The energy-saving effect of a new myosin activator, omecamtiv mecarbil, on LV mechanoenergetics in rat hearts with blood-perfused isovolumic contraction model

Koji Obata¹ · Hironobu Morita¹ · Miyako Takaki¹

Received: 13 July 2018 / Accepted: 26 June 2019 / Published online: 2 July 2019
© The Author(s) 2019

Abstract

A novel myosin activator, omecamtiv mecarbil (OM), is a cardiac inotropic agent with a unique new mechanism of action, which is thought to arise from an increase in the transition rate of myosin into the actin-bound force-generating state without increasing calcium (Ca^{2+}) transient. There remains, however, considerable controversy about the effects of OM on cardiac contractility and energy expenditure. In the present study, we investigated the effects of OM on left ventricular (LV) mechanical work and energetics, i.e., mechanoenergetics in rat normal hearts (CTL) and failing hearts induced by chronic administration of isoproterenol (1.2 mg/kg/day) for 4 weeks (ISO-HF). We analyzed the LV end-systolic pressure-volume relation (ESPVR) and the linear relation between the myocardial oxygen consumption per beat (VO_2) and systolic pressure-volume area (PVA; a total mechanical energy per beat) in isovolumically contracting rat hearts at 240- or 300-bpm pacing in the absence or presence of OM. OM did not change the ESPVR in CTL and ISO-HF. OM, however, significantly decreased the slope of VO_2 –PVA relationship in both CTL and ISO-HF, and significantly increased the mean VO_2 intercept without changes in basal metabolism in ISO-HF. These results suggested that OM improved the oxygen cost of PVA (contractile efficiency) with the unchanged LV contractility in both CTL and ISO-HF but increased VO_2 for Ca^{2+} handling in excitation–contraction (E–C) coupling in ISO-HF. We concluded that OM improves contractile efficiency in normal and failing hearts but increases O_2 consumption of Ca^{2+} handling in failing hearts in isovolumically contracting rat model.

Keywords Myosin activator · Excitation–contraction coupling · Oxygen consumption · Pressure-volume area, heart failure

Abbreviations

OM	Omecamtiv mecarbil
LV	Left ventricle
ESPVR	End-systolic pressure-volume relation
VO_2	Myocardial oxygen consumption per beat
PVA	Pressure-volume area
bpm	Beats per minute
E–C	Excitation–contraction
E_{max}	Maximum elastance
mLVV	Midrange LV volume

ANCOVA	Analysis of covariance
EDPVR	End-diastolic pressure-volume relation
ESP_{mLVV}	End-systolic pressure at mLVV
PVA_{mLVV}	PVA at mLVV
KCl	Potassium chloride
SV	Stroke volume
SET	Systolic ejection time
ATPase	Sarcoplasmic reticulum Ca^{2+}
SERCA	SR Ca^{2+} ATPase
SR	Sarcoplasmic reticulum
NCX	$\text{Na}^+/\text{Ca}^{2+}$ exchanger

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s00210-019-01685-4>) contains supplementary material, which is available to authorized users.

✉ Koji Obata
obatak@gifu-u.ac.jp

¹ Department of Physiology, Gifu University Graduate School of Medicine, 1-1 Yanagido, Gifu 501-1194, Japan

Introduction

In pharmacotherapy for heart failure, the conventional inotropic drugs such as digitalis, β -agonist, and phosphodiesterase inhibitors were sought to enhance the calcium (Ca^{2+}) handling for activating contractions. Thus, such drugs increased in myocardial

energy expenditure and the consequently increased energy expenditure can worsen a patient's prognosis in heart failure from a long-term perspective (Bers and Harris 2011).

A novel cardiac myosin activator, omecamtiv mecarbil (OM), has been reported to exert a positive inotropic action by accelerating the transition rate of myosin into the strongly actin-bound force-generating state, and decreasing the rate of actin-independent phosphate release, i.e., reducing non-productive and hence wasteful hydrolysis of ATP, without enhancing intracellular Ca^{2+} load (Teerlink 2009; Malik et al. 2011). Thus, theoretically, this drug can enhance cardiac output without increasing energy expenditure unlike conventional inotropic drugs. Previous studies reported that OM did not change minimum dP/dt, an index of diastolic function in vivo (Shen et al. 2010; Malik et al. 2011). On the other hand, in vitro, OM increased the relaxation time of cardiomyocytes (Horváth et al. 2017). The effects of OM on diastolic function and energy consumption remain controversial. Shen et al. (2010) demonstrated that chronic infusion of OM improved left ventricular (LV) function without increasing myocardial O_2 consumption (MVO_2) in systolic heart failure canine model. In contrast, Bakkehaug et al. (2015) reported that OM lead to reduced contractile efficiency and increased energy expenditure as mediated by hyperactivity in resting myosin ATPase in both of contractile and non-contractile of the hearts in mice and pigs. In addition, they showed that OM increased MVO_2 for basal metabolism in ex vivo mice hearts with unchanged MVO_2 for excitation–contraction (E-C) coupling. There are obvious discrepancies among these OM effects on cardiac function and energy expenditure, although these were reported from different animal hearts.

