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Original Article

Mechanisms of IL-6 mediated early inflammation in cardiac regeneration of neonatal mice

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Abstract: Objective: Our aim was to delve into the roles of IL-6 mediated early inflammation in cardiac regeneration of neonatal mice. Methods: A mouse model of apical heart resection was established to compare cardiac regeneration, protein positive rates, and expression levels of IL-6 in cardiac tissues, serum levels of IL-6, TNF-α and IL-1β (inflammatory cytokines) in cardiac tissues in the early period (at 1 day and 3 days) after surgery. We also examined mRNA and protein expression, phosphorylation of IL-6, Akt, and Stat3 in cardiac tissues of the mice among the normal mice (the control group and the model group), IL-6 gene knockout mice (the IL-6 knockout group), and IL-6 knockout mice whose overexpressed IL-6 vectors were transfected (the IL-6 rescue group). Results: At 21 days, regeneration of cardiomyocytes was repaired among mice in the control group, model group, and the IL-6 rescue group while scar repairs were present among mice in IL-6 knockout group. When compared with mice in control group, those in the IL-6 knockout group decreased significantly but those in model group and the IL-6 rescue group increased remarkably with regards to IL-6 protein positive rates, contents of IL-6, TNF-α, and IL-1β in serum, mRNA and protein expression of IL-6, Akt and Stat3, and protein expression levels of p-Akt and p-Stat3 (all $P<0.05$). Conclusion: IL-6 induces proliferation of cardiomyocytes and promotes cardiac regeneration in mice by mediating early inflammation in the mice.

Keywords: IL-6, early inflammation, mice, cardiac regeneration

Introduction

Patients with cardiovascular diseases including heart failure, valvular-heart disease, and myocardial infarction tend to be complicated with apoptosis and necrosis of a large number of cardiomyocytes [1]. Discovering how to promote regeneration of cardiomyocytes in patients with heart disease is essential to treatment of the disease. Mounting evidence has demonstrated that regeneration of human cardiomyocytes decreases with aging. Nevertheless, in some lower animals, hearts can be regenerated fully after partial resection [2, 3]. Moreover, previous studies on neonatal mice with cardiac injury reveal that after cardiac injury, collagen fibers were enriched in the injured sites of neonatal mice. Meanwhile, the immune/inflammatory response caused by increased blood clots and deposition of extracellular matrix promotes proliferation of cardiomyocytes and increased inflammatory cells, such as T-cells, macrophages, and neutrophils in the injured sites [4-6]. Early inflammation is generally defined as a defense response that occurs within 24 hours after injury in the body. Early inflammation has a significant effect on clearance of injured cells but its effect on cardiac regeneration in mice remains unclear [7].

According to one study, after apical resection of the heart in adult mice, acute inflammatory cytokines induced scar acceleration and proliferation of cardiomyocytes was further suppressed [8]. However, in the skeletal injury-related model, macrophages and other inflammatory cytokines accelerated regeneration of skeletal muscle by enhancing proliferation of satellite cells [9]. IL-6, an inflammatory cytokine secreted by macrophages and T-cells, plays a dominant role in anti-inflammation and trauma repair [10]. IL-6 mediated inflammatory responses contribute a lot to repair of the hearts in a zebrafish model of myocardial infarction and

are beneficial to proliferation of satellite cells in injured skeletal muscle models [11]. Phosphorylation of Akt and Stat3, acting as the downstream substrates of IL-6 signaling pathway, are associated with protein activity and play crucial roles in regeneration of hepatic and cardiac cells [12].

In our present study, we established a neonatal mice model of apical heart resection and detected inflammatory markers for proliferation of cardiomyocytes. Elevated expression of IL-6 and improved activity of early inflammatory cytokines was mediated by IL-6 after apical resection of the hearts. After IL-6 knockdown, positive rates of IL-6 protein, concentrations of inflammatory cytokines in serum, and expression of p-Akt and p-Stat3 protein decreased remarkably, suggesting that IL-6 mediated early inflammation plays a dominant role in cardiac regeneration in neonatal mice. Details have been reported in the following parts of this article.

Materials and methods

Participants

Fifty-four clean and healthy male C57BL/6 inbred strain neonatal mice aged one day and 60 IL-6 knockout mice aged one day were purchased from Jackson Laboratory in USA (https://www.jax.org/). The mice were fed according to criteria for protection and use of medical laboratory animals. Before surgery, no food and drink restrictions were made. They were fed on granular standard feeds and raised in an environment with room temperature of (22 ± 2)°C and relative humidity of (60 ± 2)%. Animal experiments were performed in accordance with the Declaration of Helsinki.

