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Effects of IL-1β and IL-18 induced by NLRP3 inflammasome activation on myocardial reperfusion injury after PCI

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Abstract. – OBJECTIVE: To investigate the effect of nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) inflammasome in the serum levels of interleukin-1β (IL-1β) and interleukin-18 (IL-18) in patients with myocardial reperfusion injury after the percutaneous coronary intervention (PCI).

PATIENTS AND METHODS: Twenty healthy controls (control group) and forty patients (treatment group) were recruited in this study. The enzyme-linked immunosorbent assay (ELISA) was used to measure the serum levels of IL-1β and IL-18 at various time points in both the control and treatment groups. Data processing and analysis were performed using the Statistical Product and Service Solution (SPSS) 22.0 software (IBM Corp, Armonk, NY, USA). Pearson's correlation coefficient test was applied in all data analyses. A difference was statistically significant when $p<0.05$.

RESULTS: The levels of IL-1β and IL-18 in the treatment group were significantly higher than those in the control group ($p<0.05$). The IL-1β level in the treatment group peaked at 0.5 h after PCI and then, gradually decreased. The multiple regression analysis showed that IL-1β level was positively correlated with levels of LDL-C and IL-18 ($p<0.05$, r=0.527 and 0.955 respectively), and negatively correlated with the HDL-C level ($p<0.05$, r=-0.34).

CONCLUSIONS: The levels of IL-1β and IL-18 significantly rose in patients with myocardial ischemia-reperfusion injury after PCI.

Key Words:
PCI, NLRP3, Myocardial reperfusion injury.

Introduction

Myocardial ischemia-reperfusion injury (I/R) refers to myocardial damage caused by recanalization of the coronary artery after myocardial ischemia for a certain period of time. I/R can trigger the onset of life-threatening arrhythmia, myocardial infarction, and acute heart failure. I/R is an acute myocardial injury associated with multiple factors, such as overproduction of reactive oxygen species (ROS), inflammation, apoptosis, calcium overload, and mitochondrial dysfunction[1,2]. As the research of I/R has been going in-depth in recent years, more and more studies have confirmed that the infiltration of the inflammatory cells plays an important role in I/R[3,4]. During myocardial ischemia-reperfusion, a variety of molecules are expressed in the myocardial cells, including pattern recognition receptors (PRRs) that trigger host inflammation. PRRs can also recognize factors that stimulate immune responses. These factors are derived from damaged tissue or necrotic cells and are referred to as damage-associated molecular patterns (DAMPs). NLRP3 belongs to intracellular PRRs, and its main function is to recognize pathogen-associated molecular patterns (PAMPs) and DAMPs inside the cells. NLRP3 is one of the most widely studied members in the 22-human nucleotide-binding oligomerization domain (NOD)-like receptor (NLR) family[5]. In sterile inflammation, NLRP3 forms a high molecular weight protein complex, i.e., the

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NLRP3 inflammasome, with apoptosis-associated speck-like protein containing a CARD (ASC) and cysteine aspartic acid protease 1 (caspase-1)[6]. The formation of the NLRP3 inflammasome further induces the release of IL-1β, IL-18, and other related inflammatory factors, thus triggering an inflammatory response. Therefore, these inflammatory factors are associated with the onset and progression of inflammatory diseases. Among them, IL-1β is one of the most widely studied cytokines in this signaling pathway[7]. In this study, the serum levels of IL-1β and IL-18 in peripheral blood of patients with myocardial ischemia-reperfusion injury were measured in order to investigate their associations with the onset and progression of myocardial ischemia-reperfusion injury.

Patients and Methods

The enrolled patients in the study were twenty healthy subjects (control group) who had physical examination in the Xuzhou Central Hospital were recruited, and 40 patients with coronary heart disease (treatment group) who were admitted to the Department of Cardiology. There were 18 males and 22 females in the treatment group who were aged 40-80 years and had an average age of (61.48 ± 10.66) years. Among the patients, there were 10 cases of unstable angina and 30 cases of acute myocardial infarction. All the patients in the treatment group were treated with PCI.

Inclusion Criteria

All patients included in this study matched the criteria for PCI treatment based on the SCAI/ACC/AHA Guideline for Percutaneous Coronary Intervention. This study was approved by the Ethics Committee of the Xuzhou Central Hospital. Patients and their families were informed, and the informed consent forms were signed.

Exclusion Criteria

(1) Patients with conditions, such as autoimmune diseases, metabolic diseases, acute and chronic infectious diseases, recent trauma and cancers; (2) patients who were using anti-inflammatory drugs; and (3) patients who had a family history of genetic diseases or infectious diseases.

