Department of Pharmacology[1], School of Pharmaceutical Science, Jilin University; FAW General Hospital[2], the Fourth Hospital of Jilin University, Changchun, P. R. China

Cardiovascular protective effects of IL-1ra-Fc-IL-18BP on experimental myocardial infarction by inhibiting oxidative stress and inflammation in a rat model

Hong Zhang[1], Huali Xu[1], Haolin Xie[2], Feng Li[1], Xiaofeng Yu[1], Dayun Sui[1]

Received March 20, 2014, accepted April 18, 2014

Dayun Sui, Ph.D, Xiaofeng Yu, Department of Pharmacology, School of Pharmaceutical Science, Jilin University, No. 1266 Fujin Rd. Changchun 130021, Jilin Province, P.R. China
suidy@jlu.edu.cn

Pharmazie 69: 769–774 (2014) *doi: 10.1691/ph.2014.4595*

In this study, we examined the cardiovascular protective effects of IL-1ra-Fc-IL-18BP on experimental myocardial infarction in a rat model. An animal model of myocardial infarction (MI) was induced by permanent ligation of the left anterior descending coronary artery (LAD) in SD rats. After surgery sixty male rats and sixty female rats were randomly divided into groups as followed: sham group, MI group, IL-1ra-Fc-IL-18BP 50, 100, 200 mg/kg treatment groups, and verapamil 5 mg/kg treatment group. IL-1ra-Fc-IL-18BP and verapamil were administered to the animals immediately after operation by intravenous injection. Treatment with IL-1ra-Fc-IL-18BP (50, 100 and 200 mg/kg) could remarkably decrease infarct size from 24.82% to 13.43% ($p < 0.05$), and decrease the activities of serum aspartate aminotransferase (AST), creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) compared with sham group ($p < 0.05$). Meanwhile, treatment with IL-1ra-Fc-IL-18BP (200 mg/kg) could significantly increase the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), but decreased the content of malondiadehyde (MDA) in serum ($p < 0.05$). Furthermore, IL-1ra-Fc-IL-18BP marablely reduced the content of calcium (Ca^{2+}) in serum ($p < 0.05$), and also decreased the levels of serum interleukin-1β (IL-1β), tumor necrosis factor (TNF-α) ($p < 0.05$). Histopathological results demonstrated the same protective effect of IL-1ra-Fc-IL-18BP. All these results above indicated that IL-1ra-Fc-IL-18BP has protective effects in myocardial infarction, improves free radicals metabolism, ameliorates myocardial calcium overload and inhibits the release of inflammatory cytokines.

1. Introduction

Myocardial infarction (MI) occurs when a coronary artery is severely blocked causing a significant reduction in blood supply and damage or death to a portion of the myocardium. According to statistics, MI is the leading cause of death worldwide, especially in the elderly (Colombo et al. 2014; Puymirat et al. 2013). Depending on the extent of heart muscle damage, the patient may experience significant disability or death (Kirchberger et al. 2014), as a result of myocardial infarction. Although modern drugs, such as calcium channel blockers, angiotensin-converting enzyme inhibitors (ACEI) have shown cardioprotective effects in both preclinical and clinical studies. In recent years, advanced studies show that inflammatory cytokines and myocardial ischemia are closely related (de Haan et al. 2013; Zeybek et al. 2011). It tips that targeting cytokine may become a new way to therapy myocardial infarction.

