Effect of interleukin-23 on inflammation response and oxidative stress in rats with myocardial ischemia reperfusion injury

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Objective: To investigate the effect of interleukin-23 (IL-23) on myocardial ischemia-reperfusion (I/R) injury. Methods: Male Sprague-Dawley rats were randomly assigned into sham operated control (SO) group, ischemia and reperfusion (I/R) group, (IL-23 + I/R) group and (anti-IL-23 + I/R) group. At 4 h after reperfusion, the serum concentration of lactate dehydrogenase (LDH), creatine kinase (CK) and the tissue MDA concentration and SOD activity were measured. The infarct size was measured by TTC staining. Apoptosis in heart sections were measured by TUNEL staining. The expression of HMGB1 and IL-17A were detected by Western Blotting and the expression of TNF-α and IL-6 were detected by Elisa.

Results: After 4 h reperfusion, compared with the I/R group, IL-23 significantly increased the infarct size, the apoptosis of cardiomyocytes and the levels of LDH and CK. Meanwhile, IL-23 significantly increased the expression of eIL-17A, TNF-α and IL-6 and enhanced both the increase of the MDA level and the decrease of the SOD level induced by I/R. IL-23 had no effect on the expression of HMGB1. All these effects were abolished by anti-IL-23 administration.

Conclusion: The present study suggested that IL-23 may promote myocardial I/R injury by increasing the inflammatory responses and oxidative stress reaction.

1. Introduction

Reperfusion therapy, such as percutaneous coronary intervention (PCI) therapy, is the most effective method for myocardial infarction. However, reperfusion itself can worsen myocardial apoptosis and necrosis, which is termed as myocardial ischemia and reperfusion (I/R) injury. It is well known that inflammation and oxidative stress are the two major mechanisms underlying myocardial I/R injury and preserve cardiac function[4,5].

IL-23, a member of IL-12 family, is a pro-inflammatory cytokine which consists of the IL-23p19 and P40 subunits. IL-23 has been shown to participate in many inflammatory diseases, such as inflammatory bowel disease, psoriasis and coronary heart disease[6-8]. There are emerging evidence suggests that HMGB1-

TLR4-IL-23/IL-17 axis plays a critical role in initiating and enhancing the inflammatory responses and ROS production during myocardial I/R[9-11]. Thus, we hypothesize that IL-23 may promote myocardial I/R injury by increasing inflammatory response and oxidative stress.

2. Materials and Methods

2.1. Animal preparation and experimental designs

The experiment protocol conformed to the Guideline for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication, revised 1996) and was approved by Institutional Animal Care and Use Committee. Male Sprague-Dawley rats (250-300 g) were randomly assigned into 4 groups receiving the following treatments:

Group 1: sham operated control (SO) (n=10): rats were subjected to surgical manipulation without the induction of myocardial ischemia.

Group 2: ischemia and reperfusion (I/R) (n=30): rats were subjected to the left anterior descending coronary artery (LAD)
occlusion for 30 min followed by reperfusion for 1 h (n=10), 2 h (n=10), 4 h (n=10).

Group 3: IL-23+I/R (IL-23+I/R) (n=15): the rats were subjected to LAD occlusion for 30 min and followed by reperfusion for 4 h. Rats were once treated with IL-23 (40 ug/kg, i.v., tail vein, Peprotech, America) 15 min before reperfusion. IL-23 was dissolved in sterile saline.

Group 4: anti-IL-23+I/R (anti-IL-23+I/R) (n=30): the rats were subjected to LAD occlusion for 30 min and followed by reperfusion for 1 h (n=10), 2 h (n=10), 4 h (n=10). Rats were treated with rats’ anti-IL-23 (0.2 mg/kg, i.v., tail vein, Boster, Wuhan) 15 min before reperfusion. Anti-IL-23 was dissolved in sterile saline.

After being anesthetized with sodium pentobarbital (45 mg/kg, ip), the rats were ventilated artificially with a volume-controlled rodent respirator at 70 strokes per minute. Rats were placed on an electric heating pad to maintain the body temperature at 37 °C.

2.4. Assessment of HMGB1 and IL-17A by Western blot

Tissue or cell lysates were resolved on SDS-polyacrylamide gels (PAGE) and transferred to polyvinylidene fluoride membranes. After being blocked with 5% non-fat milk, the membranes were probed with primary antibodies, including anti-HMGB1 (diluted 1:2 000, Baster), anti-IL-17A (diluted 1:1 000, Abcam). After incubation with the appropriate secondary antibodies, the specific bands were visualized with an ECL detection system according to the manufacturer’s instructions.

2.5. Measurement of myocardium tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) by Elisa

TNF-α and IL-6 in myocardial tissue supernatants were measured using a commercial enzyme-linked immunosorbent assay kit (Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer’s instructions. The sensitivity of the assay was 1 pg/mL for TNF-α and IL-6.