Modeling

After hypothermia anesthesia on ice, each mouse underwent surgery after the heart completely stopped beating. The apex of the heart was cut off after the heart was fixed. Timely hemostasis was performed on the mouse during surgery and suture materials were utilized to close the skin and muscle layer by layer after surgery. The air and hemathorax in the thoracic cavity of mice were extruded to prevent onset of pneumothorax. The entire surgery lasted no more than 10 minutes. After completion of surgery, the mice were placed on an electric blanket till automatic recovery of their body temperature, heartbeat, and breaths. Mice in the control group also received hypothermia anesthesia, thoracotomy, and wound suturing. After mice in the two groups recovered normal breaths and heartbeats, they were placed in cages for breastfeeding again. Behavioral scoring was employed to determine whether modeling was successful at 24 hours after surgery.

Construction of overexpression vectors

Total RNA was extracted from cardiac tissues and mRNA was purified. First-strand cDNA of IL-6 was synthesized by reverse transcription. The reverse transcription system was 20 μL including mRNA (0.1 μg), RNasin (0.5 μL; 20 U/μL), olig-dT primer (1 μg), M-MLV reverse transcriptase (1 μL; 20 U/μL), dNTP (1 μL; 10 mmoL/L), DTT (2 μL; 0.1 moL/L), and 5× RT Buffer (4 μL). Reverse-transcribed samples were water-bathed in water-bath kettles at 65 to 70°C for 10 minutes. After denaturation, samples were water-bathed at 37°C for 1 hour and then reverse transcriptase was inactivated at 99°C for 5 minutes. In the 50 μL system of PCR reaction, cDNA (0.1 μg), Taq enzyme (0.5 μL; 5 U/μL), IL-6 upstream and downstream primers (1 μg; 11 μg/μL), dNTPs (1× μL; 10 mmoL/L), $MgCl_2$ (2.5 μL; 25 mmoL/L), and 10× Buffer (5 μL) were added. Reaction conditions included 30 cycles of pre-denaturation at 94°C for 3 minutes, denaturation at 94°C for 30 seconds, renaturation at 50°C for 30 seconds, extension at 72°C for 30 seconds, followed by a thorough cycle of 72°C for 10 minutes. PCR products were purified and recovered, according to instructions on the PURIGENE kits, and ligated to pGEM-T vectors. The ligation system was 10 μL, in which vectors (0.1 μg), T4 ligase (1 μL), 10× Buffer (1 μL), and PCR products (1 μg) were added. Samples were incubated overnight at 12 to 16°C.

Randomization

The mice were stratified into 4 groups in terms of the prespecified randomization method: One-day-old neonatal mice were randomly assigned to the model group (The apical resection of the hearts under hypothermic anesthesia), control group (only thoracotomy and suture), IL-6 knockout group (construction of a IL-6 knockout mice model of apical resection), or the IL-6 rescue group (construction of a IL-6 knockout mice model of apical resection and

Table 1. qRT-PCR primer sequences

Gene	Sequence
GAPDH	F: 5'-TGTGGGCATCAATGGATTTGG-3'
	R: 5'-ACACCATGTATTCCGGGTCAAT-3'
IL-6	F: 5'-ACTCACCTCTTCAGAACGAATTG-3'
	R: 5'-CCATCTTTGGAAGGTTCAGGTTG-3'
Akt	F: 5'-AGCGACGTGGCTATTGTGAAG-3'
	R: 5'-GCCATCATTCTTGAGGAGGAAGT-3'
Stat3	F: 5'-CAGCAGCTTGACACACGGTA-3'
	R: 5'-AAACACCAAAGTGGCATGTGA-3'

injection of IL-6 overexpression plasmid into tail vein).

Hematoxylin-eosin (HE) staining

Cardiac tissues were extracted from the mice at 1 day, 3 days, 7 days and 21 days after surgery, respectively. They were fixed in 4% paraformaldehyde for 24 hours. After dehydration in 80%, 90% and 100% ethanol and n-butyl alcohol, the specimens were wax-filled in wax boxes at 60°C. After paraffin embedding, they were cut into consecutive sections at 5 μm in thickness. The sections were spread at 45°C and dewaxed in xylene after baking at 60°C for 1 hour. After hydration, they were stained in conventional hematoxylin-eosin (Beijing Solarbio, Beijing, China), dehydrated in gradient ethanol, cleared in xylene, mounted in neutral balsam, and observed for pathological changes in cardiac tissues and infiltration of inflammatory cells under the optical microscope (XP-330, Shanghai Bingyu Optical Instruments, Shanghai, China).

