Pretreatment with topiroxostat and irbesartan improves cardiac function after myocardial infarction in rats

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Abstract:

Background: Activation of angiotensin receptor type1 (AT1R) and xanthine oxidase (XO) generates reactive oxygen species (ROS), that causes cardiac dysfunction after myocardial infarction (MI). However, it remains unknown whether its inhibition could restore the cardiac function after MI. In the present study, we examined effects of irbesartan and topiroxostat on cardiac function after MI. **Methods and results:** We studied blood pressure and cardiac function in a rat myocardial infraction model using tail cuff system and echocardiography. Irbesartan and topiroxostat as well as vehicle were orally administered for 35 days to rats 7 days before MI induction. Neither irbesartan nor topiroxostat altered mean blood pressure and heart rate after MI. Treatment with either drugs significantly improved cardiac function after MI. The potency of topiroxostat to restore the cardiac function was approximately half of that of irbesartan. **Conclusions:** A non-purine XO inhibitor, topiroxostat improved cardiac function after MI, suggesting that like irbesartan, topiroxostat may be a promising drug to treat congestive heart failure after MI.

Key words:

Topiroxostat, Irbesartan, Xanthine oxidase, Myocardial infarction

Introduction

Myocardial infarction (MI) has the highest mortality rate and contributes to the development and progression of heart failure (HF)[1]. HF remains a major health problem worldwide, and there is an urgent need to develop a new therapeutic strategy[2].

Several studies have reported that reactive oxygen species (ROS) play important roles in the pathophysiology of cardiac remodeling after MI[3]. In vitro, exposure of cardiomyocytes to ROS generated by xanthine oxidase (XO), a potent enzymatic source of ROS, has been shown to promote cardiac hypertrophy and dysfunction[4]. In addition, ROS[5] caused mitochondrial injury by inhibiting the activity of various respiratory-chain enzymes, leading to a decrease in myocardial ATP production and altered glycolipid metabolism. Therefore, inhibition of XO might attenuate ROS production and protect cardiac mitochondria from oxidative damage, thereby attenuating cardiac function in congestive heart failure (CHF). Allopurinol, an authentic XO inhibitor, is used worldwide for the treatment of hyperuricemia. Several studies using both animals and humans have shown that allopurinol improves cardiac dysfunction, mechano-energetic coupling and tolerance to exercise with after MI, cardiomyopathy and HF by decreasing cardiac ROS production and increasing cardiac energy (ATP)[6-8]. However, attention

should be paid to doses of allopurinol because it has various side effects, such as allergies and liver dysfunction in patients with impaired renal function. Topiroxostat, a non-purine selective XO inhibitor, is a recently developed more potent inhibitor of XO than allopurinol, without any significant inhibitory effects on other enzymes such as aldehyde oxidase as well as purines and pyrimidine enzymes. Several studies revealed that topiroxostat protects kidney cells from apoptosis owing to its antioxidant activity in vivo[9,10]. However, the effects of topiroxostat on cardiac function after MI remains unelucidated.

Angiotensin II (Ang II) also plays a key role in the pathogenesis of myocardial repair/remodeling after MI. Ang II leads to vasoconstriction, cell growth and positive inotropic action by increasing the secretion of aldosterone through the activation of Ang II type I (AT1) receptor. AT1 signal plays a pivotal role in the progression of post-infarct left ventricular (LV) remodeling associated with CHF. Experimental studies have also shown that inhibition of RAS by AT1 blockers shows beneficial effects on rat HF or MI models[11]. Moreover, these results have been confirmed in human HF. In a clinical setting, inhibition of RAS by AT1 receptor blockers or angiotensin-converting enzyme inhibitors (ACEIs) is a standard therapy for patients with MI and CHF. Irbesartan is one of the AT1 receptor blockers, which has non-hemodynamic cardiovascular and renal protective effects. Berthonneche et al showed that irbesartan improved cardiac function and remodeling mediated by TNF-α inhibition after MI in rats[12].

In the present study, to evaluate the effects of pretreatment with topiroxostat and irbesartan prior to the induction of MI, we studied chronic effects of topiroxostat on the cardiac function and remodeling after MI and compared them to those of irbesartan.

Methods

Animals and Experimental groups

Male syngeneic Lewis rats (body weight 200 to 250 g, 8 weeks old) were obtained from Japan SLC, Inc (Hamamatsu, Japan). The experimental protocols were approved by the Institutional Animal Care and Use Committee, Faculty of Medicine, Tottori University.

