Original Article

IL-8 up-regulates proliferative angiogenesis in ischemic myocardium in rabbits through phosphorylation of Akt/GSK-3βser9 dependent pathways

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Abstract: Background: Therapeutic myocardial angiogenesis is an important compensatory mechanism in severely coronary stenosis. Previous studies demonstrated that interleukin-8 (IL-8) not only plays an important role in inflammation, but also a potent angiogenic factor through p38 mitogen-activated protein kinase (p38MAPK), nuclear factor-kappaB (NF-κB)-dependent pathway in carcinoma. Our study sought to investigate the effects of IL-8 on the angiogenesis and the underlying mechanism in the ischemic myocardium. Methods: Acute myocardial infarction animal model was established with male rabbits by directly suturing the left anterior descending branch, then lentivirus-mediated IL-8 was quarterly injected into the borderline of infarction area immediately. We employed CoCl2 induced hypoxic HUVECs for in vitro ischemia study. Left ventricular end-diastolic diameter (LVEDd) and ejection fraction (EF) were measured by echocardiography in pre-operation and at 6th week after operation. CD34 was detected with immunohistochemistry to analyse angiogenesis. Western blot was performed with regard to IL-8, protein kinase B (PKB/Akt) and Glycogen synthase kinase-3β ser9 (GSK-3βser9). For the HUVECs’ proliferation and apoptosis, multiscan spectrum reader at A570 nm and annexin V-FITC/PI staining method were used respectively. Results: The levels of IL-8, phosphorylated Akt and GSK-3β ser9 in focal myocardium significantly increased, and the over expression of IL-8 led to an increasing in angiogenesis in rabbits. Hypoxia inhibited cell proliferation and promoted apoptosis. IL-8 induced cell proliferation, phosphorylation of Akt and GSK-3β ser9 expression in HUVECs, which were attenuated by anti-IL-8 or the Akt inhibitor LY294002. Conclusions: The present results indicate that IL-8 can increase angiogenesis in myocardial infarction, which maybe through enhancing Akt and GSK-3β ser9 expression, and inhibiting myocardial apoptosis.

Keywords: IL-8, angiogenesis, ischemic myocardium, Akt/GSK-3β ser9 pathways

Introduction

Coronary atherosclerotic heart disease (CAD), usually caused by coronary stenosis and thrombus, are the leading cause of disability and death. Increasing blood supply such as percutaneous coronary intervention (PCI) as well as decreasing myocardial oxygen consumption is effective methods for relieving the related symptoms. However, increasing patients with severe CAD are not amenable to conventional revascularization therapy. Arteriogenesis, an essential component of collateral circulation formation and angiogenesis are important compensatory mechanisms of therapeutic myocardial angiogenesis in these situations [1, 2]. Angiogenesis and arteriogenesis increase the perfusion for ischemic myocardium, reserve cells survival and improve heart function.

Evidences suggest that inflammatory cytokines, chemokines and other molecules are potentially related to angiogenesis and might promote collateral vessels formation [2-5]. Even though vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and other chemokines such as monocyte chemotactic protein-1 (MCP-1) have been suggested to be the stimuli for endogenous angiogenesis [2], but the factor triggering this process and the underlying mechanism haven’t been clear yet. Interleukin-8 (IL-8) is secreted by monocytes, neutrophils,
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endothelial and tumor cells. Studies on IL-8 proposed beneficial results with the risk of myocardial infarction [6]. It was not only played an important role in inflammation, but also a potent angiogenic factor in carcinoma [3, 7]. However, whether it can facilitate angiogenesis and the underlying mechanism in ischemic myocardium was not clarified.