Sirius red staining

Cardiac tissues were extracted from the mice at 1 day, 3 days, 7 days and 21 days after surgery. They were fixed, hydrated, cut into sections, and placed in 0.2% phosphomolybdic acid hydrate for 2 minutes. Subsequently, picrosirius solution (aqueous solution of picric acid with 0.1% of Sirius red) was added and then sections were taken out and stained in a wet box for 90 minutes. After completion of staining, the picrosirius solution was removed. After the sections had been immersed in 0.01 mol/L hydrochloric acid 4 consecutive times (1 second each time), they were dehydrated in gradient ethanol and cleared in xylene for 2 minutes. The same procedure repeated 3 times in a row. Sections were mounted when xylene was still wet. After drying, images were then photographed for analysis. At 21 days, the cardiac collagen deposition was observed for assessment of cardiac regeneration.

Immunohistochemistry

Cardiac tissues were extracted from the mice at 1 day and 3 days after surgery, respectively. They were fixed with formalin, embedded in paraffin, cut into sections, and washed in distilled water. After antigen-repair under high pressure for 90 seconds, the sections were cooled at room temperature and washed with PBS. Subsequently, blocking solution (100 μL) supplemented with 5% bovine serum albumin (BSA) was added and the sections were incubated at 37°C for 30 minutes. After 100 μL of diluted IL-6 primary antibody (1:50, ab208113, Abcam, Inc., MA, USA) had been added in each well of the plate, sections were incubated overnight at 4°C and then washed with PBS. Biotinylated goat-anti-rabbit (HY90046; Shanghai Hengyuan Biological Technology, Shanghai, China) secondary antibodies working solution (diluted at 1:100) were added and sections were incubated at 37°C for 30 minutes, washed with PBS, and incubated at 37°C for 30 minutes. After adding streptavidin-peroxidase solution, they were again washed with PBS. They were stained with diaminobenzine (DAB) at room temperature, soaked in hematoxylin for 5 minutes, and flushed with tap water. After immersion in 1% hydrochloric alcohol solution for 4 seconds, the sections were processed back to blue in tap water for 20 minutes. Criterion for judging positivity of IL-6 protein was brown staining. Under a microscope at high magnification, mean optical density (OD) of IL-6 positive staining was detected with use of the image analysis software Image-Proplus (Media Cybernetics, US), followed by quantitative analysis [22].

ELISA

ELISA was performed according to instructions on the ELISA kits (eBioscience, US). ELISA kits were balanced at room temperature for 20 minutes and then some washing solution was prepared. Standard curves were plotted. The OD of each well was measured at 450 nm using BioTek Synergy 2, an automated microplate reader. The standard curves were plotted based on OD values and the contents of IL-6 (hjC14128; Shanghai Hongju Industrial, Shang-