In the present study, we would clarify the effects of OM on the relationship between mechanical work and energy consumption using equivalent E_{max} (eE_{max})-pressure-volume area (PVA)- VO_2 framework to analyze LV mechanics and energetics, i.e., mechanoenergetics, in rat normal and failing hearts using an excised, blood-perfused whole heart preparation (Takaki 2004).

Methods

Experimental animals

Isoproterenol-induced heart failure as failing hearts (ISO-HF) made as previously reported (Shibata et al. 2011; Mitsuyama et al. 2013) and normal hearts as control (CTL) were used in the present study.

Excised cross-circulated rat heart model and data analysis

The excised cross-circulated rat heart model was described in detail in the Online supplements (supplemental Fig. 1). OM

infused at a final concentration about 200–500 ng/mL into coronary circulation. Data analysis obtained from this experimental model was also described in detail in the Online supplements (supplemental Fig. 2).

Analyses of one-beat LV pressure-time curve by a logistic function

To evaluate LV end-diastolic relaxation rate or lusitropism, we used the logistic time constant derived from a logistic model to analyze LV isovolumic relaxation pressure-time curve at midrange LV volume (mLVV) (Mitsuyama et al. 2013).

Statistics

We compared the VO_2 –PVA regression lines of the volume runs between pre- and post-OM treatment in each heart by analysis of covariance (ANCOVA). Comparison of paired and unpaired individual values was performed by paired and unpaired t test, respectively. A value of $p < 0.05$ or $p < 0.01$ was considered statistically significant. All data are expressed as the mean $\pm$ S.D.

Results

Effects of OM on LV contractility in the CTL and ISO-HF

OM showed that both of LV ESPVRs and end-diastolic pressure-volume relations (EDPVRs) did not change in both CTL and ISO-HF (Fig. 1a). In fact, the mean ESP_{mLVV} and PVA_{mLVV} ($n = 6$, each) in ISO-HF showed significant decreases compared with those in CTL but did not show significant differences between pre- and post-OM treatment (Fig. 1d and e). The coronary blood flows were not significantly different between both CTL and ISO-HF in the absence or presence of OM (data not shown).

Effects of OM on VO_2 –PVA relations in the CTL and ISO-HF

OM treatment decreased the slopes of VO_2 –PVA linear relations in both of CTL and ISO-HF (Fig. 1b and c) and elevated the VO_2 intercepts of VO_2 –PVA linear relations exclusively in ISO-HF (Fig. 1c). In comparisons by paired t test between pre-

Fig. 1 Representative data of ESPVRs and EDPVRs (a), and VO_2 –PVA relations in pre- and post-OM treated same hearts in CTL (b) and ISO-HF (c), respectively. The comparison of ESP_{mLVV} (d), PVA_{mLVV} (e), slope (f), and VO_2 intercepts (g), and the means VO_2 for E-C coupling (h) and O_2 consumption for basal metabolism (i) at the pre- and post-OM treatments in CTL ($n = 5$ –6 each) and ISO-HF ($n = 4$ –6 each). Values are mean $\pm$ SD (h and i). * $P < 0.01$, pre-OM vs. post-OM. # $P < 0.05$, CTL vs. ISO-HF

and post-OM treatments, the mean slopes were significantly decreased in both CTL and ISO-HF ($P < 0.01$, Fig. 1f). The

mean VO_2 intercepts were significantly increased in ISO-HF ($P < 0.01$, Fig. 1g) but not in CTL. These results indicate that

Fig. 2 Representative data of normalized pressure-time curves at mLVV in the CTL at 300 bpm (a), 240 bpm (b) pacing, and ISO-HF (c) at 240-bpm pacing in the absence (closed circle) and the presence (opened circle) of OM (289–316 ng/mL in the blood). The comparison of the mean logistic time constants at mLVV in the CTL at 300-bpm pacing ($n = 8$), 240-bpm pacing ($n = 7$), and ISO-HF at 240-bpm pacing ($n = 8$) in the absence (solid column) and presence of OM (open column) (211–357 ng/mL in the blood) (d). Values are mean $\pm$ SD. * $P < 0.01$, # $P < 0.05$, OM (+) vs. OM (–)

OM markedly improves the cost of PVA or its reciprocal shows contractile efficiency, i.e., the energy transduction rate of mechanical work per ATP.