Methods

After the subjects were enrolled, their clinical data, such as gender, age, mean arterial pressure (MAP), body mass index (BMI), fasting serum glucose (FPG), and blood lipids (LDL-C, HDL-C), were collected. In the day after enrollment at 6:00 am, 20 ml of peripheral venous blood was drawn from patients in the control group. For patients in the treatment group, 20 ml of venous blood was drawn at four-time points, i.e., 0.5 h before PCI surgery and 0.5 h, 3 h, and 3 days after the surgery. The whole blood (4 mL) was centrifuged, and the supernatant was collected and stored at -80°C. The serum levels of IL-1β and IL-18 were measured by the enzyme-linked immunosorbent assay (ELISA) in strict accordance with the kits' instruction.

Statistical Analysis

Data processing and analysis were performed using the SPSS 22.0 (IBM Corp., Armonk, NY, USA) software. All measurement data were tested for normality and homogeneity of variance. Independent data that conformed to normal distribution and passed the test for homogeneity of variance were expressed as mean ± standard deviation (±s). An independent sample *t*-test was used to compare the differences between the two groups. Count data were expressed as percentage and tested using the x^2-test. The test for spherical symmetry was conducted on the measurement data at different time points that had a normal distribution. If the data had spherical symmetry, then, the repeated measures analysis of variance was conducted. If the data did not have spherical symmetry, especially if epsilon (ε) was less than 0.75, the Greenhouse-Geisser (G-G) correction was applied to correct the spherical symmetry coefficient. The Bonferroni test was used for pairwise comparisons. The Pearson correlation analysis was conducted for all data. A difference was statistically significant if $p<0.05$.

Results

Clinical Data

There was no significant difference in gender, age, BMI, and FPG between the two groups ($p>0.05$). As shown in Table I, in the treatment group, the low-density lipoprotein cholesterol (LDL-C) level was higher, whereas the high-density lipoprotein (HDL-C) level was lower compared to the control group ($p<0.05$).

Comparison of Serum Levels of IL-1β and IL-18 Between the Two Groups Before PCI

As shown in Table II, the levels of IL-1β and IL-18 before PCI were significantly higher in the

Table I. Clinical data of the subjects.

Item		Control group	Treatment group	T/x^2-value	*p*-value
Subject number		20	40		
Gender	Male	9	18	1.913	0.725
	Female	11	22		
Age (year)		62.30±10.19	61.48±10.66	0.287	0.775
MAP (mm Hg)		92.20±2.63	94.18±4.16	-1.936	0.058
LDL-C (mM)		1.98±0.21	2.52±0.52	-4.425	<0.001
HDL-C (mM)		2.11±0.24	1.54±0.26	8.174	<0.001
FPG (mM)		5.62±0.55	5.80±0.52	-1.285	0.204
BMI (kg/m^2)		24.77±0.69	24.83±0.70	-0.333	0.741

treatment group than those in the control group (p<0.001).

Comparison of IL-1β and IL-18 Levels at Different Time Points in the Treatment Group

In terms of the IL-1β level, the values at 0.5 h and 3 h after operation were higher than that at 0.5 h before operation (p<0.05); the value at 3 days after operation was lower than that at 0.5 h before operation (p<0.05); the values at 3 h and 3 days after operation were lower than that at 0.5 h after operation (p<0.05), and the value at 3 days after operation was lower than that at 3 h after operation (p<0.05). The results were shown in Table III.

Correlations of IL-1β and IL-18 with Other Factors

Pearson correlation analysis showed that IL-1β was positively correlated with LDL-C, HDL-C, and IL-18 (p<0.05). Multivariate regression analysis was performed with age, gender, LDL-C, MAP, IL-18, and HDL-C as independent variables and IL-1β as the dependent variable. The results indicated that LDL-C and IL-18 were positively correlated with IL-1β, with correlation coefficients of 0.527 and 0.955 (p<0.05), respectively (See Table IV), while HDL-C was negatively correlated with IL-1β, with a correlation coefficient of -0.685 (p<0.05, See Table V).

Discussion

Percutaneous coronary intervention (PCI) is a non-surgical procedure used to open blocked coronary arteries aimed to rescue infarcted myocardium, save patient life, and reduce patient mortality. However, myocardial reperfusion itself induces further myocardial cell death, known as myocardial reperfusion injury[8-10]. The inflammatory response is an important component of healing after injury, as it is the reperfusion injury after myocardial infarction. During acute myocardial infarction (AMI), sudden blockage of blood flow in the coronary arteries leads to ischemic injury and myocardial cell death. When the cells become necrotic and die, interleukin-1 is released immediately, recruiting nearby inflammatory factors, and further aggravating the inflammatory response[11,12]. The multifaceted mechanisms of myocardial I/R injury include ATP depletion, Ca^{2+} overload, and ROS generation. Many reports suggest that ROS plays an important role in the pathogenesis of myocardial I/R injury as mediators of myocardial stunning, myocardial apoptosis, and reperfusion arrhythmias[13-16].

The inflammatory response is the stress defense response of the body tissue to the injury factor. The NLRP3 inflammatory body is a member of the innate immune system and is an important component of the inflammatory response[17,18]. When I/R occurs in the myocardium, NLRP3 is

Table II. Serum levels of IL-1β and IL-18 before PCI in the two groups.