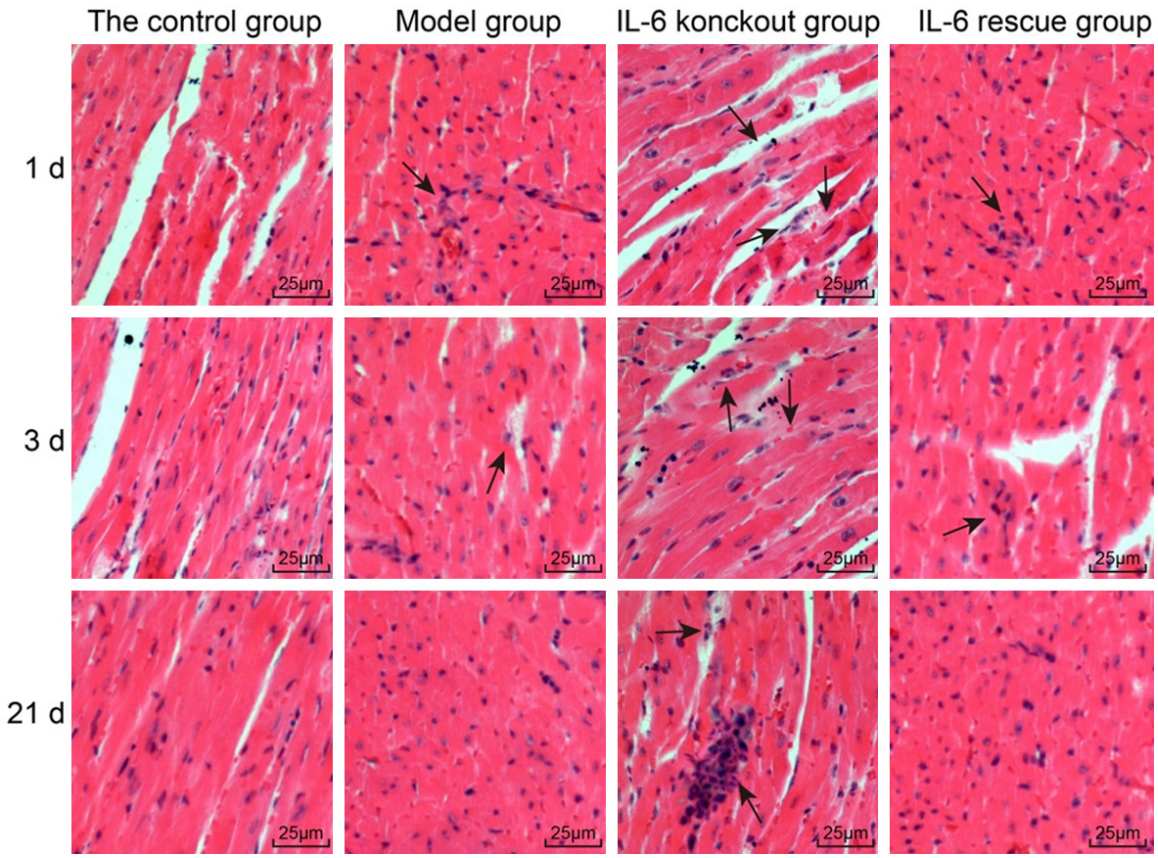

Figure 1. HE staining of cardiac tissues of mice in each group at 1, 3, 21 d (400x). The arrows denote inflammatory cells and scars; in **Figure 1**, well-arranged myocardial cells, clear boundaries between cardiac tissues, and no infiltration of inflammatory cells were observed among the mice in the control group; evident infiltration of inflammatory cells after modeling and successful cardiac regeneration at 21 d were noted among the mice in the model group and the IL-6 rescue group; nevertheless, no cardiac regeneration, but the major manifestation of scar repairs, was seen in the mice in the IL-6 knockout group.

hai, China), IL-1β (interleukin-1β, hj-C14087; Shanghai Hongju Industrial, Shanghai, China), and tumor necrosis factor-α (TNF-α, hj-C14134; Shanghai Hongju Industrial, Shanghai, China) in the blood of mice in all the groups. Standard curves were detected at 1 day and 3 days after surgery, respectively.

qRT-PCR

Cardiac tissues were taken from the mice at 1 day and 3 days after surgery, respectively. They were then stored in a refrigerator at -80°C. Total RNA was extracted from cardiac tissues after they had been taken out from the refrigerator. RNA was diluted at 20 folds and measured for purity. In accordance with instructions on the reverse transcription kits (Beijing TransGen Biotech, Beijing, China), cDNA template synthesis via reverse transcription reaction on a PCR amplifier and design of the primers for IL-6, Akt and Stat3 mRNA were conducted by Shanghai Bioengineering, China (**Table 1**). cDNA was taken for real-time fluorescence quantitative PCR, following the instructions on the SYBR®Premix Ex TaqTM II kits (TaKaRa, Dalian, China), on the reaction system of 20 μL, containing SYBR Premix (10 μL), cDNA template (2 μL), upstream and downstream primers (0.6 μL) and sterile solution (6.8 μL). RT-PCR was performed using Type 7500 fluorescent quantitative PCR (ABI, US). mRNA expression was normalized to GAPDH and the reaction conditions were as follows: pre-degeneration at 95°C for 30 seconds, degeneration at 95°C for 30 seconds, annealing for 20 seconds, extension at 72°C for 30 seconds, followed by 45 successive cycles. Subsequently, mRNA expression was detected in IL-6, Akt, and Stat3. The ratio between target gene expression of the model group and that of the control group was calculated by $2^{-\Delta\Delta Ct}$ method. The formula was: $\Delta\Delta CT=\Delta Ct_{Model\ group}-\Delta Ct_{Control}$

