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Original Article

Effects of interleukin-37 on cardiac function after myocardial infarction in mice

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Abstract: Background: Interleukin-37 (IL-37) is a new discovered member of the interleukin family and plays anti-inflammatory effect in some inflammatory disease. A recent study found that IL-37 elevated significantly in peripheral blood of patients with acute myocardial infarction. We aimed to explore the effect IL-37 on cardiac function after mice myocardial infarction (MI) and its mechanism. Methods: Acute MI mouse model was established and divided into three groups: sham group, MI group and IL-37 treatment group. MPO expression was detected by immunohistochemistry; NF-κB signaling pathway was tested by Western blot; and cardiac function was measured by echocardiography. Results: Compared with MI mice, IL-37 treatment showed an obvious decrease of MPO expression, suppression of p-p65 expression, and improved cardiac function by decreasing left ventricular shortening fraction (LVFS). Conclusion: IL-37 may improve MI mice cardiac function via inhibition of inflammatory NF-κB signaling pathway.

Keywords: Interleukin-37, myocardial infarction, cardiac function, inflammation

Introduction

Interleukin-37 (IL-37) is a newly discovered member of the interleukin family. It mainly expresses in the brain, kidney, heart, peripheral blood mononuclear cells, and dendritic cells, etc. It can inhibit inflammation and immune reaction [1, 2]. Previous study reported that the proinflammatory factor tumor necrosis factor α (TNFα), IL-1β, and IL-18 can up-regulate IL-37 expression [3]. It was also found that IL-37 exhibits anti-inflammatory effect in sepsis and liver ischemia-reperfusion model [4]. All of the abovementioned studies suggest that IL-37 presents anti-inflammatory and protect effects in inflammatory diseases. More importantly, recent study reveals that IL-37 level increased obviously in peripheral blood of acute myocardial infarction (MI) patients [5]. This indicates IL-37 may be associated with acute myocardial infarction. Our study aimed to explore the effect of IL-37 on cardiac function after mice MI and its mechanism.

Materials and methods

Acute MI mouse modeling and grouping

45 C57BL/6 male mice at 11-12 weeks and weighted 25 g were obtained from Vital River co., LTD (Beijing, China). Mice were randomly divided into three groups: control group, MI group, and MI + IL-37 group. MI modeling process was as follows: The mouse was anaesthetized by 50 mg/kg sodium pentobarbital. Surgery was conducted under aseptic conditions. An incision was made through the skin on the fourth rib and the heart was exposed. An 8-0 sterile surgical suture was used to ligature the left coronary artery. When the mouse myocardium changed white and ST segment sustained elevation can determine the success of myocardial infarction model establishment. 2 μg IL-37 (R&D Company, USA) was intraperitoneal injected to the mice in the IL-37 treatment group on the first day after MI modeling and sustained for 1 week. The coronary artery was only threaded but not ligatured by suture on the mice in the sham surgery group.

Mice were used for all experiments, and all procedures were approved by the Animal Ethics Committee of our hospital.

Ultrasonic cardiac function evaluation

Echocardiography was performed using a Siemens 520 ultrasound machine and a 15L8 ultrasonic probe. After a short-axis 2-D image of

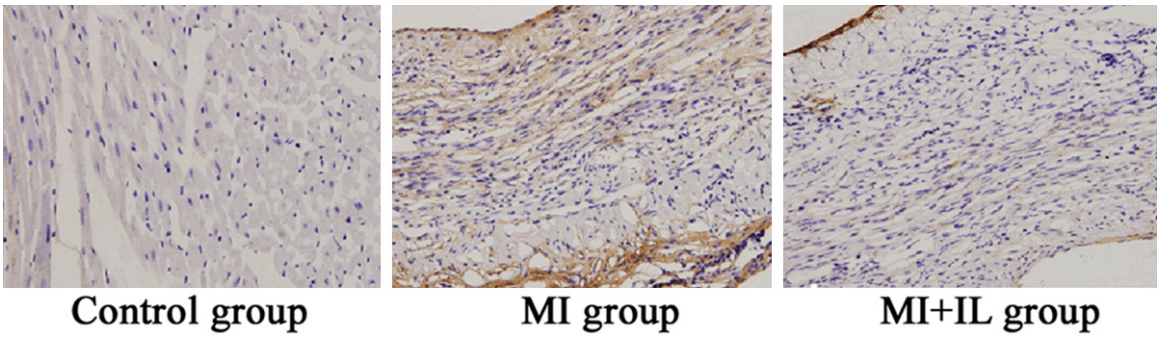

Figure 1. MPO expression in each group (Bar = 100 μm).