**Methods**

**Experimental model of acute myocardial infarction**

40 healthy male Chinese rabbits (Purchased from the zoology department of Xiangya Medical college) weighting 2.0 to 2.5 kg were used for experiments. AMI model was induced as described previously [8]. Briefly, male rabbits were anesthetized with 3% pentobarbital Sodium (1 ml/kg IV). Under aseptic conditions, heart was exposed through a percordial incision at the 2nd and 3rd ribs, and a 6/0 silk suture (Ethicon, Norderstedt, Germany) was carefully placed around the left anterior descending branch (LAD) 2-3 mm from the ostium of LAD, resulting in regional ischemia and necrosis. Successful ischemia was verified by change in the color of the LV myocardium at the 1st hour and the ST segments in percordial leads were elevated after LAD was sutured for 6 hour. Sham control rabbits were in same procedure except the silk was sutured. After completion of the surgical procedure, the wound was closed. The rabbits were kept in rooms at 24±2°C, fed a standard diet, and allowed free access to tap water. Animals were killed after 6 weeks for biochemical and histological studies. Tissues around the infarction area were collected, immediately immersed in liquid nitrogen, and stored at -80°C for further studies.

The animal procedures were performed conform the NIH guidelines (Guide for the care and use of laboratory animals) on the protection of animals used for scientific purposes. This protocol was reviewed and approved by the Ethical Committee of Xiangya Hospital, Central South University.

**Construction of lentivirus-mediated IL-8**

IL-8 gene was constructed into the lentivirus vector using a lentivirus expressing system by using PCR from the purchased cDNA library as described previously [9]. Directional exchanged the IL-8 gene into Age I enzyme cutting production (pGC-FU Vector), then transformed to bacterial competent cells. The target plasmid and two auxiliary plasmids were co-transinfected into 293T cells. Primer was designed according to Genebank (http://www.ncbi.nlm.nih.gov) and the manufacturer’s instructions (Gikai Co, Shanghai, China). PCR was performed with the following specific primers: IL8-Age I-R: TCACATGGTGCGACCGGTGAATTCTCAGCCCTCTTC; IL8-Age I-F: GAGGATCCCCGGGTACCGGTCGCCACCATGACTTCCAAGCTGGCCG; EGFP-N-R: CGTCGCCGTCCAGCTGACCAG; Ubi-F: GGATATATGTAATTTCAGTG. PCR reactions were performed under the following conditions: 1 cycle at 94°C for 5 minutes, followed by 30 cycles at 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds. The final elongation was extended to 10 minutes at 72°C. Then the pGC-FU-IL8 was tagged with enhanced green fluorescent protein (EGFP) and confirmed as following.

The lentivirus pGC-FU-IL8-EGFP plasmid was confirmed with Western Blotting, real time PCR. The expression of pGC-FU-IL8-EGFP-tagged plasmid in ischemia myocardium was detected under fluorescence microscope.

**Cell culture**

Human umbilical vein endothelial cells (HUVECs, CRL-2873) purchased from cell culture center of Central South University, were cultured in DMEM containing 10% FBS (Gibco Co, USA), at 37°C under 5% CO₂ as previously described after resuscitation [10]. For anoxia, HUVECs were cultured with DMEM contained CoCl₂ at a concentration of 200 μmol/L for 48 h.

**Experimental protocols**

Rabbits were divided randomly into four groups: (1) Control group: Silk was only placed around LAD except be sutured and 10 μl normal saline (NS) was quarterly injected around the vessel; (2) AMI group: Silk was sutured around LAD and 10 μl NS was quarterly injected around the infarction area; (3) Plasmid group: After silk was sutured around LAD, 10 μl lentivirus vector (2.00E+8 TU/ml) without IL-8 was quarterly injected around the infarction area; (4) Treatment group: After silk was sutured around
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LAD, 10 μl lentivirus vector (2.00E+8 TU/ml) transferred with IL-8 gene was quarterly injected around the infarction area.

Confluent CRL-2873 cells incubated in medium containing 10% FBS alone or with CoCl\textsubscript{2} 200 μmol/L for 48 h were prepared. In subsequent experiments, HUVECs were incubated with either the recombinant human IL-8 (Ximei Biotech Co, Shanghai) 10 ng/ml alone, or co-exposed with Anti-IL-8 (Ximei Biotech Co, Shanghai) 10 ng/ml or LY294002 (Biyuntian Biotech Co, Shanghai) 20 μmol/L for 48 hours. All experiments were triplicated.