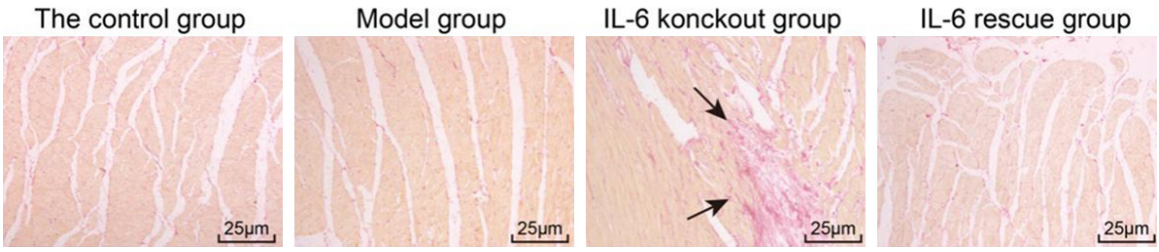

Figure 2. Sirius red staining at 21 d (400x). Cardiac morphology of the mice was normal in the control group; in the model group and the IL-6 rescue group, there was no significant collagen deposition, and the development and cardiac morphology were normal. In the IL-6 knockout group, there was obvious collagen deposition and scars in the hearts of mice.

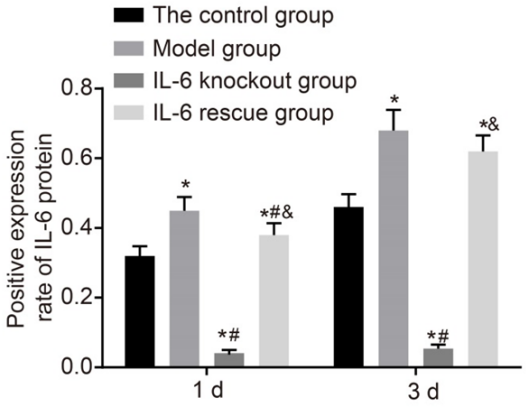

Figure 3. Positive expression of IL-6 protein (1 d and 3 d, n=6). *P<0.05 compared with the control group at different time points; #P<0.05 compared with the model group at different time points; &P<0.05 compared with the IL-6 knockout group at different time points; the IL-6 protein was still expressed in the mice in the IL-6 knockout group, suggesting that the protein expression of IL-6 was not completely knocked out in the mice of the IL-6 knockout group though most of the protein expression of IL-6 was reduced and released. The protein expression of IL-6 was recovered in the mice of the IL-6 rescue group.

$_{group}$. Ct denoted total amplification cycles when the real-time fluorescence intensity reached the pre-specified threshold. mRNA expression in IL-6, Akt and Stat3 in cardiac tissues of the mice at 1 day and 3 days after surgery was detected three consecutive times.

Western blot

Cardiac tissues were taken from hearts of the mice and placed in a prepared centrifuge tube. They were centrifuged at 13,000 r/min for 15 minutes, followed by collection of supernatants as protein samples. Concentrations of the protein samples were determined by BCA method and then the protein samples were stored in a refrigerator at -20°C. Diluted primary antibodies IL-6, Akt, Stat3, p-Akt and p-Stat3 (Abcam, Cambridge, MA, USA) were added. Membranes were incubated overnight at 4°C and washed with PBST (10 min*3 times) the next day. Subsequently, goat anti-rabbit polyclonal secondary antibodies (ab7312, Abcam, Cambridge, MA, USA) were diluted in 5% skim milk agitated at room temperature for 1 hour. After that, membranes were washed again with PBST solution 3 times (15 minutes each time). Proteins were imaged by injection of contrast medium and via Bio-Rad gel imaging system (MG-8600, Thmorgan Biotechnology, Beijing, China). The IPP7.0 software (Media Cybernetics, Singapore) was applied for a semi-quantitative analysis. Protein expression was normalized to GAPDH. Ratio between the gray value of the target band and that of the reference band was described as relative expression level of proteins. Levels of IL-6, Akt, Stat3, p-Akt and p-Stat3 protein in cardiac tissues of the mice were detected at 1 day and 3 days after surgery.

