European Review for Medical and Pharmacological Sciences 2017; 21: 1837-1842

Correlation study between interleukin-6 levels and coronary reflow

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Abstract. – OBJECTIVE: To analyze the correlation between local interleukin-6 (IL-6) levels in different parts of blood vessel and the record of Thrombolysis in Myocardial Infarction (TIMI) frame count (TFC) after myocardial ischemia-reperfusion (IR) model.

MATERIALS AND METHODS: Establishment of IR model in Yorkshire pigs, the pigs were divided into two groups (n=6). Experiment group pigs were administrated with Shenfu injection (SF) intravenously (1 mL/kg), control group was given saline injection. The blood reflowed after 30 min. TIMI was recorded to evaluate the coronary blood flow and myocardial perfusion. IL-6 levels in venous sheath, coronary sinus, artery sinus, and proximal coronary artery were determined by ELISA.

RESULTS: The records of TIMI in experimental group were higher than that in control group. The difference was statistically significant ($p < 0.05$). The level of IL-6 increased obviously compared with control group after reperfusion ($p < 0.05$). Shenfu injection reduced the level of IL-6. IL-6 level at the coronary sinus was positively correlated with TIMI in experimental group ($p = 0.03$, $R^2 = 0.97$) but not in control group.

CONCLUSIONS: IL-6 levels were significantly increased after reperfusion, which aggravated myocardial injury. IL-6 may be associated with coronary reflow, but further study is needed.

Key Words:
IR model, Interleukin-6, Coronary reflow, Reperfusion, TIMI frame count.

Introduction

Cardiovascular disease devastatingly affects the health and economics of healthcare in the world, which accounts for 50% of all deaths in several developed countries, more than 50% in Africa and Western and Southeast Asia[1]. Patients with coronary artery disease were treated with revascularization usually[2], but they have a high risk to catch a thrombosis after surgery[3]. Coronary artery occlusion dramatically reduces blood flow to the portion of the myocardium, which profoundly impairs the energy metabolism. Therefore, the methods and opportunities to identify influencing factors on patients, will make the aggressive antithrombotic treatment possible and be helpful to study the relationship between inflammation and the early coronary angiography[4-7]. Thrombolysis in Myocardial Infarction (TIMI) frame count (TFC) is initially defined as the number of cine-frames required for contrast to come up a standardized distal coronary landmark in the culprit vessel[8]. Currently, as a clinical commonly tool, TIMI was used to assess quantitative indexes of coronary blood flow. This technique counts the cineangiographic frames numbers from initial contrast opacification of the proximal coronary artery to opacification of distal arterial landmarks and is corrected for the length of left anterior descending coronary artery (LAD)[9,10]. Iinterleukin-6 (IL-6) is a reliable inflammation marker and its role is involved in the progression of myocardial injury[11]. Dysfunction derived from different etiologies, in long-term heart failure syndrome[11,12]. Karpiński et al[13] studied that IL-6 concentration in plasma elevated was positively correlated with the record of TFC in patients with slow coronary flow (SCF). Although TFC has been demonstrated to be more precisely to predict the outcome of thrombolytic therapy after myocardial infarct, no study has

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examined the relationship between TFC and IL-6 after ischemia-reperfusion. In the present study, after the successful myocardial IR injury, we systematically measured the IL-6 levels in venous sheath, coronary sinus, artery sinus, and proximal coronary artery, combined with the TFC to assess the correlation of IL-6 and coronary artery reflow. The purpose was to reveal whether IL-6 level could be taken as a predictor for the coronary artery reflow after myocardial injury.