To investigate the effect and underlying mechanism of IL-8 on HUVECs’ proliferation and apoptosis, in the following experiment, Cells were divided into 5 groups and cultured for 48 h as following described: (1) Control group: Cells were cultured in DMEM containing 10% FBS; (2) hypoxia group: Cells were incubated with 200 μmol/L CoCl\textsubscript{2}; (3) IL-8 group: Cells that pretreated with the 200 μmol/L CoCl\textsubscript{2} for 48 h were incubated with 10 ng/ml IL-8; (4) Anti-IL-8 group: As identical to group 3 with the addition of 10 μg/ml anti-IL-8 was added; (5) LY294002 group: As identical to group 3 with the addition of 20 μM LY294002 was added. The experiments were triplicated.

To gain further mechanistic insights into the signaling mechanisms responsible for IL-8 induced angiogenesis and inhibited apoptosis in HUVECs. CRL-2873 cells were divided into 8 groups and all cultured in DMEM containing 10% FBS for 48 h as following described (1) Control group: Cells were cultured as above mentioned; (2) Hypoxia group: Cells were incubated with 200 μmol/L CoCl\textsubscript{2}; (3) IL-8 group: Cells were incubated with 10 ng/ml IL-8; (4) IL-8 + Hypoxia group: Cells that pretreated with the 200 μmol/L CoCl\textsubscript{2} for 48 h were incubated with 10 ng/ml IL-8; (5) Anti-IL-8 group: Cells were cultured with 10 ng/ml IL-8 and 10 μg/ml anti-IL-8; (6) Anti-IL-8 + Hypoxia group: Cells that pretreated with the 200 μmol/L CoCl\textsubscript{2} for 48 h were cultured with 10 ng/ml IL-8 and 10 μg/ml anti-IL-8; (7) LY294002 group: Cells were cultured with 10 ng/ml IL-8 and 20 μM LY294002; (8) LY294002 + Hypoxia group: Cells that pretreated with the 200 μmol/L CoCl\textsubscript{2} for 48 h were cultured with 10 ng/ml IL-8 and 20 μg/ml LY294002. The experiments were replicated.

**Echocardiography**

Echocardiography (Envisor HD, Probe 7.5 MHz) was performed during 1\textsuperscript{st} and 6\textsuperscript{th} week of post-operation respectively. M-mode and two-dimensional ultrasonography were used to measure ejection fraction (EF), left ventricular (LV) fraction shortening (LVFS), LV end-systolic diameter (LVESd), and LV end-diastolic diameter (LVEDd) at parasternal long axis view.

**Tissue harvest and hematoxylin-eosin (H-E) staining**

Rabbits were anesthetized with 3% pentobarbital Sodium (1 ml/kg IV) and were killed at the 6\textsuperscript{th} week of post-operation according to the ethical committee require. Tissues near the infarction area in identical place for all animals were harvested and placed in a microcentrifuge tube, immediately submerged in liquid nitrogen and stored for further analysis. The formalin-fixed and paraffin-embedded cross-sections tissues were cut into 5-μm serial sections and subsequent were stained with hematoxylin-eosin and examined microscopically for histological examination.

**Immunohistochemistry analysis**

Immunohistochemistry was performed as described previously [11]. The formalin-fixed and paraffin-embedded cross-sections tissues were cut into 3 μm sections. For analyses of angiogenesis, embedded tissue sections were incubated with rabbit monoclonal anti-CD34 antibody (1:1000) at 4°C overnight, followed by sequential incubations of rabbit anti-mouse antibody (HRP-Polymer anti ms/rb IgG) for 20 minutes at room temperature. Then sections were color-developed by DAB and sequentially counterstained with hematoxylin. The positive signals of CD34 were stained with orange-yellow. Percentage of angiogenesis was count in area that bearing positive CD34 signals was analyzed under microscope as previously described [12, 13].

**Western blot assay**

Western blot was performed as described previously for IL-8, protein kinase B (PKB/Akt) and Glycogen synthase kinase-3\textsuperscript{ser9} (GSK-3\textsuperscript{ser9}) detection [9]. Briefly, Tissues were separated and cellular proteins were separated by 12%
sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto NC membranes (PIERCE, USA). The NC membranes were incubated with the appropriate primary antibody rabbit anti-pAKT (dilution with 1:300, Santa Cruz Biotechnology), rabbit anti-IL-8 (dilution with 1:300, Abcam Biotechnology), rabbit anti-pGSK-3β<sup>ser9</sup> (dilution with 1:400, Milipore Biotechnology) and mouse anti-GAPDH (dilution with 1:1000, ProMab Biotechnology) respectively for 2 hours. Then the membranes were incubated with peroxidase-conjugated secondary antibodies for 1 hour, followed by the enhanced chemiluminescence (ECL) system. Finally the signals were quantified.