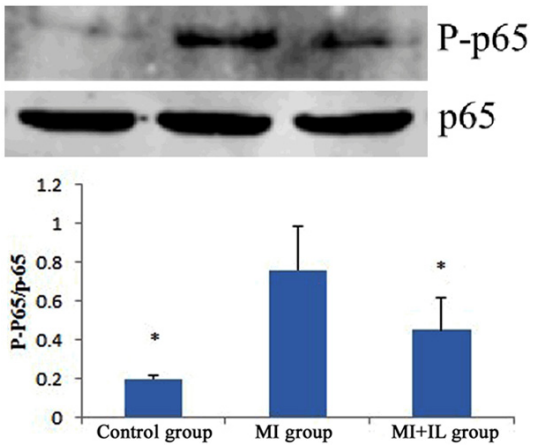

Figure 2. IL-37 inhibition on NF-κB signaling pathway in acute MI mice. *Compared with MI group, $P < 0.05$.

the left ventricle was obtained at the level of the papillary muscles, 2-D guided M-mode images were acquired at a sweep speed of 100 mm/s and stored digitally. Left ventricular end diastolic diameter (LVEDd) and left ventricular end-systolic diameter (LVESd) were measured. Left ventricular shortening fraction was measured as follows: LVFS = (LVEDd-LVESd)/LVEDd × 100.

Immunohistochemistry

The mice were euthanatized at 1 week after modeling and the heart tissue was fixed in 10% formalin. Sections (5 μm thick) were cut and mounted onto adhesive-coated glass slides. Slides were endogenous peroxidase blocked with 3% hydrogen peroxide and incubated with the primary antibody MPO (concentration 1:200; Abcam, UK) overnight at 4°C. Then the slides were incubated with the secondary antibody (Jackson, UK) for 1 h. after DAB treatment, the coverslips were then applied to specimens using routine pathological procedures.

Western blot

Total protein was scraped from the left heart. Proteins were separated on 10% SDS-PAGE and transferred to membrane. Then the membrane was blocked with 5% non-fat milk and incubated with primary antibodies P-P65, P-65, and β-actin (Cell Signaling, USA) at 1:1000. The proteins were detected with hemiluminescence reagents and NIH Image J software was applied for further analysis.

Statistical analysis

All statistical analyses were performed using SPSS13.0 software (Chicago, IL). Numerical data were presented as means and standard deviation (± SD). All of the statistical indexes were tested by normality. Differences between means were analyzed using SNK test or one-way ANOVA. $P < 0.05$ was considered as statistical significant.

Results

IL-37 inhibits MPO expression in MI mice

Compared with the sham surgery mice, immunohistochemical results revealed that MPO expression increased significantly, while IL-37 treatment can effectively inhibit MPO expression in MI mice (**Figure 1**).

IL-37 inhibits NF-κB signaling pathway in MI mice

Western blot showed that p-p65 overexpressed in the myocardium of MI mice, which indicated the activation of NF-κB signaling pathway. IL-37

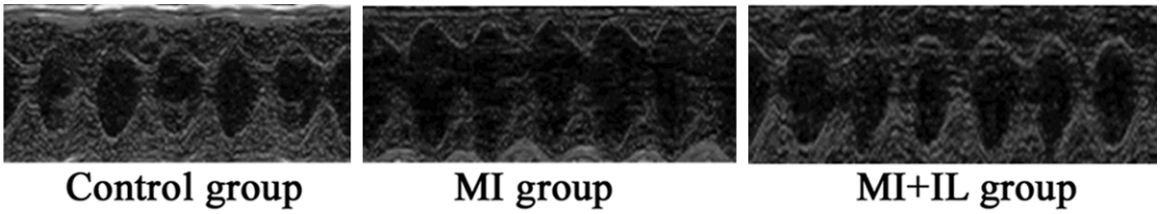

Figure 3. Mice M type ultrasonic in each group.

