Original Article
The expression and role of LC3II and inflammatory cytokines in in-stent restenosis after percutaneous coronary intervention in patients with coronary heart disease

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Received March 25, 2019; Accepted June 11, 2019; Epub July 15, 2019; Published July 30, 2019

Abstract: Objective: To investigate the expression and role of microtubule-associated protein 1A/1B-light chain 3-II (LC3II) and inflammatory cytokines in in-stent restenosis after percutaneous coronary intervention (PCI) in patients with coronary heart disease (CHD). Methods: We recruited 64 CHD patients with in-stent restenosis in the observation group and an additional 64 CHD patients without in-stent restenosis as the control group. The expression levels of LC3II in the two groups were measured and compared. Changes in the serum levels of interleukin-6 (IL-6) and interleukin-8 (IL-8) were evaluated. The levels of nitric oxide (NO) and vascular endothelial growth factor (VEGF) were also compared between the two groups. Results: The serum levels of IL-8, IL-6, NO and VEGF in the observation group were higher than those in the control group (all P < 0.05). The expression level of LC3II in the observation group was higher than that in the control group (P < 0.05). The correlations were positive between LC3II levels and the levels of IL-8, IL-6, NO, and VEGF (all P < 0.001). Conclusion: The serum levels of IL-8, IL-6, NO, and VEGF in CHD patients with in-stent restenosis after PCI were significantly different from those in CHD patients without in-stent restenosis. These changes are involved in the pathophysiological process of in-stent restenosis and may be associated with autophagy mediated by the expression of LC3II.

Keywords: Coronary heart disease, in-stent restenosis, inflammation, autophagy

Introduction
Studies have confirmed that coronary heart disease (CHD) is the leading cause of human death. Although it is decreasing in developed countries, the occurrence of CHD shows an upward trend in developing countries on an annual basis [1]. In China, the incidence of CHD is rising as the issue of population aging increases. Studies have shown that CHD caused approximately 100 deaths in every 100,000 Chinese people in the past ten years and was the major cause of death among Chinese residents. In addition, CHD can result in disability, posing a heavy economic burden on the society [2].

Percutaneous coronary intervention (PCI) is the major treatment for CHD. PCI can treat CHD by restoring the blood supply from coronary arteries, thereby relieving clinical symptoms or preventing the occurrence of adverse events [3, 4]. However, a previous study showed that restenosis or even occlusion would occur in one quarter to half of the patients within 5-10 years after PCI, degrading their prognosis and quality of life [5]. Currently, research has confirmed that diabetes, endothelial cell injury, and inflammatory response are the major reasons for the occurrence of stenosis in PCI patients, but the specific mechanisms have not been described [6].

Recent studies have shown that autophagy plays an important role in the in-stent restenosis caused by vascular endothelial injury after PCI treatment, and have confirmed that there is excessive levels of autophagy, instead of physiological levels of autophagy that would effectively protect the vascular endothelium, but
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instead aggravates vascular endothelial injury [7, 8]. Moreover, autophagy can affect vascular endothelial injury through its effect on the inflammatory response. Therefore, this study explored the role of LC3II-mediated autophagy and inflammatory cytokines in in-stent restenosis of PCI patients, providing new theoretical basis for the long-term prevention of in-stent restenosis in PCI patients and identifying potential therapeutic targets.

Materials and methods

General information

We recruited 64 patients with in-stent restenosis (6 months after PCI) who underwent PCI from January 2016 to December 2017, in the Department of Cardiology at The First Hospital of Qiqihar as the observation group, and an additional 64 patients without in-stent restenosis who underwent surgery in the same period as the control group. Inclusion criteria: Patients whose follow-up angiography showed that the in-stent restenosis was ≥ 50% stenosis (< 50% stenosis was not considered as in-stent restenosis, and one or more coronary arteries with ≥ 50% stenosis was diagnosed as in-stent restenosis); patients who had never received PCI before the study; patients had no history of myocardial infarction; patients whose left ventricular ejection fraction ≥ 55%; patients aged < 80 years old. Exclusion criteria: Patients complicated with valvular disease; patients who failed to regulate blood glucose levels within a normal range; patients complicated with other organ dysfunction; patients with poor treatment compliance; patients complicated with immune system diseases; patients who took steroid hormones; patients who had infections in the two weeks before the study. This study was approved by the Ethics Committee of The First Hospital of Qiqihar and all participants signed informed consent before participating in the study.

Measurements

From each patient, fasting peripheral venous blood (6-8 mL) was drawn into blood collection tubes with anticoagulants. The specimens were then centrifuged at 3,000 r/min to collect the supernatant as serum. Protein extraction solution (Protein Extraction Kit, BestBio, Shanghai, China) was added to the serum samples. After mixing evenly, the samples were placed at 4°C for 5 min, then centrifuged at 1,400 r/min for 10 min. The supernatant, which was serum protein, was collected into pre-cooled sterilized centrifuge tubes and stored at -80°C. The enzyme-linked immunosorbent assay kit (Santa, USA) and Infinite F50 Absorbance Microplate Reader (Tecan, Switzerland) were used in the two groups to detect the serum levels of interleukin-8 (IL-8), interleukin-6 (IL-6), nitric oxide (NO) and vascular endothelial growth factor (VEGF) according to the manufacturers’ instructions. The reagent kit (Jiangsu Jianglai Biotechnology Co., Ltd., China) was used to detect the serum levels of LC3-II in the two groups.