Effects of OM on O_2 consumptions for E–C coupling and basal metabolism in the CTL and ISO-HF

To clarify the mechanisms of the increased VO_2 intercepts in OM-treated ISO-HF, we performed cardiac arrest by KCl infusion to obtain O_2 consumption for basal metabolism. OM treatment did not change the O_2 consumption for basal metabolism in both of CTL and ISO-HF (Fig. 1i) but significantly increased the VO_2 for E–C coupling exclusively in ISO-HF ($P < 0.01$, Fig. 1h). These results indicate that OM increases VO_2 intercepts mediated via the increase of VO_2 for E–C coupling in ISO-HF.

Effects of OM on lusitropism in the CTL and ISO-HF

We compared the normalized LV pressure-time curves and logistic time constants between pre- and post-OM treatments in the same hearts of each of CTL and ISO-HF to reveal the effects of OM on LV lusitropism. OM prolonged the duration

of LV relaxation time in a CTL at 300-bpm pacing (Fig. 2a) and an ISO-HF at 240-bpm pacing (Fig. 2c) but not in a CTL at 240-bpm pacing (Fig. 2b). The mean post-OM logistic time constants elongated in both CTL at 300-bpm pacing ($P < 0.05$) and ISO-HF at 240-bpm pacing ($P < 0.01$) but not in CTL at 240-bpm pacing (Fig. 2d).

Discussion

In the present study, we found that the administration of OM did not change the ESPVR and EDPVR, ESP, and PVA at mLVV in both of CTL and ISO-HF, indicating that OM does not affect the LV contractility in both normal and failing hearts. Previous *in vivo* studies showed that OM improved stroke volume (SV) and cardiac output related to the extension of systolic ejection time (SET) in rat or canine failing hearts (Malik et al. 2011; Shen et al. 2010; Malik and Morgan 2011). In addition, Bakkehaug et al. (2015) reported that the OM increased cardiac output through the extension of SET in swine failing hearts. These results suggested that OM would exert positive inotropic effects by extending the SET without changing cardiac contractility. The results from clinical trials

showing OM significantly increases SET in a dose-dependent manner in both healthy volunteers and patients with systolic heart failure (Cleland et al. 2011; Teerlink et al. 2011) might support this suggestion.

The most significant effect of OM would be an enhancing cardiac function without increasing intracellular Ca^{2+} and energy expenditure unlike conventional inotropic drugs. Shen et al. reported that OM treatment increased stroke work over 60% with no increase in O_2 demand, resulting in a 25–30% increase in cardiac efficiency (Teerlink 2009; Shen et al. 2010). In contrast, Bakkehaug et al. (2015) reported that OM decreased the contractile efficiency evaluated by the reciprocal of the slope of PVA–MVO₂ linear relation with the unchanged stroke work, indicating that OM leads to significant O_2 waste. In addition, OM increased basal metabolic VO₂. In the present study, our experimental model has an isovolumic contracting hearts without ejection, i.e., no cardiac output, which is a possible reason for giving the discrepancy from previous studies. We found that OM obviously improves the contractile efficiency in both normal and failing hearts with the unchanged ESPVR, indicating that OM leads to apparent O_2 saving with the unchanged LV contractility (Fig. 1a, d–f). This discrepancy in the effects of OM on contractile efficiency might be generated by the difference among experimental models. In the present study, the reason why OM decreased O_2 consumption in spite of the unchanged PVA at the same preload is still unknown. OM might have decreased the increasing rate of the number of myosin head binding actin filaments and/or reduced non-productive ATP hydrolysis (the rate of actin-independent phosphate release) under increasing LVV in isovolumic contracting hearts. In addition, the effects of OM on contractile efficiency appear to exert in both normal and failing hearts regardless of cardiac myosin isoform. Because it is well known that myosin isoform shift, for example, from the faster alpha-myosin isoform to slower beta-myosin isoform in rodents occurs in failing hearts. Our recent study demonstrated that the slope of VO₂–VA relations in the hearts of cardiac sarcoplasmic reticulum Ca^{2+} ATPase (SERCA) 2a-overexpressed transgenic rats significantly smaller than that in wild-type rats, which indicates that the increased SERCA protein improved the efficiency of chemo-mechanical energy transduction (Zhang et al. 2012). Thus, the interaction between Ca^{2+} handling in E–C coupling and cross-bridge cycling may be related to the mechanism providing contractile efficiency.