Statistical analysis

Data analyses were performed with use of SPSS statistical software, version 18.0 (IBM Corp, Armonk, NY, USA). Measurement data are expressed as mean ± SD. One-way ANOVA with post hoc Bonferroni tests were utilized for comparisons among more than two groups. P<0.05 was deemed as statistically significant.

Results

HE staining

Histopathological changes were observed among the mice in each group (**Figure 1**). At 1 day

Table 2. Expression of associated inflammatory cytokines in the blood of mice (1 day and 3 days after surgery)

Time	Inflammatory cytokine	Control group	Model group	IL-6 knock-out group	IL-6 rescue group
1 d	IL-6 (pg/mL)	42 ± 3.02	82 ± 6.19*	35 ± 1.87$^{*,\#}$	65 ± 5.33$^{*,\&}$
	TNF-α (pg/mL)	95 ± 4.49	163 ± 6.26*	89 ± 3.27$^{*,\#}$	137 ± 5.12$^{*,\&}$
	IL-1β (pg/mL)	38 ± 1.25	63 ± 5.32*	30 ± 2.73$^{*,\#}$	47 ± 3.67$^{*,\&}$
3 d	IL-6 (pg/mL)	38 ± 3.21*	62 ± 5.78*	11 ± 1.02$^{*,\#}$	53 ± 4.63$^{*,\&}$
	TNF-α (pg/mL)	73 ± 6.32	139 ± 7.32*	68 ± 3.68$^{*,\#}$	102 ± 5.29$^{*,\&}$
	IL-1β (pg/mL)	24 ± 1.63	51 ± 4.67*	19 ± 0.96$^{*,\#}$	38 ± 3.02$^{*,\&}$

Note: *P<0.05 compared with the control group; $^{\#}$P<0.05 compared with the model group; $^{\&}$P<0.05 compared with the IL-6 knockout group.

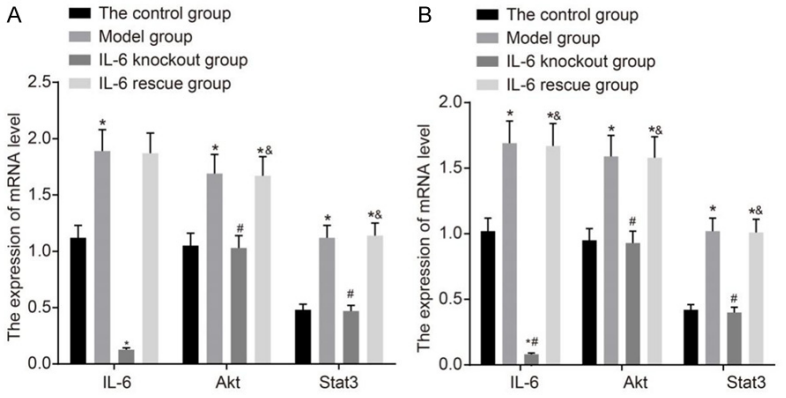

Figure 4. Expression level of mRNA of associated genes in the cardiac tissues. A: 1 d after surgery; B: 3 d after surgery; *P<0.05 compared with the control group; $^{\#}$P<0.05 compared with the model group; $^{\&}$P<0.05 compared with the IL-6 knockout group.

after resection of apical tissues, the wound sites of mice in the model group and IL-6 rescue group were covered with blood clots and accompanied by concomitant infiltration of inflammatory cells. At 3 days, visible blood clots disappeared and evident infiltration of inflammatory cells was noted in the new tissues. There was a clear boundary between new and original cardiac tissues in mice. Morphological changes of the hearts were different insignificantly between the IL-6 rescue group and control group. At 21 days, among mice in the IL-6 knockout group, visible scars without evident infiltration of inflammatory cells were observed in apical sites of the hearts and there were clearly-defined boundaries between injured and normal cardiac tissues.

Sirius red staining

Histopathological results were observed among the mice in each group (**Figure 2**). At 21 days after surgery, the hearts were healthily developed and heart morphology was well-arranged among the mice in the control group. Among those in the model group and IL-6 rescue group, heart morphology was normal. The tissues were continuous with no clear collagen deposition in the tissues. Among those in IL-6 knockout group, visible scars at the apical parts of the heart, a lot of collagen deposition in the tissue space, and a clearly-defined boundary between injured and normal cardiac tissues were found, suggesting that scar repair was the major repair mode of the apical tissues among mice in the IL-6 knockout group.