Irbesartan and topiroxostat as well as vehicle were orally administered to rats 7 days before creation of MI. Rats were randomly allocated into four groups: (1) Sham group (n=5) (2) MI + vehicle group (n=4) (3) MI + topiroxostat group (n =5), and (4) MI + irbesartan group (n=5). Topiroxostat (0.5 mg/kg)[13] (in MI + topiroxostat group), irbesartan (30 mg/ kg)[12] (in MI + irbesartan group) or vehicle (0.5 mL) (in sham group and MI + control group) were administered to rats once per day from day -7 to day 35 by gavage using a stomach tube.

Induction of myocardial infarction

Rats were anaesthetized by inhalation of isoflurane (3-5%; DS Pharma Animal Health, Osaka, Japan), intubated and mechanically ventilated via tracheal cannula connected to a constant volume ventilator (60 cycles/min, 1 mL/100 g). Left thoracotomy and pericardiotomy were performed, and the left main anterior coronary artery was completely ligated 1-2 mm from its origin with a 6-0 polypropylene suture[12] on day 0. Coronary occlusion was verified by the rapid occurrence of akinesia and discoloration in the area at risk.

In vivo measurement of blood pressure and heart rate

Systolic and diastolic blood pressure (BP) and heart rate (HR) were measured by a tail cuff system (BP-98A, Softron, Tokyo, Japan) on the day -7 (before MI operation and just before drug administration), day 0 (just before MI operation), days 7, 21 and 35. The mean arterial pressure (MAP) was calculated from measured systolic and diastolic BP.

Echocardiographic analysis

Cardiac function and LV morphology were evaluated with transthoracic echocardiography using the LOGIQ P5 system with a 12-MHz probe (12 L, GE Healthcare, Fairfield, CT). Echocardiography was performed under anesthesia with isoflurane (3-5%) on days 0, 7, 21 and 35. We used the images of mid-papillary short-axis (SAX) views of the LV for analysis of LV end diastolic dimension (LVEDD), LV end systolic dimension (LVESD), anterior wall thickness (AWT), and fractional shortening (FS). All measurements were made in triplicate and averaged by two independent experienced examiners in a blinded fashion.

Statistical Analysis

Comparisons of the cardiac function, MAP and HR among multiple groups were determined by one-way ANOVA with the Tukey-Kramer test. All data are expressed as the mean ± S.E.M.; P<0.05 was considered statistically significant. Comparisons within a group were made by repeat measures one-way ANOVA followed by the Bonferroni multiple comparison post test analysis when the global test was significant. Two-way ANOVA was used to compare effects of topiroxostat, irbesartan and vehicle, followed by Bonferroni post tests. Unpaired t-test was performed for comparison between groups.

Results

Effects of topiroxostat and irbesartan on MAP and HR

There were no changes in MAP either in the Sham or the MI + topiroxostat group during the entire period. In the MI + vehicle group, MAP trended to decrease on day 7 compared to that on day -7. In the MI+ Irbesartan group, MAP
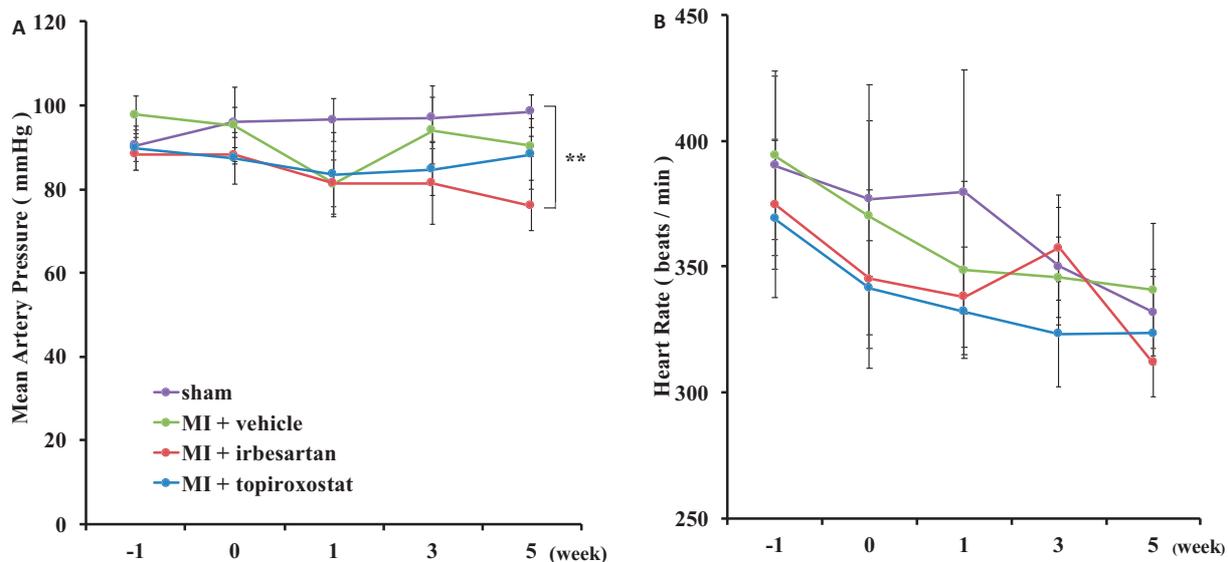
Figure 1. Effects of topiroxostat and irbesartan on the blood pressure before and after myocardial infarction.
Red: irbesartan, blue: topiroxostat. Green; vehicle, purple: sham
*: p<0.05 vs sham
**: p<0.01 vs sham