Recently, it was reported that OM increased the open probability of ryanodine receptor channel and the Ca^{2+} sensitivity of force production (Nánási et al. 2017; Nagy et al. 2015), suggesting that a Ca^{2+} leak from the sarcoplasmic reticulum (SR) may modify the inotropic effect of OM. On the other hand, OM did not increase the Ca^{2+} transient though it increased rat and canine cardiac myocyte shortening (Malik et al. 2011; Horváth et al. 2017) and OM did not change the

VO₂ for Ca^{2+} handling in E–C coupling in ex vivo mouse hearts (Bakkehaug et al. 2015). In the present study, we found that the VO₂ for Ca^{2+} handling in E–C coupling was increased in ISO-HF but not CTL after OM treatment (Fig. 1h). This increase might be related to the deterioration of Ca^{2+} uptake due to the SERCA2a downregulation in failing hearts. The increased intracellular Ca^{2+} with a Ca^{2+} leak from SR by OM is dominantly removed from the cytosol via $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX), rather than Ca^{2+} uptake via the SERCA2a to SR. Although NCX per se does not consume ATP to remove cytosolic Ca^{2+} in exchange with Na^+ influx (stoichiometry of $3\text{Na}^+ : 1\text{Ca}^{2+}$), Na^+ influx must be pumped out by Na^+/K^+ -ATPase, with a stoichiometry of $3\text{Na}^+ : 2\text{K}^+ : 1\text{ATP}$, resulting in the net stoichiometry of $1\text{Ca}^{2+} : 1\text{ATP}$. In contrast, SERCA2a removes cytosolic Ca^{2+} based on stoichiometry of $2\text{Ca}^{2+} : 1\text{ATP}$ (Takaki 2004; Mitsuyama et al. 2013). Thus, it is a possible mechanism that OM increased the VO₂ in E–C coupling only in failing hearts.

OM significantly increased in LV relaxation time in failing hearts at 240-bpm pacing (higher 240-bpm pacing is impossible in failing hearts) or CTL at 300-bpm pacing, which would be associated with the impaired extrusion of the increased intracellular Ca^{2+} from the cytosol (Fig. 2). Recently, Horváth et al. (2017) demonstrated that high-frequency pacing in the presence of OM and/or high concentration of OM decreased canine cardiomyocyte shortening and OM dose-dependently prolonged canine cardiomyocyte relaxation time with the unchanged Ca^{2+} transient amplitude. These results suggest that any hearts with a higher heart rate, especially in diastolic dysfunction, would be susceptible to any detrimental effects of OM.

Finally, we concluded that OM improved the efficiency for converting chemical energy into mechanical work with the unchanged LV contractility in isovolumic contracting rat heart model. In the present failing hearts, OM increased the VO₂ for Ca^{2+} handling in E–C coupling. Therefore, upon the clinical application of OM, we must perform a careful pharmacotherapy for the patients with higher heart rate, i.e., tachycardia, and/or with diastolic dysfunction, to be sufficient for the ventricular filling and the coronary flow.

Author contributions KO and MT conceived and designed research. KO conducted experiments and obtained the data. HM and MT supervised the present study. KO and MT performed the data analysis and drafted the manuscript.

Funding information This work was supported in part by grants-in-aid nos. 22790216 and 25460283 for the Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan and by Novartis Pharma Research Grants to K.O.

Compliance with ethical standards

Conflict of interest The authors declare that they have no of conflict of interest.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