Immunohistochemistry

IL-6 positivity of mice in the four groups is shown in **Figure 3**. At 1 and 3 days, positive rates of IL-6 were substantially decreased in the IL-6 knockout group (P< 0.05) but remarkably increased in the model group and IL-6 rescue group as compared with that in the control group (both P<0.05). The positive rate significantly reduced in the IL-6 knockout group (P<0.05) but differed insignificantly in IL-6 rescue group when compared with the model group (P>0.05). Rate was strikingly increased in the IL-6 rescue group compared to IL-6 knockout group (P<0.05).

ELISA

Expression of associated inflammatory cytokines was detected in the supernatant of cells (**Table 2**). At 1 day and 3 days after surgery, contents of IL-6, TNF-α and IL-1β of the mice were considerably lowered in the IL-6 knockout group (all P<0.05) but significantly increased in the model group and IL-6 rescue group (all P<0.05). Compared with model group, the contents were decreased remarkably in the IL-6 knockout group (all P<0.05) but differed insig-

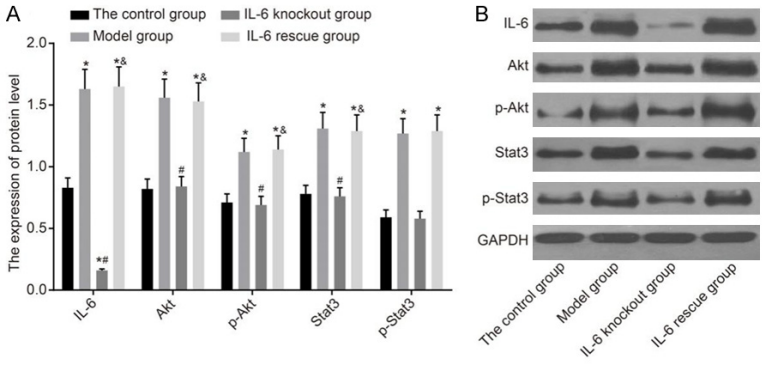

Figure 5. Protein expression levels of associated genes in the cardiac tissues of mice at 1 day after surgery. A: Bar chart for the protein expression; B: Diagram of protein bands. *P<0.05 compared with the control group; #P<0.05 compared with the model group; &P<0.05 compared with the IL-6 knockout group.

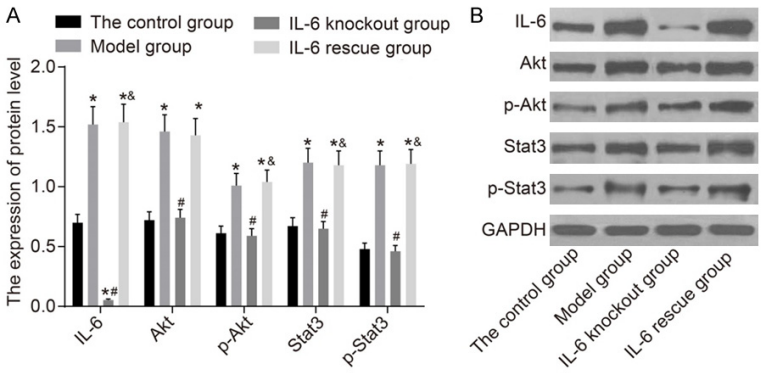

Figure 6. Protein expression levels of associated genes in the cardiac tissues of mice at 3 days after surgery. A: Bar chart for the protein expression; B: Diagram of protein bands. *P<0.05 compared with the control group; #P<0.05 compared with the model group; &P<0.05 compared with the IL-6 knockout group.

nificantly from those in IL-6 rescue group (all P>0.05). Contents were remarkably higher in the IL-6 rescue group than in IL-6 knockout group (all P<0.05).

qRT-PCR

Figure 4 shows mRNA expression of associated genes in the cardiomyocytes. At 1 day and 3 days, mRNA expression in IL-6, Akt and Stat3 of mice in the IL-6 knockout group was decreased substantially (all P<0.05) but increased remarkably in the model group and IL-6 rescue group compared with the control group (all P<0.05). mRNA expression was decreased strikingly in the IL-6 knockout group (all P<0.05) but differed slightly from those in IL-6 rescue group when compared with the model group (all P>0.05). mRNA expression was much higher in the IL-6 rescue group than in IL-6 knockout group (all P<0.05).