Materials and Methods

Establishment of Myocardial Ischemia-reperfusion Model in Yorkshire Pigs

12 healthy 4-month-old Yorkshire pigs (Yantai Longda breeding Co, Ltd, Shandong, China), 30-40 kg, male or female, were administrated orally with 300 mg minced aspirin (Bayer Health Care, Pharmaceutical Inc., Whyppany, NJ, USA) dissolved in water 1 h before surgery. This experiment meets the requirements of Animal Ethics. All the necessary components were preassembled on a sterile tray ready for use. Briefly, after anesthesia, the pig was fasted on the operating Digital Subtraction Angiography (DSA) bench and connected with multi-channel physiological instrument and coronary pressure monitor. After povidone-iodine disinfection of inguinal region, 1% lidocaine (Jincheng Hayes Pharmaceutical Co, Ltd, China) was applied to the area for local anesthesia underneath the inguinal ligament for later puncture. After a skin incision along the inguinal, the local tissue was segregated. According to Salinger's method, the right femoral artery and femoral vein were punctured. The guidewire (6F, Suzhou High Wire Medical Devices Co, Ltd, China) was carefully inserted into arterial sheath. 7000 Unit intravenous heparin (Kunming Jida Pharmaceutical Co, Ltd, China) was intrathecal administrated. Sending guidewire (BMW, Suzhou High Wire Medical Devices Co, Ltd, Suzhou, China) to the distal left anterior descending artery (LAD), we performed angiogram procedure. The middle part of the anterior descending branch, nearly 1/3 to the middle of the vessel was designated as target vessel. Then the multi-purpose arterial catheter (5F, Arrow International, Inc., Wayne, PA, USA) was sent to the coronary sinus ostium along the venous sheath. After angiogram, blood perfusion was recovered. Blood flow recovery was confirmed by angiography. LAD TIMI frame counts were evaluated.

Detection of IL-6

After the IR model, the pigs were divided into control group (n=6) and experimental group (n=6). The experimental pigs were administered 30-34 mL (1 ml/kg) Shenfu injection (Ya'an 39 Pharmaceutical Co, Ltd, Chengdu, Sichuan, China) intravenously within 2 min after the model. Shenfu injection (Shenfu solution isn't known in Europe since it is a traditional/Chinese medicine, not approved by EMA), the main component is red ginseng. Ginseng can be used for the treatment of hemorrhagic shock[14]. Shenfu has been widely regarded as an effective therapeutic approach in clinic for its protective effects on ischemia/reperfusion injury[15-17]. The control group received the same amount of saline. The change of TFC was recorded. IL-6 level was determined from venous sheath, coronary sinus, artery sinus, and proximal coronary artery by ELISA (BioSource International, Camarillo, CA, USA).

Statistical Analysis

SAS9.12 (SAS Inc., Chicago, IL, USA) was used to perform statistical analysis. Measurement data were presented as mean ± standard deviation. Correlation analysis was utilized to analyze data. The comparison of measurement data between the two groups was performed by using the *t*-test. A value of $p < 0.05$ was considered statistically significant.

Results

TIMI Frame Count was Increased in Treatment Group

Comparison results of TIMI blood flow frame count in two groups were shown in Table I. There was no significant difference of TFC in the two groups at the moment of blood opening immediately ($p > 0.05$). However, after 30 min following blood flow opening, TFC were significantly higher in experiment group than that in control group ($p < 0.05$).

Different Expression of IL-6 in Different Parts of Blood Vessels

IL-6 level was measured at different time courses and difference locations. There was no significant difference of IL-6 between the two groups in femoral vein before surgery. However,

Table I. TFC in control and experimental group.

	Before surgery	Blood open (immediately after)	Blood open (30 min later)
Experimental	23.5 ± 3.42	44.5 ± 6.76	37.0 ± 6.88*
Control	22.25 ± 5.12	45.25 ± 4.99	56.5 ± 16.42*
p	0.63	0.83	0.02

IL-6 levels in the experimental group were significantly lower than those in the control group after surgery ($p < 0.05$) (Figure 1A). IL-6 level in experiment group was significantly lower than those in the control group at the time of blockage, immediately after opening and 30 min after opening ($p < 0.05$) (Figure 1B, C, D). IL-6 is an inflammation marker; these results revealed SFI treatment inhibited the inflammatory response in experimental group but not in control group. After operation, the level of IL-6 increased continuously. Consequently, myocardial injury was aggravated (Figure 1).