Interleukin-1 (IL-1) and interleukin-18 (IL-18) are potent pro-inflammatory cytokines in inflammation-related diseases, such as arthritis, atherosclerosis, myocardial infarction and myocardial ischemia-reperfusion injury (Dinarello 1996, 2006). Their actions are regulated by the IL-1 receptor antagonist (IL-1ra) and the IL-18 binding protein (IL-18BP). IL-1ra-Fc-IL-18BP is a recombinant human fusion protein. It contains an amino-terminal segment that specifically binds to IL-18 and a carboxy-terminal sequence of IL-1ra that binds to the IL-1 receptor. The Fc portion of human IgG1 in the fusion protein links the two segments and is capable of dimerizing. IL-1ra-Fc-IL-18BP is not a soluble form of either chain of the IL-18 receptor but rather a constitutively secreted, high-affinity and specific inhibitor of IL-18. IL-1ra-Fc-IL-18BP is currently applied in clinical trials for the treatment of rheumatoid arthritis and severe psoriasis, but the results have not yet been published (Liu et al. 2011), and it is rarely reported that IL-1ra-Fc-IL-18BP has an effect on cardiovascular diseases. The aim of our study was to explore the cardiovascular protective effects of IL-1ra-Fc-IL-18BP on experimental myocardial infarction in a rat model.

2. Investigations and results

2.1. Effects of IL-1ra-Fc-IL-18BP on myocardial infarct size and serum AST, CPK, LDH activity

Based on the examination of nitro-blue tetrazolium (NBT) hearts, a typical myocardial ischaemic zone was observed in the

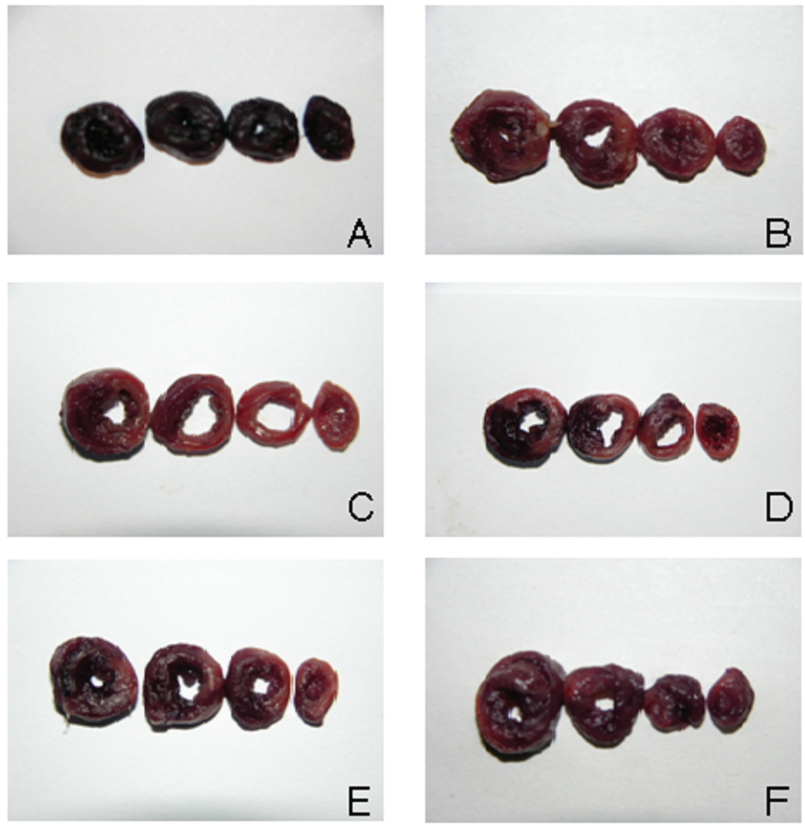

Fig. 1: IL-1ra-Fc-IL-18BP on infarct zone in rats stained by NBT. The tissues of ventricles were excised and cut into transverse slice and incubated for 20 min in 0.05% nitrotetrazolium blue chloride (NBT) at 37 °C. White color means ischemic myocardium and dark red color means non-ischemic myocardium. A: Sham group; B: MI group; C: IL-1ra-Fc-IL-18BP 50 mg/kg; D: IL-1ra-Fc-IL-18BP 100 mg/kg; E: IL-1ra-Fc-IL-18BP 200 mg/kg; F: Verapamil 5 mg/kg.