on day 35 significantly decreased compared to that in the Sham group (**Figure 1A**). There was no significant difference in changes in HR among all groups during the entire period (**Figure 1B**).

Effects of topiroxostat and irbesartan on cardiac changes

Both LVEDD and LVESD significantly increased after MI, while AWT and FS significantly decreased in the MI + vehicle, MI + irbesartan and MI + topiroxostat groups compared to those in the Sham group (**Figure 2**). In the MI + irbesartan group, LVEDD significant decreased compared to that in the MI + vehicle group (on day 7 and day 35) and MI + topiroxostat group (on day 35) (**Figure 2A**). In the MI + irbesartan group, LVESD also significantly decreased compared to that in the MI + vehicle group (from day 7 to day 35), and the MI + topiroxostat group (on day 7 and day 35) (**Figure 2B**). There was no significant difference in AWT after MI among the 3 groups (MI + vehicle, irbesartan and topiroxostat groups) (**Figure 2C**).

FS significantly increased in the MI + irbesartan group, compared to that in the MI + vehicle group on days 21 and 35. In the MI + topiroxostat group, FS also significantly increased compared to that in the MI + vehicle group at the same time points (**Figure 1D and F**). However, FS in the MI + topiroxostat group was significantly lower than that in the MI + irbesartan group (FS on day 35; 39.1% in the sham group, 13.8% in the MI + vehicle group, 16.8% in the MI + topiroxostat group, 21.6% in the MI + irbesartan group).

Discussion

In this study, we demonstrated the effects of an XO inhibitor topiroxostat and an AT1 receptor blocker irbesartan started 7 days before MI (day -7), on cardiac function and remodeling up to day 35 (42 days observation period). To our knowledge, this study is the first report that directly compared effects of two drugs on cardiac function and remodeling after MI.

The most prominent finding is that topiroxostat significantly improved LV function (FS), although it did not show protective effects on LV remodeling (LVEDD and LVESD). The precise mechanisms on the protective action of topiroxostat on cardiac function without protective action on remodeling remain unknown.

In vitro studies using isolated hearts have shown that progressive development of HF is associated with increased myocardial XO levels, resulting in an increase in cardiac ROS[13,14]. It has been reported that XO-derived ROS could interfere with nitric oxide regulation of myocardial energetics[15] and depressed myocardial excitation-contraction coupling[16]. Allopurinol has been reported to decrease myocardial oxygen consumption and increase cardiac contractility and mechanical efficiency[17]. In the present study, topiroxostat significantly increased cardiac contractility (estimated by FS after MI), indicating that reduction in ROS production by topiroxostat may restore nitric oxide regulation of myocardial energetics.