- Bakkehaug JP, Kildal AB, Engstad ET, Boardman N, Næshheim T, Rønning L, Aasum E, Larsen TS, Myrmet T, How OJ (2015) Myosin activator omecamtiv mecarbil increases myocardial oxygen consumption and impairs cardiac efficiency mediated by resting myosin ATPase activity. *Circ Heart Fail* 8:766–775. <https://doi.org/10.1161/CIRCHEARTFAILURE.114.002152>
- Bers DM, Harris SP (2011) Translational medicine: to the rescue of the failing heart. *Nature* 473:36–39. <https://doi.org/10.1038/473036a>
- Cleland JG, Teerlink JR, Senior R, Nifontov EM, Mc Murray JJ, Lang CC, Tsyrlin VA, Greenberg BH, Mayet J, Francis DP, Shaburishvili T, Monaghan M, Saltzberg M, Neyses L, Wasserman SM, Lee JH, Saikali KG, Clarke CP, Goldman JH, Wolff AA, Malik FI (2011) The effects of the cardiac myosin activator, omecamtiv mecarbil, on cardiac function in systolic heart failure: a double-blind, placebo-controlled, crossover, dose-ranging phase 2 trial. *Lancet* 378:676–683. [https://doi.org/10.1016/S0140-6736\(11\)61126-4](https://doi.org/10.1016/S0140-6736(11)61126-4)
- Horváth B, Szentandrassy N, Veress R, Almássy J, Magyar J, Bányász TA, Papp Z, Nánási PP (2017) Frequency-dependent effects of omecamtiv mecarbil on cell shortening of isolated canine ventricular cardiomyocytes. *Naunyn Schmiedeberg's Arch Pharmacol* 390:1239–1246. <https://doi.org/10.1007/s00210-017-1422-z>
- Malik FI, Morgan BP (2011) Cardiac myosin activation part 1: from concept to clinic. *J Mol Cell Cardiol* 51:454–461. <https://doi.org/10.1016/j.yjmcc.2011.05.006>
- Malik FI, Hartman JJ, Elias KA, Morgan BP, Rodriguez H, Brejc K, Anderson RL, Sueoka SH, Lee KH, Finer JT, Sakowicz R, Baliga R, Cox DR, Garard M, Godinez G, Kawa S, Kraynack E, Lenzi D, Lu PP, Muci A, Niu C, Qian X, Pierce DW, Pokrovskii M, Suehiro I, Sylvester S, Tochimoto T, Valdez C, Wang W, Katori T, Kass DA, Shen YT, Vatner SF, Morgans DJ (2011) Cardiac myosin activation: a potential therapeutic approach for systolic heart failure. *Science* 331:1439–1443. <https://doi.org/10.1126/science.1200113>
- Mitsuyama S, Takeshita D, Obata K, Zhang GX, Takaki M (2013) Left ventricular mechanical and energetic changes in long-term isoproterenol-induced hypertrophied hearts of SERCA2a transgenic rats. *J Mol Cell Cardiol* 59:95–106. <https://doi.org/10.1016/j.yjmcc.2013.02.012>
- Nagy L, Kovács Á, Bódi B, Pásztor ET, Fülöp GÁ, Tóth A, Édes I, Papp Z (2015) The novel cardiac myosin activator omecamtiv mecarbil increases the calcium sensitivity of force production in isolated cardiomyocytes and skeletal muscle fibres of the rat. *Br J Pharmacol* 172:4506–4518. <https://doi.org/10.1111/bph.13235>
- Nánási P Jr, Gaburjakova M, Gaburjakova J, Almássy J (2017) Omecamtiv mecarbil activates ryanodine receptors from canine cardiac but not skeletal muscle. *Eur J Pharmacol* 809:73–79. <https://doi.org/10.1016/j.ejphar.2017.05.027>
- Shen YT, Malik FI, Zhao X, Depre C, Dhar SK, Abarzúa P, Morgans DJ, Vatner SF (2010) Improvement of cardiac function by a cardiac myosin activator in conscious dogs with systolic heart failure. *Circ Heart Fail* 3:522–527. <https://doi.org/10.1161/CIRCHEARTFAILURE.109.930321>
- Shibata M, Takeshita D, Obata K, Mitsuyama S, Ito H, Zhang GX, Takaki M (2011) NHE-1 participates in isoproterenol-induced downregulation of SERCA2a and development of cardiac remodeling in rat hearts. *Am J Physiol Heart Circ Physiol* 301:H2154–H2160. <https://doi.org/10.1152/ajpheart.00483.2011>
- Takaki M (2004) Left ventricular mechanoenergetics in small animal. *Jpn J Physiol* 54:175–207. <https://doi.org/10.2170/jjphysiol.54.175>
- Teerlink JR (2009) A novel approach to improve cardiac performance: cardiac myosin activators. *Heart Fail Rev* 14:289–298. <https://doi.org/10.1007/s10741-009-9135-0>
- Teerlink JR, Clarke CP, Saikali KG, Lee JH, Chen MM, Escandon RD, Elliott L, Bee R, Habibzadeh MR, Goldman JH, Schiller NB, Malik FI, Wolff AA (2011) Dose-dependent augmentation of cardiac systolic function with the selective cardiac myosin activator, omecamtiv mecarbil: a first-in-man study. *Lancet* 378:667–675. [https://doi.org/10.1016/S0140-6736\(11\)61219-1](https://doi.org/10.1016/S0140-6736(11)61219-1)
- Zhang GX, Obata K, Takeshita D, Mitsuyama S, Nakashima T, Kikuta A, Hirabayashi M, Tomita K, Vetter R, Dillmann WH, Takaki M (2012) Evaluation of left ventricular mechanical work and energetics of normal hearts in SERCA2a transgenic rats. *J Physiol Sci* 62:221–231. <https://doi.org/10.1007/s12576-012-0200-4>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.