According to literature and instruction, CRL-2873 cells were lysed and subsequent 15 µl sample were used to separate protein by 12% SDS-PAGE. Protein was transferred onto NC membranes (PIERCE, USA). After blocking with 5% skim milk in TBST at room temperature for 2 h, The NC membranes were incubated with the primary antibody rabbit anti-pAKT (dilution with 1:400, Santa Cruz Biotechnology), rabbit anti-pGSK-3β<sup>ser9</sup> (dilution with 1:400, Milipore Biotechnology), mouse anti-caspase-3 (dilution with 1:400, Santa Cruz Biotechnology) and mouse anti-GAPDH (dilution with 1:1000, ProMab Biotechnology) respectively for 2 hours. Then subsequent incubated with peroxidase-conjugated secondary antibodies for 1 hour, followed by the enhanced chemiluminescence (ECL) system. Finally the signals were quantified.

In vitro CRL-2873 cell proliferation and apoptosis assay

CRL-2873 cells were cultured in 96-well plates (1×10<sup>4</sup>/well) for 24 h. IL-8, Anti-IL-8, LY294002 were added to the cell culture medium respectively and incubated for 48 h, subsequent incubated with 50 µl 1×MTT for 4 h at 37°C.
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Absorbance was measured on Multiscan spectrum reader at A_{570} nm. The proliferation percentage (%) was presented as compared with OD of control group. All experiments were repeated three times and mean absorbance was presented.

Apoptosis was analysed by annexin V-FITC/PI staining method. Briefly, HUVECs were cultured in 6-well plates (5×10^5/well) and incubated with IL-8, Anti-IL-8, LY294002 respectively. After labelled by annexin V-FITC and PI (ADL Co, USA), the cells were analysed using a flow cytometer and CELL Quest software.

Statistical analysis

The data were presented as mean value ± standard deviation (X ± S.D.). Analyzed with SPSS (version 12). Comparisons between groups were performed by one-way ANOVA and Student-Newman-Keuls-test. Statistical significance was considered to be indicated by a two-tailed value of P<0.05.

Results

Establishment of acute myocardial infarction rabbit model and transfection of lentivirus-mediated IL-8

In the present study, 40 male rabbits were used, animals were randomly divided into control, AMI, plasmid and treatment group, and 33 survived duration of the 6-week study. 7 rabbits were died during operation and in 24 h after the operation for cardiac arrest, ventricular fibrillation, heart failure or pneumothorax. Two rabbits showed negative transfection were ruled out. Finally there were 9, 8, 7 and 7 animals in control, AMI, plasmid and treatment group respectively. All rabbits in plasmid and treatment group showed positive transfection signal in cardiac muscle by detection under fluorescence microscope (Figure 1), however no difference was found between the two groups. No signal was detected in control and AMI group.

Regional morphology and change of Left ventricular volumes and EF

Myocardial necrosis and fiber proliferation were shown in infarction area under H-E staining (data was not showed). After AMI was induced for 1 and 6 weeks, LVEDd and LVESd were significantly increased, concomitantly LVFS and LVEF were decreased in all groups as compared with control group (Tables 1, 2). Cardiac function was not improved in Plasmid group as compared with AMI group at 1st and 6th week (52.3±4.12 mm VS 50.6±2.98 mm; 31.8±3.01 mm VS 31.1±2.97 mm, respectively). Though EF was improved in Treatment group at 6th week, no statistical significance was shown as compared with AMI group (35.3±3.27% VS 31.1±2.97%) and Plasmid group (35.3±3.27% VS 31.8±3.01%).