In contrast, topiroxostat could not ameliorate cardiac remodeling after MI. We recently showed that topiroxostat (3 μmol/L) prevented LV dysfunction and facilitated recovery from arrhythmias using an ischemia-reperfusion (I/R) model of rat heart. Topiroxostat inhibited XO activity to a much
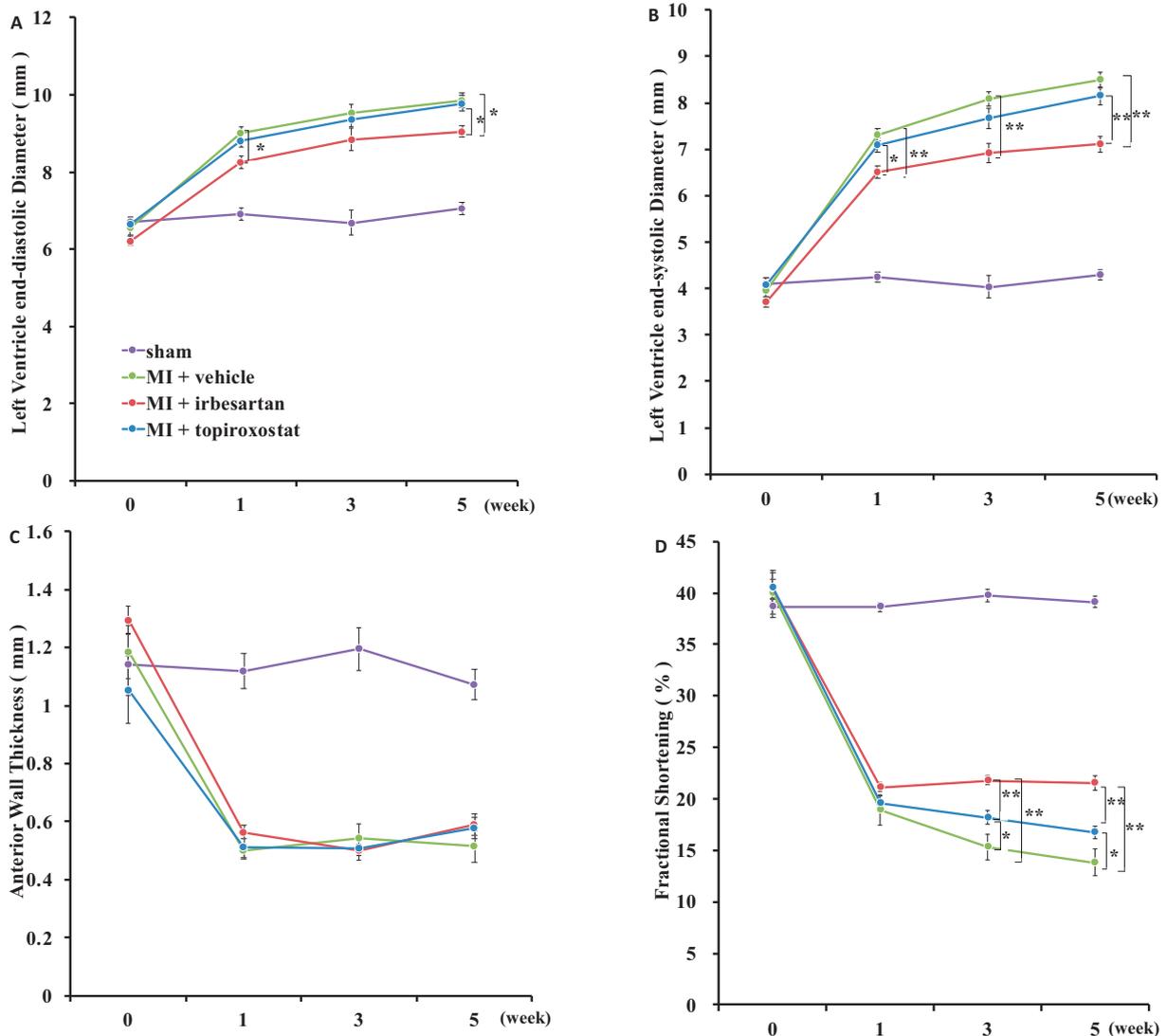
Figure 2. Effects of topiroxostat and irbesartan on the cardiac function before and after myocardial infarction.

Panel A: Effects of topiroxostat and irbesartan on FS before and after myocardial infarction. Note that topiroxostat and irbesartan significantly improved FS.

Panel B: Effects of topiroxostat and irbesartan on LVEDD before and after myocardial infarction. Note that irbesartan significantly improved LVEDD.

Panel C: Effects of topiroxostat and irbesartan on LVESD before and after myocardial infarction. Note that irbesartan significantly improved LVESD.

Panel D: Effects of topiroxostat and irbesartan on AWT before and after myocardial infarction. Note that both agents did not improve AWT.