MI group, white for ischemic myocardium and dark red for non-ischemic myocardium (Fig. 1). As shown in Fig. 2, the infarct size presented as a ratio of the weight of ischemic zone over ventricular mass being $24.82 \pm 6.72\%$ in the MI group and statistically significant compared to sham group. The infarct sizes were $15.77 \pm 5.67\%$, $14.09 \pm 4.32\%$ and $13.43 \pm 4.85\%$, respectively after treatment of IL-1ra-Fc-IL-18BP.

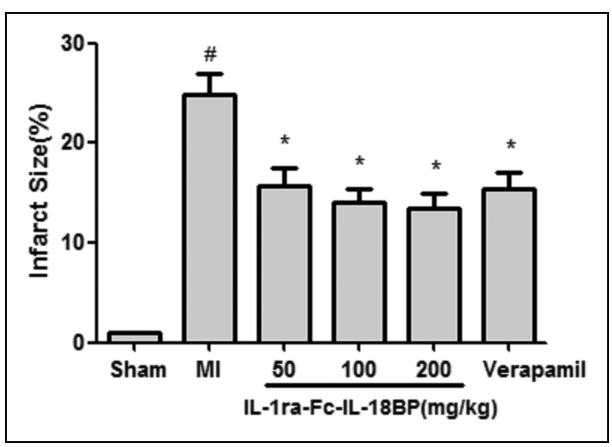

Fig. 2: Effects of IL-1ra-Fc-IL-18BP on myocardial infarct size. The tissues of ventricles were excised and cut into transverse slice and incubated for 20 min in 0.05% NBT at 37 °C. White color means ischemic myocardium and dark red color means non-ischemic myocardium. The ischemic myocardium was cut and weighted. The infarct size as a percent of the ventricular mass was calculated as: weight of ischemia zone/ total weight of ventricular × 100%. Myocardial infarct size is expressed as percentage of region of ventricle. Data are expressed mean ± SD for each group. $^{\#}P < 0.05$ vs. Sham group $^{*}P < 0.05$ vs. MI group.

AST, CPK and LDH release from the cardiomyocytes reflects cellular injury or tissue necrosis and membrane permeability. The amount of those enzymes were much higher in the MI group. The amount of AST, CPK and LDH release were significantly decreased in the IL-1ra-Fc-IL-18BP groups ($p < 0.05$) (Table). Our results indicated that IL-1ra-Fc-IL-18BP showed cardioprotection.

2.2. Effects of IL-1ra-Fc-IL-18BP on the activities of SOD, GSH-Px and the content of MDA in serum

As shown in Fig. 3, the activities of antioxidant enzymes SOD and GSH-Px were significantly decreased, while the content of MDA, an index of lipid peroxidation, was increased significantly in the MI group compared with the sham group. Treatment with IL-1ra-Fc-IL-18BP (50, 100 and 200 mg/kg) could increase the serum activities of SOD (240.4 ± 36.0, 252.9 ± 44.0 and 257.8 ± 35.6 U/ml, Fig. 3A), and that of GSH-Px (1235.8 ± 194.2, 1306.5 ± 256.5 and 1352.8 ± 221.4 μmol/L, Fig. 3B). However, IL-1ra-Fc-IL-18BP decreased the serum content of MDA (5.45 ± 1.96, 4.37 ± 1.56 and 4.24 ± 0.74 nmol/ml, Fig. 3C).