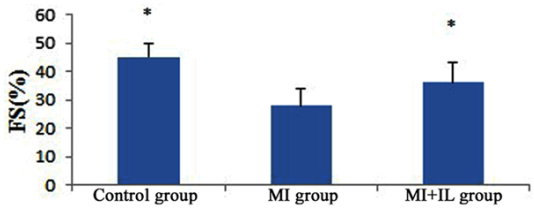

Figure 4. IL-37 improve mice cardiac function after acute MI. *Compared with MI group, $P < 0.05$.

treatment can inhibit p-p65 expression, suggesting it can efficiently suppress the inflammatory NF-κB signaling pathway activation caused by MI ($P < 0.05$) (**Figure 2**).

IL-37 improves cardiac function in acute MI mice

Echocardiographic results presented that compared to control mice, FS decrease significantly in acute MI mice. IL-37 treatment can improve cardiac function and delay FS reduction ($P < 0.05$) (**Figures 3** and **4**).

Discussion

IL-37 is a newly discovered member of the interleukin family in recent years with anti-inflammatory and immune inhibitory effect [2]. Several studies confirm IL-37 plays an important role in many inflammatory diseases. IL-37 protects from inflammation mainly Literature by inhibiting a series of inflammatory factors expression in the LPS induced mice septic shock model. It has been verified that IL-37 exhibit its anti-inflammatory and immune inhibitory effect through inhibiting inflammatory cytokines such as IL-1β, IL-18, and TNFα secreted by peripheral blood mononuclear cells, dendritic cells, macrophages, and epithelial cells. For example, it can enhance IL-18BP inhibition on IL-18 after combining with IL-18BP. The exact mechanism of anti-inflammatory effect of IL-37 may be related to its combination with Smad3 in the nucleus to form complex to regulate inflammatory cytokines transcription [3].

Recent studies reported that IL-37 level increased significantly in the MI patients' peripheral blood [5]. The rise of plasma IL-37 level in coronary heart disease may be caused by inflammatory activation, while raised IL-37 can inhibit inflammatory cytokines expression. This clinical study suggested that IL-37 is an important anti-inflammatory cytokine in the process of acute MI. Although IL-37 exhibits protecting role for inflammatory disease, its specific receptor and related signaling transduction is still unclear. The rise of plasma IL-37 in acute MI patients might be a compensatory reaction of the body, while whether exogenous IL-37 has the treatment function for acute MI and its related mechanism is ambiguous.

Serious myocardial ischemia caused by acute MI can activate autoimmunity, which recruits a large number of inflammatory cells to the infarction area and releases plenty of cytokines participating in inflammatory response [6, 7]. It was shown that excessive inflammatory cytokine may produce toxic effect on myocardial cells that accelerating myocardial cell apoptosis and eventually damaging the heart function [8-10]. Numerous inflammatory cells infiltration may also release abundant inflammatory mediators such as MPO participating in inflammation. The release of these inflammatory mediators took part in myocardial cells necrosis and apoptosis, and endothelial cell dysfunction [11, 12]. MPO is mainly secreted by polymorphonuclear leukocytes (PMN) such as neutrophils, monocytes, and part of macrophages [13]. MPO is the marker of neutrophil activation, and its level and activity represent the function and state of PMN [14]. It was also reported that MPO enzyme is an independent predictor for acute coronary artery syndrome closely associated with acute MI [15, 16]. Our study indicated the anti-inflammatory effect of IL-37 by inhibit-

ing MPO expression in acute MI mice. We also proved the activation of NF-κB signaling pathway after MI, and the inhibition of this signaling pathway can improve cardiac function after MI and prognosis [17-19]. NF-κB is a kind of important nuclear transcription factor, as it not only plays an important role in inflammation, but also relates to myocardial cell apoptosis and myocardial remodeling process after MI [20-22]. It regulates many proinflammatory cytokines transcription such as TNFα, IL-6, and monocyte chemotactic protein (MCP-1). All of these cytokines can cause myocardial cell hypertrophy and apoptosis, and affect the myocardial systolic function leading to ventricular remodeling occurrence and heart failure [23, 24]. Our research used recombinant IL-37 to treat acute MI in mice. A week treatment can effectively inhibit inflammatory NF-κB signaling pathway activation, and ultimately can efficiently improve cardiac function. It illustrated that exogenous IL-37 can effectively inhibit inflammation caused by acute MI, and eventually improve cardiac dysfunction caused.

In conclusion, IL-37 can improve mouse cardiac function after MI by inhibiting inflammation. IL-37 might be a potentially effective treatment for acute MI.

Disclosure of conflict of interest

None.

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