Item	Control group	Treatment group	T value	*p*-value
Subject number	20	40		
IL-1β (ng/L)	14.47±1.73	26.82±1.56	-27.852	<0.001
IL-18 (ng/L)	50.06±2.84	98.39±4.33	-45.208	<0.001

Table III. Comparison of IL-1β and IL-18 levels at different time points in the treatment group.

		IL-1β (ng/L)	IL-18 (ng/L)
Time points	0.5 h before PCI	20.62±6.48	74.25±24.81
	0.5 h after PCI	50.74±3.88[a]	128.05±9.07[a]
	3 h after PCI	37.75±3.29[ab]	106.69±7.02[ab]
	3 days after PCI	15.98±1.54[abc]	54.61±4.48[abc]
Mauchly sphericity test		<0.001	<0.001
ε		0.643	0.427
Statistic		F(1.930, 75.265)=620.166	F(1.281, 496.961)=209.658
p		<0.001	<0.001

Notes: [a]$p<0.05$, compared with 0.5 h before operation; [b]$p<0.05$, compared with 0.5 h after operation; [c]$p<0.05$, compared with 3 h after operation.

activated by various factors (such as potassium outflow, increased ROS, lysosomal destabilization, etc.), resulting in the production of a large number of proinflammatory factors IL-1β and IL-18, which are released outside the cell[19]. As an important member of the interleukin-1 family, IL-1β is a typical proinflammatory cytokine. It is mainly produced by activated innate immune cells, such as macrophages and monocytes, and plays a key role in the early stage of inflammation. IL-1β is released during ischemia, triggering neutrophil infiltration into the myocardium. After reperfusion, under the synergistic action of IL-1β with other cytokines and complements, neutrophils are subsequently activated and interact with endothelial cells, generating ROS and aggravating myocardial injury[20]. Myocardial apoptosis was proved to be an outcome of myocardial I/R injury. IL-1β mediates the changes in Bak and Bcl-X gene transcription by activating AP-1 and NF-κB pathways and is involved in the myocardial I/R injury promoting myocardial apoptosis[21-23].

In this study, the expression levels of IL-1β and IL-18 were measured in patient serum after PCI, as well as in healthy controls. The results showed that the levels in the treatment group were significantly higher than those in the control group, and the differences were statistically significant ($p<0.05$). It indicates that when I/R occurs in the myocardium, it may cause the NLRP3 inflammatory corpuscle pathway to be activated, resulting in the production and release of pro-inflammatory cytokines IL-1β and IL-18 into the blood, causing myocardial damage. In the treatment group, the levels of IL-1β and IL-18 peaked at 0.5 h after PCI, then gradually decreased, and returned to

Table IV. Correlation between serum level of IL-1β and other factors.

		MAP	LDL-C	HDL-C	FSG	BMI	Age	IL-18
IL-1β	r	0.225	0.527	-0.685	0.174	0.094	0.028	0.955
	p	0.084	<0.001	<0.001	0.184	0.476	0.834	<0.001

Notes: [a]$p<0.05$, compared with 0.5 h before operation; [b]$p<0.05$, compared with 0.5 h after operation; [c]$p<0.05$, compared with 3 h after operation.

Table V. Multivariate linear regression analysis of correlation between IL-1β and clinical indicators.

						95% CI	
Variable	β	SE	β'	*t*	*p*-value	Upper limit	Lower limit
LDL-C	1.703	0.768	0.124	2.217	0.033	0.144	3.263
IL-18	0.229	0.021	0.877	10.819	<0.001	0.186	0.272

normal levels after 3 days. The findings suggested that inflammation may be at its high level within 3 days after myocardial I/R injury. The mechanism still needs to be further verified by animal experiments. Pearson correlation analysis showed that IL-1β was positively correlated with LDL-C, HDL-C, and IL-18 ($p<0.05$). Multivariate regression analysis was performed with age, gender, LDL-C, MAP, IL-18, and HDL-C as independent variables and IL-1β as the dependent variable. The results indicated that LDL-C and IL-18 were positively correlated with IL-1β with correlation coefficients of 0.527 and 0.955 ($p<0.05$), respectively, while HDL-C was negatively correlated with IL-1β with a correlation coefficient of -0.685 ($p<0.05$). The findings reported in this study suggested that the combined measurement of the serum levels of IL-1β and IL-18 in patients after PCI may have clinical significance in evaluating myocardial injury after I/R. In this study, the clinical effects of the serum markers IL-1 and IL-18 downstream of NLRP3 inflammasome on myocardial I/R were investigated. The mechanism of action and expression of the gene/protein still require further animal experiments.

Conclusions

This study demonstrated that the levels of IL-1β and IL-18 were significantly elevated in I/R myocardium after PCI. Combined detection of serum IL-1β and IL-18 levels in patients after PCI may have clinical significance for the prevention and treatment of myocardial injury after I/R.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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