2.3. Effects of IL-1ra-Fc-IL-18BP on the content of Ca^{2+} in serum

As shown in Fig. 4, serum Ca^{2+} contents of MI group (3.08 ± 0.63 mmol/L) were found to be statistically significant higher ($p < 0.05$) than that of the sham group (2.47 ± 0.38 mmol/L). With the treatment of IL-1ra-Fc-IL-18BP, the Ca^{2+}

Table 1: Effect of IL-1ra-Fc-IL-18BP on the activities of aspartate aminotransferase (AST), creatine phosphokinase (CPK), lactate dehydrogenase (LDH) in serum

Group	Infarct size (%)	AST (U/L)	CPK (U/L)	LDH (U/L)
Sham	0.97 ± 0.22	174.9 ± 73.6	594.3 ± 213.0	405.6 ± 140.6
MI	24.82 ± 6.72#	259.0 ± 59.9#	846.6 ± 212.1#	647.0 ± 200.8#
IL-1ra-Fc-IL-18BP				
50 mg/kg	15.77 ± 5.67*	246.8 ± 68.7	750.9 ± 199.3	579.2 ± 203.8
100 mg/kg	14.09 ± 4.32*	207.7 ± 37.5*	681.3 ± 129.5*	474.9 ± 114.5*
200 mg/kg	13.43 ± 4.85*	199.4 ± 61.9*	636.7 ± 163.1*	459.8 ± 148.8*
Verapamil	15.40 ± 5.07*	205.3 ± 44.5*	651.9 ± 174.6*	475.7 ± 94.6*

#$P < 0.05$ vs. Sham group *$P < 0.05$ vs. MI group

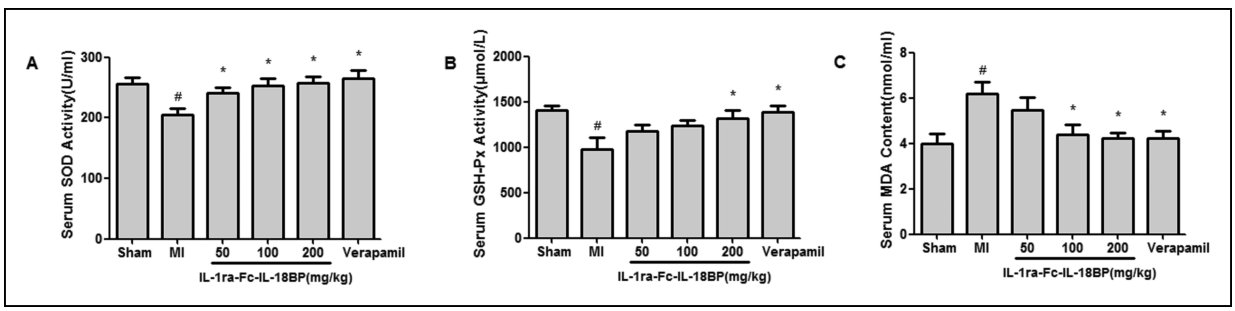

Fig. 3: Effects of IL-1ra-Fc-IL-18BP on the activities of superoxide dismutase (SOD) (A), glutathione peroxidase (GSH-Px) (B), and the content of malondialdehyde (MDA) (C) in serum. Blood sample was collected from abdominal aorta was taken to measure the activities of SOD, GSH-Px and the content of MDA by using diagnostic kits. Data are expressed mean ± SD for each group. #P < 0.05 vs. Sham group *P < 0.05 vs. MI group.

content in serum were found to be 2.60 ± 0.37 2.34 ± 0.29 and 2.26 ± 0.30 mmol/L, respectively.

2.4. Effects of IL-1ra-Fc-IL-18BP on inflammatory cytokines

As shown in Fig. 5, compared with the rats in sham group, the content of inflammatory cytokines IL-1β, TNF-α were increased in the MI group. Treatment with IL-1ra-Fc-IL-18BP (200 mg/kg) could inhibit the increase in IL-1β, TNF-α content compared with the MI group ($p < 0.05$). IL-1ra-Fc-IL-18BP had a dose-dependent effect because the high dose of IL-1ra-Fc-IL-18BP showed a more significant inhibition than the low dose.