Red: irbesartan, blue: topiroxostat. Green; vehicle, purple: sham

LVEDD: Left ventricular end diastolic dimension, LVESD: Left ventricular end systolic dimension, AWT: anterior wall thickness, FS: fractional shortening

*: $p < 0.05$, **: $p < 0.01$

greater extent than allopurinol[18]. Sugiyama et al. indicated that the minimum and maximum plasma concentrations of topiroxostat were estimated to be 0.93 and 7.1 μmol/L, respectively, which are reached by oral administration of topiroxostat at 20 and 180 mg/day[19]. Topiroxostat concentration in the I/R model at 3 μmol/L was within its clinical concentration. In this study, we orally administered of topiroxostat at 0.5 mg/kg/day in rats. We chose this dose because there was a possibility of nephropathy at 1 mg/kg/day[20]; thus, we prioritized safety and reduced the dosage considerably. The

dosage of 0.5 mg/kg/day was very low compared to the clinical dosage (2.5-3 mg/kg/day). Thus, one of the possible reasons that topiroxostat did not show a protective effect on cardiac remodeling in this study may be under-dosage of topiroxostat. In addition, topiroxostat has reported to show mechanism-based and structure-based inhibition of XO without any inhibitory actions on other enzymes. In the MI model, the mechanisms of LV remodeling include multiple factors, such as TNF-α, NF-κB, and activator protein-1 (AP-1) not only ROS. The different mechanisms between I/R

model and MI model was one of the possible reasons why topiroxostat showed the protective effect on cardiac function without any protective action in the MI model.

Irbesartan prevented LV remodeling starting from an early phase (day 7) to the chronic phase (day 35) and improved LV function in the chronic phase. On day 35, MAP was lower in the irbesartan group than in the sham group. It is generally accepted that Ang II promotes cardiac remodeling via AT1 receptors, which increase in the heart after MI. Ang II activates various transcription factors, such as TNF-α, NF-κB and AP-1. Irbesartan is a high selective and long-acting AT1 receptor blocker. Berthonneche et al showed that administration of irbesartan in the early phase (7 days after MI) improved cardiac function and cardiac geometry mediated by inhibition of myocardial TNF-α generation in an MI rat model[12]. Watanabe et al showed that irbesartan exerted antifibrotic and anti-inflammatory effects by inhibiting the activation of NF-κB, AP-1 and NOX4 28 days after MI induction simulating cardiorenal syndrome in a rat model[21]. In addition, Schafer et al indicated that irbesartan restored aortic eNOS expression and reduced aortic superoxide formation in CHF 10 weeks after MI induction in rats[22]. We have also reported that irbesartan chronically suppressed LV remodeling after MI, which may be related to reduced TNF-α, NF-κB, AP-1 and MAP[21].

It is interesting to compare the potency of topiroxostat to protect cardiac function to that of the AT1 receptor blocker, irbesartan, a standard treatment for CHF. Although the cardioprotective effect of topiroxostat on the MI heart was weaker than that of irbesartan, it significantly improved cardiac function compare to the vehicle group. Since its mechanism on improving cardiac function is different from that of irbesartan, a combination of topiroxostat and irbesartan may accentuate their protective action on cardiac function after MI.

Our study has several limitations. First, it is unclear whether the dosages of topiroxostat and irbesartan were appropriate and equipotent. There are not many reports regarding appropriate doses of topiroxostat in a rat model. As mentioned above, we prioritized safety and reduced the dosage considerably. Topiroxostat dosage of 0.5 mg/kg/day may be too low. More studies are required about topiroxostat dosage. We used irbesartan at 30 mg/kg/day[12], which was within the no-observed-adverse-effect level and much higher than the clinical dosages (3-3.5 mg/kg/day). Second, we studied MAP, HR and a few echocardiographic parameters, but did not study changes in cytokine levels, hemodynamics, oxidative stress and uric acid level. Further studies are needed to clarify the detailed mechanisms. Third, we did not measure the marker of oxidative stress such as TBARS to evaluate the effect of topiroxostat and irbesartan on oxidative stress. Fourth, in the present study, we examined whether pretreatment with topiroxostat and irbesartan prior to MI could improve cardiac dysfunction after MI. However, we did not evaluate why there was no significant difference in the size of MI among groups because of experimental limitations.

In conclusion, a non-purine XO inhibitor, topiroxostat, improved cardiac function after MI. Although it was shown that topiroxostat had different mechanisms for the cardiac protective effect compared to irbesartan and it exerted lesser cardiac protective effects than irbesartan, topiroxostat will be a potential drug for improving cardiac functions after MI.

Conflicts of Interest
Dr. I. Hisatome reported receiving lecturer's fee from Sanwa Kagaku Kenkyusho Co. Ltd, Feizer Co. Ltd. and Fuji Yakuhin Co. Ltd., and research grants from Dainippon Sumitomo Pharmaco. Co., Teijin Pharma, Fuji Yakuhin Co. Ltd and Sanwa Kagaku Kenkyusho Co. Ltd.

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