2.6. MDA and SOD activity assay

Measurement of myocardium malondialdehyde (MDA) concentration and superoxide dismutase (SOD) activity in myocardial tissue were measured using commercialized assay kits in accordance with the manufacturer’s instructions (Nanjing Jiancheng Bioengineering Institute, China), as described in the previous study. The former was used as indexes of oxygen free radical and the latter as lipid superoxide level in the myocardium, respectively.

2.7. Apoptosis assay

In situ cardiac myocyte apoptosis was examined by the TdT-mediated dUTP nick end labelling (TUNEL) assay (Boehringer Mannheim, Indianapolis, IN, USA) as described previously[12]. Five slides from each block were evaluated for percentage of apoptotic cells by using the TUNEL assay. Then 5 watch fields were chosen randomly under microscope on each section. Positive brown cells and total cells were counted by MIAS4.0 (Medical Image Analysis System, Beijing Bingyang Keji Corp., China). The 5 fields were averaged for each block. Then the blocks were averaged for each heart to obtain one number for each heart. Apoptosis index (positive cells/total cells 100%) was used as the indicator of apoptosis.

2.8. Statistical analysis

Statistical analysis was performed with the SPSS 21.0 (SPSS Inc., Chicago, IL, USA). All values were expressed as mean ± SD. One-way ANOVA, Welch or t-test was used for comparisons among groups and the Least-significant Difference or Dunnett T3 was used for post-hoc multiple comparisons. Statistical significance was defined as P<0.05.

3. Results

3.1. Infarct Size

After 4 h reperfusion, the infarct size in the IL-23+ I/R group was significantly increased when compared with the I/R group (P<0.05).
Administration of anti-IL-23 significantly decreased the infarct size compared to that in the I/R group (P<0.05) (Figure 1).

### 3.2. LDH and CK levels

After 4 h reperfusion, both LDH and CK levels in the I/R group were significantly increased compared with that in the SO group (both P<0.05). IL-23 further increased the level of LDH and CK compared with the I/R group (both P<0.05). Administration of anti-IL-23 abolished the effects of IL-23 on the increase of LDH and CK levels compared to that in the IL-23+I/R group and the I/R group, respectively (all P<0.05) (Figure 2A).

Additionally, both LDH and CK in the I/R group and the anti-IL-23+I/R group showed an increasing trend from 1 h reperfusion to 4 h reperfusion. Compared to the I/R group, the high expression levels of LHD and CK at 2 h and 4 h in the anti-IL-23 group were significantly decreased, respectively (both P<0.05) (Figure 2B).

### 3.3. HMGB1 and IL-17A levels

After 4 h reperfusion, the HMGB1 and IL-17A levels in the I/R group were significantly increased compared with those in the SO group (both P<0.05). IL-23 further increased the level of IL-17A (P<0.05), but not HMGB1 (P>0.05) compared with the I/R group. Administration of anti-IL-23 abolished the effect of IL-23 on the increase of IL-17A levels compared with that in the I/R group (P<0.05), but it had no effect on the expression level of HMGB1 (P>0.05) (Figure 3A).

Additionally, both HMGB1 and IL-17A levels in I/R group showed an increasing trend from 1 h to 4 h reperfusion (all P<0.05). This increasing trend of IL-17A expression was inhibited by anti-IL-23 (P<0.05 after 2 h and 4 h reperfusion). However, anti-IL-23 did not affect the expression of HMGB1 (all P>0.05) (Figure 3B and 3C).

### 3.4. TNF-α and IL-6 levels

After 4 h reperfusion, the TNF-α and IL-6 levels in the I/R group was significantly increased compared with those in the SO group (both P<0.05). IL-23 further increased the level of TNF-α and IL-6 compared with the I/R group (both P<0.05). Administration of anti-IL-23 abolished the effects of IL-23 on the increase of TNF-α and IL-6 levels compared to that in the IL-23+I/R group and the I/R group, respectively (all P<0.05) (Figure 4A).

Additionally, both TNF-α and IL-6 in the I/R group and the anti-IL-23+I/R group showed an increasing trend from 1 h reperfusion to 4 h reperfusion. Compared to the I/R group, the high expression...
levels of TNF-α and IL-6 at 2 h and 4 h in the anti-IL-23 group were significantly decreased, respectively (both \( P<0.05 \)) (Figure 4B).

![Figure 4](image)

**Figure 4.** Effect of IL-23 on TNF-α and IL-6 during I/R. ◆ \( P<0.05 \), vs. SO group; # \( P<0.05 \), vs I/R group.

### 3.5. MDA and SOD activity

After 4 h reperfusion, the MDA level in the I/R group was significantly increased while the SOD level was significantly decreased compared with those in the SO group (\( P<0.05 \)). Both the increase of the MDA level and the decrease of the SOD level were significantly enhanced by IL-23 (both \( P<0.05 \)). Administration of anti-IL-23 abolished the effects of IL-23 on the increase of the MDA level and the decrease of the SOD level compared to that in the IL-23+I/R group and the I/R group, respectively (all \( P<0.05 \)) (Figure 5A).

