pk}) prior to and following OM incubation in donor and HF skinned myocardium

Group	T_{rec} (msec)	T_{pk} (sec)
Donor		
Pre-OM	66.25±4.03	2.79±0.08
0.5μM OM	73.00±5.41	3.44±0.14 *
1.0μM OM	67.33±6.09	4.84±0.24 *
HF		
Pre-OM	103.96±13.98	2.75±0.23
0.5μM OM	152.63±12.60 *†	4.55±0.27 *†
1.0μM OM	167.92±17.93 *†	6.66±0.29 *†

Values are expressed as mean ± S.E.M. 12 skinned myocardial preparations (3 fibers each from 4 hearts) were used for both the groups.

* different vs. the corresponding Pre-OM group;

† different vs. the corresponding donor group. Asterisks indicate $P < 0.05$.