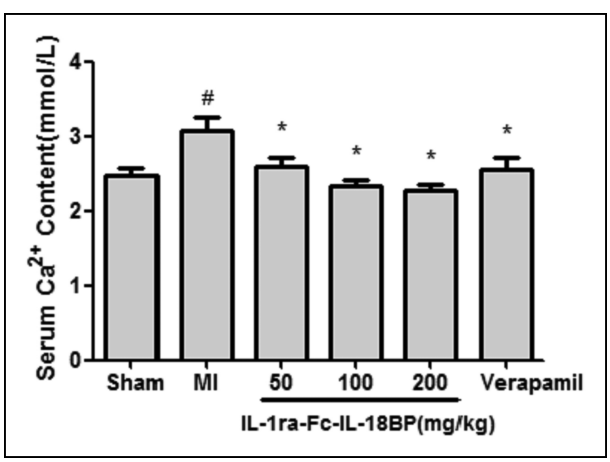

Fig. 4: Effects of IL-1ra-Fc-IL-18BP on the content of calcium (Ca^{2+}). Blood sample was collected from abdominal aorta was taken to measure the content of Ca^{2+} by using diagnostic kits. Data are expressed mean ± SD for each group. #P < 0.01 vs. Sham group *P < 0.05 vs. MI group.

2.5. Histopathological examination of cardiac tissues

As shown in Fig. 6, the heart tissues in the infarct control group showed myocardial cell loss, widespread myocardial structure disorder, myocardium fragment, hyperaemia and leukocyte infil-

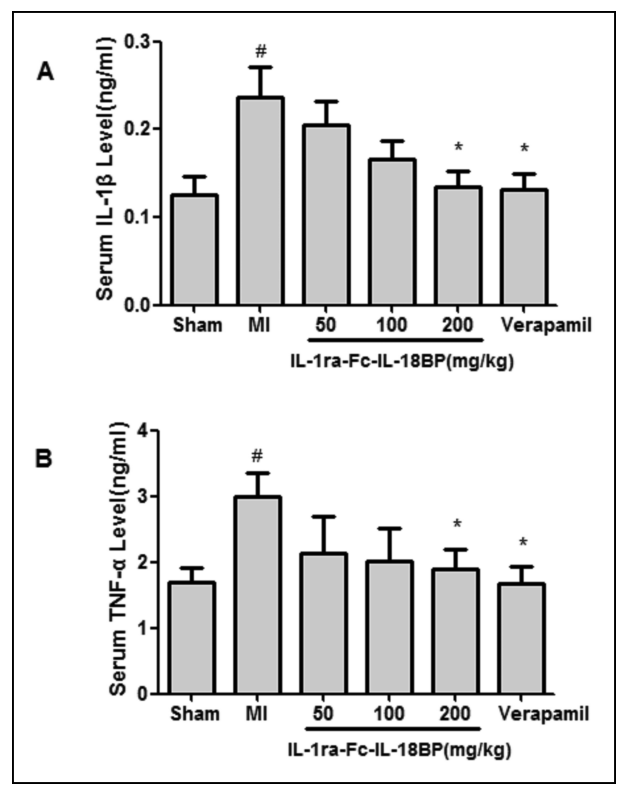

Fig. 5: Effects of IL-1ra-Fc-IL-18BP on the content of interleukin-1β (IL-1β), tumor necrosis factor (TNF-α) in serum. Blood sample was collected from abdominal aorta for the estimation of the content of IL-1β, TNF-α in serum according to the instructions. Data are expressed, mean ± SD for each group. #P < 0.05 vs. Sham group *P < 0.05 vs. MI group.