Statistical analysis

All the data were statistically processed using SPSS 22.0 software package. Measurement data are expressed as mean ± standard deviation (X ± sd). Comparison of measurement data between the two groups was based on the t-test. Comparison of rates between the two groups was based on the chi-square test. Pearson correlation analysis was applied to evaluate the correlations between the level of LC3II and the levels of IL-6, NO, VEGF and IL-8. P < 0.05 was considered statistically significant.

Results

Comparison of baseline characteristics

The results of this study showed that there were no significant differences between the
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The serum level of LC3II protein in the observation group was significantly higher than that in the control group (P < 0.05), indicating that the level of autophagy in the observation group was higher than that of the control group. See Figure 1.

Comparison of LC3II protein levels

The serum levels of IL-6 and IL-8 in the observation group were significantly higher than those in the control group (both P < 0.05), indicating that the inflammatory response was possibly involved in the development of in-stent restenosis in PCI patients after the surgery. See Figure 2.

Comparison of IL-6 and IL-8 levels

The results of this study showed that the serum levels of NO and VEGF in the observation group were higher than those in the control group (both P < 0.05), indicating that intravascular injury in CHD patients complicated with diabetes was more severe than that in the control group, as shown in Figures 3 and 4.

Comparison of serum levels of NO and VEGF

In the control group (both P < 0.05), indicating that the inflammatory response was possibly involved in the development of in-stent restenosis in PCI patients after the surgery. See Figure 2.

Comparison of serum levels of NO and VEGF
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Prevention of in-stent restenosis after PCI is a common challenge facing both physicians and patients. Although PCI patients currently receive aspirin and antiplatelet drugs and other such measures as lowering cholesterol and controlling blood pressure and glucose levels to prevent in-stent restenosis, a number of CHD patients still suffer restenosis after PCI [9, 10]. Consequently, the chest tightness, chest pain and other symptoms occur again due to ischemia, affecting the patient’s health while reducing the effect of PCI. Moreover, these clinical symptoms pose greater psychophysiological and economic burdens on the patients. To this end, it is of great significance to study the occurrence of in-stent restenosis in CHD patients after PCI [11-14].

Studies have shown that autophagy plays an important role in the development of cardiovascular disease. Autophagy, the process by which cells recycle cytoplasm, dispose of excess or defective organelles, and convert them into energy supply, helps relieve myocardial ischemic and hypoxic injury. However, excessive levels of autophagy can lead to cell damage, indicating that there are both benefits and risks to the process of autophagy [15, 16]. Recent studies have shown that autophagy is important in the development of coronary artery stenosis and restenosis after PCI. It may be involved in the above pathophysiological processes via the aggravation of local inflammatory response and vascular injuries [17]. Nevertheless, autophagy is associated with the inflammatory response and vascular injury to a certain extent. Therefore, this study explored the role of autophagy in restenosis in CHD patients after PCI.

LC3II is one of the proteins representative of autophagy in the body. The results of this study showed that the LC3II levels in the observation group were higher than that in the control group, indicating that the level of autophagy was higher in PCI patients with in-stent restenosis than that in PCI patients without in-stent restenosis, which is consistent with the conclusions of previous studies [18].

Furthermore, autophagy inhibits the functioning of neutrophils. At the early stage of revascularization, the inflammatory response heightens, resulting in the increase of inflammatory cytokines, IL-6 and IL-8. Autophagy can effectively reduce the levels of inflammatory cytokines, but over-elevated autophagy worsens the inflammatory response as a result of damage to endothelial cells. The results of this study showed that the levels of IL-6 and IL-8 in the observation group were significantly higher than those in the control group, indicating that over-elevated autophagy increases the levels of inflammatory cytokines and thus participates in the pathophysiological process of in-stent restenosis after PCI, which is consistent with the results of previous studies [19].

Studies have confirmed that functional damage of the coronary endothelium is also an important factor for the formation of in-stent restenosis, and the related literature has also confirmed that autophagy can engage in vascular endothelial injury by affecting the levels of NO and VEGF [20]. Our study showed that the levels of NO and VEGF in the observation group were significantly higher than those in the control group, indicating that over-elevated autophagy increases the levels of inflammatory cytokines and thus participates in the process of coronary restenosis after PCI, which corroborates the results of previous studies [21].

By further analyzing the correlations between levels of autophagy and inflammatory response and VEGF, our study found that there are positive correlations between LC3II and the above-mentioned indicators. Autophagy could be considered as a potential therapeutic target for the prevention and treatment of in-stent restenosis after PCI.

### Discussion

The results of this study showed that there were significant positive correlations between the LC3II level and the levels of IL-8, IL-6, NO and VEGF (all \( P < 0.001 \)). See Table 2.

### Table 2. Correlation analysis between LC3II level and other related factors

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Correlation coefficient</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-8</td>
<td>0.901</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.929</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>NO</td>
<td>0.907</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>VEGF</td>
<td>0.912</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

*Note: LC3II, microtubule-associated protein 1A/1B-light chain 3-II; IL-8, interleukin-8; IL-6, interleukin-6; NO, nitric oxide; VEGF, vascular endothelial growth factor.*

**Correlation analysis between LC3II level and levels of IL-8, IL-6, NO and VEGF**

The results of this study showed that there were significant positive correlations between the LC3II level and the levels of IL-8, IL-6, NO and VEGF (all \( P < 0.001 \)). See Table 2.
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prevention of in-stent restenosis. However, this study is a single-center study with a small sample size. Therefore, the verification is to be performed in the future by conducting multi-center studies with enlarged sample size. The moderate levels of autophagy also warrant assessments by more accurate indicators.

Disclosure of conflict of interest

None.

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References


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