Western blot

Figures 5 and **6** show protein expression of associated genes in cardiac tissues of the mice. At 1 day and 3 days, protein expression levels of IL-6, Akt, p-Akt, Stat3 and p-Stat3 of the mice were significantly lower in the IL-6 knockout group (all P<0.05) but increased remarkably in the model group and IL-6 rescue group as compared the control group (all P<0.05). Protein expression level was lower in the IL-6 knockout group (all P<0.05) but different insignificantly from those in the IL-6 rescue group when compared with model group (all P>0.05). Protein expression rose remarkably in the IL-6 rescue group compared to IL-6 knockout group (all P<0.05).

Discussion

Multiple reports have been involved with roles of IL-6 mediated signaling pathways in tissue or organ regeneration of stem cells and skeletal muscle but studies on the roles of early acute inflammation in improving regeneration of cardiomyocytes are controversial [13, 14]. IL-6, a B-cell differentiation factor, has multiple pleiotropic effects as it is primarily produced by B cells, monocytes, macrophages, T cells, fibroblasts, interstitial cells, and endothelial cells. IL-6 activates downstream PI3K and STAT3 in the downstream pathways by binding to its receptor, IL-6R. As a crucial factor in inflammatory response, IL-6 contributes much to cell regeneration [15].

In our present study, after pathological examination of myocardial tissues of mice in each group, we found that among the mice in the model group and IL-6 rescue group, after resec-

tion of apical tissues of the hearts, the wounds were covered with blood clots and complicated by infiltration of inflammatory cells. Among the mice in the IL-6 knockout group, at 21 days after surgery, no infiltration of inflammatory cells was observed at the wounds of apical hearts that were primarily covered with fibrotic scars, suggesting that hearts of the neonatal mice could not achieve complete regeneration after resection of apical tissues of the hearts with IL-6 knockdown and the major mode of tissue repair was scar repair. Our results were also confirmed by Burt et al. when they explored association between proliferation of cardiomyocytes and secretion of inflammatory cells in neonatal mice [16].

The positivity of IL-6 protein of mice in the four groups was detected by immunohistochemistry. IL-6 protein positive rates of mice in the model group and IL-6 rescue group were significantly higher than those in the control group and IL-6 knockout group. ELISA detection revealed the expression of associated inflammatory cytokines in the blood of the neonatal mice. When compared with the control group, contents of IL-6, TNF-α and IL-1β (associated inflammatory cytokines) in the supernatants of mice in the model group and IL-6 rescue group rose remarkably at 1 day after surgery. They declined gradually whereas those of the mice in the IL-6 knockout group changed mildly. Considering pathological myocardial repair, these indexes indicate that early inflammatory response is a major marker for trauma repair in neonatal mice after cardiac injury.

Mounting evidence has demonstrated that IL-6 plays key physiological roles in regulation of multiple inflammatory pathways. Akt and Stat3, acting as decisive downstream substrates of IL-6, are closely associated with functions of IL-6 [17-20]. Detection of the mRNA expression of associated genes in the cardiac tissue of mice by QRT-PCR revealed that expression of mRNA in IL-6, Akt, and Stat3 of mice was substantially reduced in the IL-6 knockout group compared with the control group whereas expression was increased remarkably in the mice of the model group and IL-6 rescue group. Western blot suggested that protein expression of associated genes in the cardiac tissues of mice rose strikingly at 3 days in the model group and IL-6 rescue group, suggesting that IL-6 did not regulate proliferation of cardiomyocytes of cardiac-apex-resected mice via a single pathway, such as by proliferating pre-existed cardiomyocytes or triggering cardiac stem cells, but by concomitantly regulating downstream substrates of Akt and Stat3, thereby promoting proliferation of cardiomyocytes [21-23].

Nevertheless, our present study has some limitations. We did not compare postoperative cardiac functions between IL-6 knockout mice and those in the control group at the same time points. Moreover, we did not quantify proliferation of cardiomyocytes in mice. Therefore, future studies will be focused on clarification and improvement of the mechanisms for cardiac regeneration in mice.

In conclusion, we explored association between IL-6 mediated early inflammation and cardiac regeneration in neonatal mice model of apical resection. We substantiated that IL-6 mediated Akt and Stat3 inflammatory pathways promote proliferation of cardiomyocytes and accelerate cardiac regeneration. This study provides some evidence for treatment of heart-related diseases and we hope that it will bring some insight into medical research on cardiac regeneration.

Disclosure of conflict of interest

None.

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