IL-8 improved angiogenesis around the myocardial infarction area

To test whether IL-8 overexpression was associated with increased angiogenesis, the immunohistochemistry analysis against CD34 was performed and evaluated microvessel density (MVD) around the myocardial infarction. As shown in Figure 2, IL-8-transfected group displays enhanced MVD. MVD around the infarction area were increased at 6th week as com-

Table 1. Left ventricular volumes and ejection fraction at 1st week

<table>
<thead>
<tr>
<th>Group</th>
<th>LVEDd (mm)</th>
<th>LVESd (mm)</th>
<th>FS (%)</th>
<th>EF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=9)</td>
<td>7.3±0.46</td>
<td>4.3±0.39</td>
<td>38.9±5.29</td>
<td>76.2±1.88</td>
</tr>
<tr>
<td>AMI (n=8)</td>
<td>11.2±1.49*</td>
<td>8.4±1.22*</td>
<td>25.1±3.29*</td>
<td>50.6±2.98*</td>
</tr>
<tr>
<td>Plasmid (n=7)</td>
<td>10.9±1.33*</td>
<td>7.9±0.97*</td>
<td>25.9±5.31*</td>
<td>52.3±4.12*</td>
</tr>
<tr>
<td>Treatment (n=7)</td>
<td>9.8±1.13*</td>
<td>7.7±1.09*</td>
<td>26.8±3.57*</td>
<td>51.2±4.96*</td>
</tr>
</tbody>
</table>

*P<0.01 as compared with Control group.

Table 2. Left ventricular volumes and ejection fraction at 6th week

<table>
<thead>
<tr>
<th>Group</th>
<th>LVEDd (mm)</th>
<th>LVESd (mm)</th>
<th>FS (%)</th>
<th>EF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=9)</td>
<td>13.1±1.56</td>
<td>9.1±1.58</td>
<td>33.9±3.93</td>
<td>73.5±4.38</td>
</tr>
<tr>
<td>AMI (n=8)</td>
<td>18.3±1.64*</td>
<td>15.3±0.97*</td>
<td>15.3±1.35*</td>
<td>31.1±2.97*</td>
</tr>
<tr>
<td>Plasmid (n=7)</td>
<td>17.9±1.61*</td>
<td>15.9±1.34*</td>
<td>15.8±2.85*</td>
<td>31.8±3.01*</td>
</tr>
<tr>
<td>Treatment (n=7)</td>
<td>16.8±1.55*</td>
<td>13.6±1.02*</td>
<td>17.3±2.52*</td>
<td>35.3±3.27*</td>
</tr>
</tbody>
</table>

*P<0.01 as compared with Control group; #P<0.05 as compared with Control group.
IL-8 facilitates angiogenesis in ischemic myocardium through Akt/GSK-3β_\text{ser9}

Compared with control group (P<0.01). Angiogenesis was increased in Treatment group as compared with both the Plasmid and AMI group (P<0.01). Nevertheless, no significant difference was shown between the Plasmid and AMI group. These results implied that IL-8 overexpression could result in the development of therapeutic angiogenesis.

To evaluate the underlying mechanism of enhanced angiogenesis, the IL-8, phosphorylated Akt and GSK-3β_\text{ser9} were detected by Western blot assay. As showed in Figure 3. After AMI was induced for 6 weeks, the expres-

Figure 2. IL-8 improved therapeutic angiogenesis around the myocardial infarction area (×200). MVD increased obviously after the AMI was constructed, however, lentivirus vector transferred with IL-8 gene can boost more angiogenesis around the area. *P<0.01 as compared with Control, AMI and Plasmid group; #P<0.01 as compared with Control group. A: Control group (n=9); B: AMI group (n=8); C: Plasmid group (n=7); D: Treatment group (n=7).
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**Figure 3.** Expression of IL-8, phosphorylated Akt and GSK-3βser9 in rabbit myocardium. Expression of IL-8 (1), phosphorylated Akt (2) and GSK-3βser9 (3) around the infarction area were significantly increased at 6th week as compared with control group (P<0.01), which was enhanced by injection of lentivirus-transfected IL-8. *P<0.01 as compared with Control, AMI and Plasmid group; #P<0.01 as compared with Control group. A: Control group (n=9); B: AMI group (n=8); C: Plasmid group (n=7); D: Treatment group (n=7).