Additionally, both the increase of the MDA level and the decrease of the SOD level were enhanced in the I/R group and the anti-IL-23+I/R group from 1 h reperfusion to 4 h reperfusion, but it did not reach statistical significance in the anti-IL-23+I/R group. Anti-IL-23 inhibited the increase of the MDA level and the decrease of the SOD level at 1 h, 2 h and 4 h, respectively, compared to the I/R group (all \( P<0.05 \)) (Figure 5B).

![Figure 5](image)

**Figure 5.** Effect of IL-23 on MDA and SOD during I/R. ◆ \( P<0.05 \), vs. SO group; # \( P<0.05 \), vs I/R group.

### 3.6. Myocardial cell apoptosis

After 4 h reperfusion, the myocardial apoptosis index in the I/R group was significantly increased compared with that in the SO group (\( P<0.05 \)). IL-23 further increased the apoptosis index compared with the I/R group (\( P<0.05 \)). Administration of anti-IL-23 abolished the effects of IL-23 on the increase of apoptosis index compared to that in the IL-23+I/R group and the I/R group, respectively (both \( P<0.05 \)) (Figure 6).

![Figure 6](image)

**Figure 6.** Effect of IL-23 on apoptosis during I/R. ◆ \( P<0.05 \), vs. SO group; # \( P<0.05 \), vs I/R group.
4. Discussion

In the present study, we demonstrated in I/R rats that IL-23 induced an increase in myocardial infarct size, cardiomyocyte apoptosis index and high levels of LDH and CK. IL-23 also aggravated the expression of inflammatory cytokines including IL-6 and TNF-α and the production of ROS induced by I/R. These effects were abolished by anti-IL-23 administration. These results indicate that IL-23 may promote myocardial I/R injury by increasing the inflammatory responses and oxidative stress reaction.

IL-23, a pro-inflammatory cytokine, is secreted by activated dendritic cells (DC) and phagocytic cells in response to pathogens stimulation and TLR ligands[13]. Its receptor is widely expressed in T cells, natural killer T (NKT) cells, NK cells, macrophages cells, monocytes cells and DC [13, 14]. IL-23 has been reported as an essential factor in the pathogenesis of many autoimmune inflammatory diseases such as psoriasis, inflammatory bowel diseases and rheumatoid arthritis and cancer[15-17]. There are mounting studies demonstrating that IL-23 plays a major role in myocardial I/R injury through HMGB1-TLR4+ IL-23/IL-17 axis[9-11]. In the present study, we found that IL-23 could significantly increase the expression of IL-17A but not HMGB1, and anti-IL-23 could significantly decrease the expression of IL-17A but had no effect on HMGB1 expression, consistent with previous findings[9-11]. IL-17 and IL-23 are also increased in myocardial tissue during I/R[9-11].

But the high continuous expression of IL-17 relied on the present of IL-23[18], as IL-23 could maintain and enhance Th17 to secret IL-17 initiated by IL-12[19]. These results indicated that IL-23 might play a critical role in myocardial I/R injury. Previous studies have demonstrated that IL-17 could promote the expression of Th1 cytokines including IL-6 and TNF-α and the production of ROS through activating P38 mitogen-activated protein kinase (P38-MAPK) signaling pathway in atherosclerosis models[20-22]. It is well known that IL-6 and TNF-α are the classic cytokines that aggravate myocardial I/R injury. In the present study, we found that anti-IL-23 could significantly decrease the expression of IL-6 and TNF-α and ROS production. It might be partly due to that anti-IL-23 inhibited the expression of IL-17. Recent studies demonstrated that IL-23 could not only activate JAK2/STAT3 pathway which induced IL-17, IL-22, GSF expression and stabilized Th17 cells, but also activate JAK2/STAT4 pathway which induced IL-6 and TNF-α expression[15,23-25]. Thus, our findings further confirmed that IL-23 could aggravate myocardial I/R injury by promoting IL-6 and TNF-α expression and ROS production.

Apoptosis is an important mechanism causing an amount of cardiomyocyte necrosis in myocardial I/R[26]. And it could be enhanced by ROS and cytokine[27-29]. The present study demonstrated that IL-23 could aggravate cardiomyocyte apoptosis, as confirmed by elevated apoptosis index.

In conclusion, our study revealed IL-23 could worsen myocardial I/R injury and this was associated with increased inflammatory responses and ROS production. Therapeutic strategy targeting IL-23 might be a potential approach to attenuate myocardial injury induced by I/R.

References