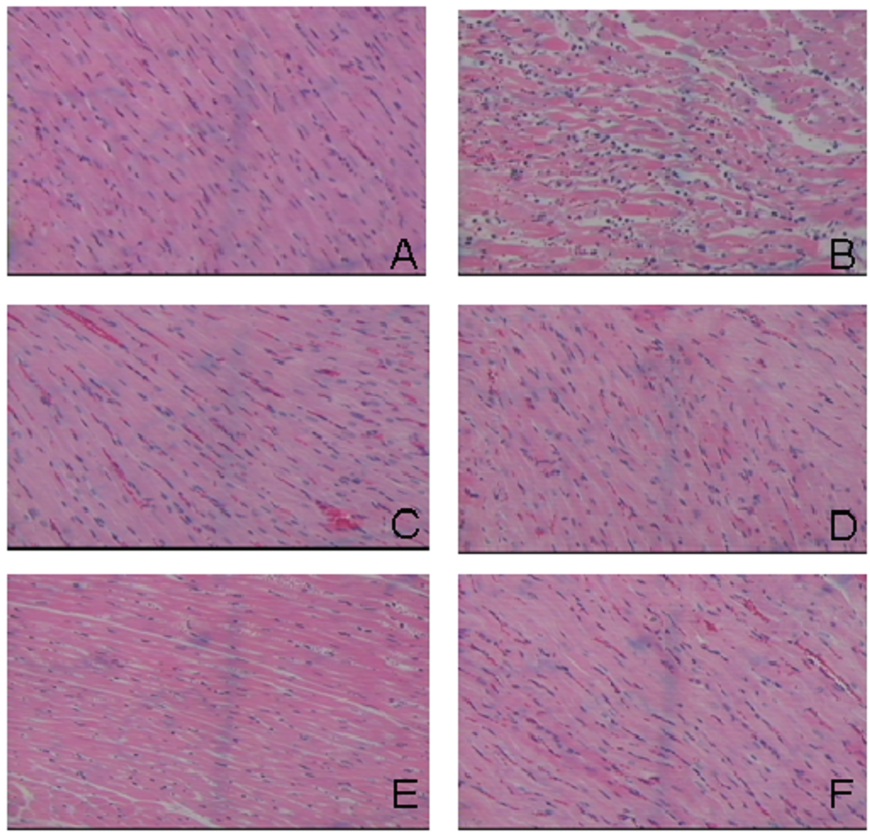

Fig. 6: Representative haematoxylin-eosin (H&E) pathological photomicrographs of left ventricular tissue. The cardiac apex was excised and fixed with 10% formalin for subsequent H&E staining. The sections were examined under light microscope, and then photomicrographs were taken. (magnification × 200), A: Sham group; B: MI group; C: IL-1ra-Fc-IL-18BP 50 mg/kg; D: IL-1ra-Fc-IL-18BP 100 mg/kg; E: IL-1ra-Fc-IL-18BP 200 mg/kg; F: Verapamil 5 mg/kg.

tration. Treatment with IL-1ra-Fc-IL-18BP (100 and 200 mg/kg) significantly attenuated the pathophysiological changes in the cardiac muscle fiber. IL-1ra-Fc-IL-18BP (50 mg/kg) had rarely an effect.

3. Discussion

In the present study, we demonstrated that IL-1ra-Fc-IL-18BP elicited a significant cardioprotective effect on myocardial infarction induced by permanent ligation of the left anterior descending coronary artery.

Infarct size is used for evaluation of left ventricular function. It is also an important parameter for evaluation the effectiveness of cardiovascular drugs in the treatment of ischemic heart disease (Dupliakov et al. 2013). Rats treated with IL-1ra-Fc-IL-18BP (100 and 200 mg/kg) significantly decreased the infarct size after MI in rats (Fig. 1).

Myocardial infarction induces cell membrane to permeate or rupture, which results in the leakage of the AST, CPK and LDH into blood (Howie-Esquivel and White 2008). Hence, the AST, CPK and LDH activities in serum reflect the alterations of membrane integrity and the degree of myocardial injury. Consequently, the activities of myocardial enzymes in blood serum increase, so changes in serum myocardial enzymes are considered to be a measure of impairment produced by myocardial ischemia (Kelley et al. 2009). Our results showed a significant increase in the activities of AST, CPK and LDH in MI rats. Treatment with IL-1ra-Fc-IL-18BP (100 and 200 mg/kg) significantly decreased the activities of AST, CPK and LDH in serum (Table). These results suggest that the protective effects of IL-1ra-Fc-IL-18BP against MI may be produced by elevating cardiomyocyte membrane stability, to decrease the out leakage of enzymes and by reducing the activities of these enzymes.