**Figure 4.** Effect of IL-8 on cell proliferation and apoptosis in HUVECs. Cells’ proliferation was inhibited (A) and apoptosis was induced (B) when incubated with 200 µmol/L CoCl2. Incubated with 10 ng/ml IL-8 ameliorated the reaction, however, those were attenuated when incubated with anti-IL-8 or LY294002 simultaneously. All experiments are triplicated. *P<0.01 As compared with Control group (the 1st lane); △P<0.01 As compared with Hypoxia group (the 2nd lane); #P<0.01 As compared with IL-8 group (the 3rd lane).
IL-8 facilitates angiogenesis in ischemic myocardium through Akt/GSK-3β

IL-8 induced cell proliferation and inhibited apoptosis in HUVECs, which were reversed by anti-IL-8 and the Akt inhibitor LY294002

CoCl₂ was used as a properly hypoxic inducer in cells via boosting hypoxia inducible factor 1 alpha (HIF-1α) and heme oxygenase-1 (HO-1) expression. CRL-2873 cells were pretreated with the CoCl₂ at 50, 100, 200 and 400 μmol/L for 24 h. The proliferation was exaggerated when incubated with CoCl₂ at 50, 100 μmol/L, however, inhibited with 200 and 400 μmol/L, which was reversed when incubated with 10 ng/ml IL-8 for 48 h (data was not showed). As showed in Figure 4. Pretreated with the 200 μmol/L CoCl₂ for 24 h can significantly induce apoptosis and decrease cell survival rate as compare with control (13.56±0.65 VS 2.36±0.08, P<0.01). Cultured with 10 ng/ml IL-8 can attenuate the effects of survival rate and apoptosis that induced by CoCl₂ (9.17±0.32 VS 13.56±0.65, P<0.01), however, the effects were be attenuated by simultaneously incubating with 10 μg/ml anti-IL-8 (13.53±0.57 VS 9.17±0.32, P<0.01) or 20 μM LY294002 (15.13±0.37 VS 9.17±0.32, P<0.01).
IL-8 facilitates angiogenesis in ischemic myocardium through Akt/GSK-3βser9

As shown in Figure 5. Hypoxia induced by CoCl2 significantly down-regulated phosphorylation of Akt (143.40±6.22 VS 322.68±6.11, P<0.01) and GSK-3βser9 (97.42±4.28 VS 223.68±7.05, P<0.01) expression, meanwhile up-regulated the expression of Caspase3 (308.35±5.60 VS 80.50±3.12, P<0.01). IL-8 can up-regulated phosphorylation of Akt (376.50±4.09 VS 322.68±6.11, P<0.05; 376.50±4.09 VS 143.40±6.22, P<0.01, respectively) and GSK-3βser9 (281.13±9.79 VS 223.68±7.05, P<0.05; 281.13±9.79 VS 97.42±4.28, P<0.01, respectively) expression, and down-regulated the expression of Caspase3 (63.8±4.26 VS 80.50±3.12, P<0.05; 63.8±4.26 VS 308.35±5.60, P<0.01, respectively) obviously in normal or hypoxic cells as compared with control respectively (P<0.01). However, those effects can be attenuated both in normal and hypoxic cells by simultaneously cultured with 10 µg/ml anti-IL-8 (P<0.01) or 20 µM LY294002 (P<0.01). Those imply that the effect of IL-8 on HUVECs’ proliferation and apoptosis maybe through the pathway of IL-8 receptor, subsequent phosphorylation of Akt and GSK-3βser9.

Discussion

There are three key findings in this experiment. First, the expression of IL-8, Akt and GSK-3βser9 were elevated in focal ischemic myocardium. Second, IL-8 can further promote the expression of Akt, GSK-3βser9 and increase the angiogenesis in ischemic myocardium. Third, IL-8 can increase the expression of Akt and GSK-3βser9, inhibit Caspase3 through IL-8 receptor and Akt/GSK-3βser9/Caspase3 pathway both in normal and hypoxic cultured HUVECs, which may facilitate cell proliferation and inhibit apoptosis.