Severe oxidative stress could be induced by myocardial infarction in the myocardium of rats (Misra et al. 2009). Overproduction of reactive oxygen species and/or the depletion of the antioxidants in the defense system may result in a lipid peroxidative process and affect the pathogenesis of myocardial infarction (De Rosa et al. 2010). Free radical scavenging enzymes such as SOD and GSH-Px are the first line cellular defense against oxidative stress, eliminating reactive oxygen radicals. MDA is a major lipid peroxidant end product, increased MDA content indicates activation of the lipid peroxidative process (Azizova et al. 2009). Our study demonstrated that IL-1ra-Fc-IL-18BP treatment could reduce MDA content elevation and increase the activities of SOD and GSH-Px in myocardial infracted rats (Fig. 3). The findings indicated that the cardioprotective property of IL-1ra-Fc-IL-18BP was associated with its antioxidant activity, at least in part.

Oxidative stress due to the formation of hydrogen peroxide leading to lipid peroxidation and sulfhydryl group oxidation during myocardial ischemia seems to be one of the mechanisms that may produce membrane defects and result in intracellular calcium overload and cardiac contractile dysfunction in the stunned myocardium (Dhalla et al. 1999). The intracellular accumulation of Na^+ and Ca^{2+} plays a key role in ischemia-induced myocardial injury that may be manifest as left ventricular (LV) mechanical dysfunction, arrhythmia, or infarction (Clanachan 2006). Our results demonstrated that IL-1ra-Fc-IL-18BP treatment could reduce Ca^{2+} content elevation. In the present study, Ca^{2+} content was significantly higher in MI group compared

with the sham group. Administration with IL-1ra-Fc-IL-18BP at a dose of 50, 100 or 200 mg/kg reduced Ca^{2+} content (Fig. 4). The findings indicated that the cardioprotective property of IL-1ra-Fc-IL-18BP was associated with ameliorating myocardial calcium overload.

Inflammation plays a crucial role in the pathophysiology of myocardial infarction. The inflammasome is a large multi-protein complex that is formed in the cytosol in response to danger signals; it drives the proinflammatory cytokine IL-1β. Research indicates that the inflammasome is a key player in the disease processes of sterile inflammation. In particular, MI is accompanied by significantly increased cytokine levels, including the cytokine IL-1β, TNF-α for at least several hours to weeks after occurrence of the event (Francis et al. 2003; Takahashi 2011). We selected the MI rat heart model to explore the inflammation targeting properties of IL-1ra-Fc-IL-18BP because proinflammatory cytokines, such as TNF-α, IL-1, IL-6 and IL-18 are induced at the site of myocardial injury (Dinarello 2001; Frangogiannis 2006). Treatment with IL-1ra-Fc-IL-18BP (100 mg/kg) could inhibit the increase in IL-1β, TNF-α content compared with MI group (Fig. 5). The results suggested that IL-1ra-Fc-IL-18BP could inhibit the release of inflammatory cytokine, thus play a role in protecting the myocardial ischemia. In summary, IL-1ra-Fc-IL-18BP could provide significant cardioprotective effects against myocardial infarction injury in rats. The mechanisms might be attributed to improve free radicals metabolism, ameliorate myocardial calcium overload and inhibit the release of inflammatory cytokine. These results indicated that IL-1ra-Fc-IL-18BP might be an effective protective agent for preventing and treating myocardial infarction.