Collateral circulation formation is an important compensatory mechanism in severe CAD not amenable to currently available interventions, especially coronary arteries were diffuse and chronic total occlusion. Approaches to induce coronary collateralization may be an essential therapeutic method. However, the underlying mechanisms have not been clarified. Gene, protein and cellular methods and so on, had been attempted to induce collateral vessel formation [5]. Various angiogenic growth factors and progenitor cells can enhance collateral vessel formation. Previously therapeutic angiogenesis attempts were mostly focused on application of VEGF or FGF to augment angiogenesis and collateralization formation [2]. IL-8 is well known as an inflammatory and proangiogenic marker, has been proved to associating with proliferation, migration, angiogenesis and chemosensitivity in wound healing and various cancers [7]. IL-8 gene silencing with small interfering RNAs can decreases tumor growth through antiangiogenic mechanisms in ovarian carcinoma [14]. The expressions of IL-8, vascular endothelial growth factor receptor 1 (VEGFR-1) and IFN-gamma were significantly higher in thromboangiitis obliterans patients, which imply those may play a role in neovascularization [3]. Overexpression of IL-8 has been detected in CAD patients [6, 15, 16]. IL-8 is associated with circulating CD133+ progenitor cells in acute myocardial infarction [17], which may play a role in post-myocardial angiogenesis and reserve cardiac function. So we assume IL-8 may facilitate angiogenesis in ischemic heart. Leukocyte antigen CD34, the human hematopoietic progenitor cell antigen, was expressed on endothelial abluminal microprocesses during angiogenesis, and can be used for measure neovascularization by immunohistochemical analysis which was identical to CD31 [13]. In this experiment, IL-8 and MVD around the infarction area were obviously increased at 6th week of post-myocardial infarction. Directly injection with lentivirus-mediated IL-8 around the infarction area can facilitate further angiogenesis. IL-8 display enhanced angiogenic activity in this animal experiment. Previous studies showed endothelial cells play an essential role in angiogenesis by promoted proliferation and inhibited apoptosis. To clarify whether IL-8 can directly affect the endothelial cells’ proliferation and apoptosis, we incubated endothelial cells with IL-8 and demonstrated a significant up-regulation of survival and down-regulation of apoptosis that induced by hypoxia, which were attenuated by anti-IL-8. Therefore, IL-8 can directly facilitate angiogenic formation through promoting proliferation and inhibiting apoptosis in HUVECs, which may play a role in reserving cardiac function.

IL-8 can promote neovascularization, however, the underlying signal pathway is not clearly clar-
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Stimuli such as IL-1, TNF-α, ischemia, hypoxia/anoxia and cellular stresses can induce expression of IL-8. In our experiment, IL-8 increased with hypoxia induced by CoCl2 and ischemia. Its receptors include CXCR1, CXCR2 and Duffy antigen receptor. The proangiogenic activity occurs predominantly following binding to CXCR2, but CXCR1 appears to contribute as well through an independent activity [18]. Chen et al showed that IL-8 may mediate angiogenesis of endometrium through p38 mitogen-activated protein kinase (p38MAPK), nuclear factor-kappaB (NF-κB)-dependent pathway [19]. Document has showed that IL-8 stimulate phosphorylation of phosphoinositide 3-kinase (PI3K) as well as the downstream factors such as Akt when combined with CXC receptor [20]. GSK-3β is a ubiquitously downstream factor of PI3K/Akt signaling pathway that phosphorylates cellular substrates and thereby regulates a variety of cellular functions [21]. The activity of GSK-3β is oppositely regulated by Phosphorylation of GSK-3βser9 and GSK-3βtyr216. In this study, the expression of phosphorylated Akt and GSK-3βser9 were significantly increased and further promoted by IL-8 around infarction area and in HUVECs, which was blocked by administring the Akt inhibitor LY294002 and anti-IL-8. The changes were accordance with MVD, cell proliferation and apoptosis. Thus, IL-8 may facilitate angiogenic formation through PI3K/Akt/GSK-3β/Caspase signaling pathway.

In conclusion, we showed that, IL-8 could increase the therapeutic angiogenesis in myocardial infarction rabbits, which maybe via inducing phosphorylation of Akt and GSK-3βser9, and inhibiting Caspase3 expression that facilitating proliferation and inhibiting apoptosis of HUVECs. Understanding the pathways of angiogenesis may promote the potential of therapeutic angiogenesis.

Disclosure of conflict of interest

None.

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