4. Experimental

4.1. Experimental animals

The experimental protocol used in this study was reviewed and approved by the Animal Care and Use Committee of Jilin University and in accordance with the National Institutes of Health guidelines for the use of experimental animals. Male and female SD rats weighing 230 g to 250 g (12 to 14 wk old) were provided by the Experimental Animal Center of Jilin University. All animals were allowed free access to food and water and maintained at 22–24 °C under a cycle of 12 h:12 h light-dark.

4.2. Drugs and reagents

IL-1ra-Fc-IL-18BP was produced by Harbin Pharmaceutical Group Bioengineering Co., Ltd., China. IL-1ra-Fc-IL-18BP was dissolved in normal saline to the desired concentration before use. Nitrotetrazolium blue chloride (NBT) was purchased from Shanghai Qianjin Chemical Agent Factory, China. Commercial kits for SOD, GSH-Px, MDA, Ca^{2+} were purchased from Nanjing Jiancheng Bioengineering Institute, China. IL-1β, TNF-α RIA kits were bought from Cogent Rui Biotechnology Co., Ltd., Beijing, China.

4.3. In vivo myocardial infarction in rats

The myocardial infarction animal model was established by ligation of the left anterior descending (LAD) coronary artery, as described previously (Yang et al. 2010; Zhou et al. 2012). Briefly, rats were anaesthetized with urethane (1.0 g/kg, i.p.), they were artificially ventilated using a volume-regulated respirator. The thorax was opened, the heart was exteriorized, and the left coronary artery was ligated 2-3 mm beneath its origin between the pulmonary artery conus and left atrium using a 6-0 Prolene suture. The heart was returned and the thorax was closed. Sham group underwent the same surgical procedure without LAD ligation.

The surviving sixty male rats and sixty female rats were randomly divided into groups as follows: sham group, MI group, IL-1ra-Fc-IL-18BP 50, 100, 200 mg/kg treatment groups, and verapamil 5 mg/kg treatment group. IL-1ra-Fc-IL-18BP and verapamil were administered to the animals immediately after operation by intravenous injection.

4.4. Determination of infarct size

After 24 h of ligation, the heart was excised from the thorax rapidly and the greater vessels were removed. The left ventricle was separated from the heart and weighed. It was sliced parallel to the atrioventricular groove and the 3 mm thick slices were incubated in 0.5% nitrotetrazolium blue chloride (NBT) solution prepared in pH 7.38 phosphate buffer for 20 s at 37 °C (Xu et al. 2013). White color means ischemic myocardium and dark red color means non-ischemic myocardium. The ischemic myocardium was cut and weighed. The infarct size as a percent of the ventricular mass was calculated as: weight of ischemia zone/total weight of ventricular × 100%. Myocardial infarct size is expressed as percentage of region of ventricle.

4.5. Biochemical analysis of serum

After 24 h of ligating, rats were anesthetized with pentobarbital sodium (30 mg/kg, ip) and blood samples were collected from each group from the abdominal aorta for the estimation of the activities of AST, CPK, LDH, SOD, GSH-Px, and the content of MDA, Ca^{2+}, IL-1β, TNF-α in blood serum. The serum AST, CPK and LDH activities were measured on COBAS-FAR A automatic biochemical analyzer (Germany). The activities of SOD, GSH-Px and the content of MDA, Ca^{2+}, IL-1β, TNF-α in serum were measured using diagnostic kits according to the manufacturer's instructions.

4.6. Assessment of histopathology of myocardium

For light microscopic evaluation, tissue sections from the left ventricles were fixed in phosphate buffered 10% formalin solution. The specimens embedded with paraffin were cut into 5 μm thick sections and stained with hematoxylin–eosin (Wang et al. 2010). The sections were examined by an experienced observer who was blind to the treatment under light microscope and then photomicrographs were taken.

4.7. Statistical analysis

All the grouped data were statistically evaluated with student-test. P values of less than 0.05 were considered to indicate statistical significance. All these results were expressed as mean ± SD.

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