Correlation Analysis Between IL-6 and Coronary Reflow

We analyzed the correlation between IL-6 and coronary reflow. Results were shown in Tables II and III. There was a positive correlation between

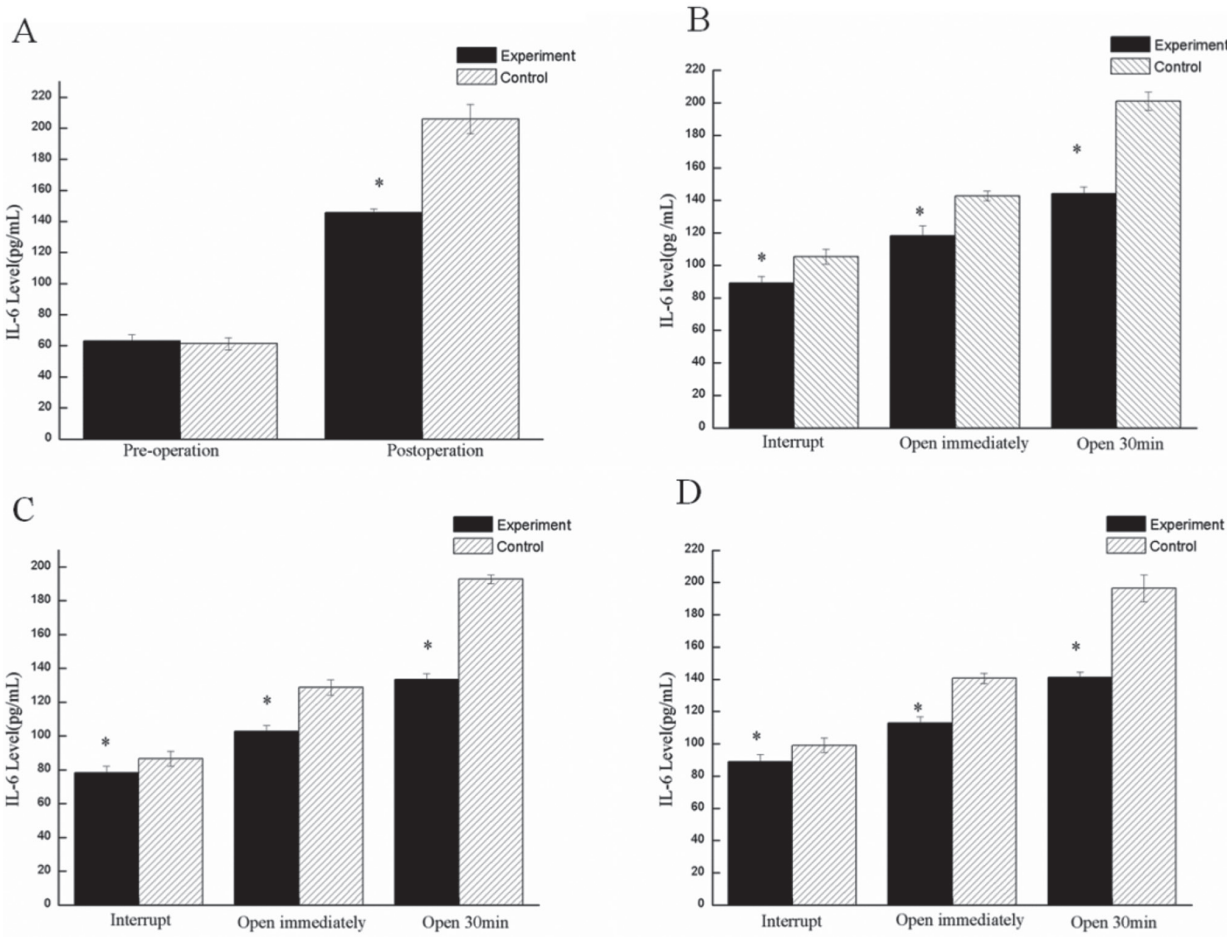

Figure 1. IL-6 level in different time courses. ***A,*** IL-6 expression in femoral vein. ***B,*** IL-6 expression in coronary sinus. ***C,*** IL-6 expression in coronary artery sinus. ***D,*** IL-6 expression in distal coronary artery. Compared with the control group, *$p < 0.05$.

Table II. Correlation analysis of IL-6 and TFC in experimental group.

	p	R^2
Coronary sinus	0.03	0.97
Coronary artery sinus	0.14	0.91
Coronary artery proximal	0.13	0.91

IL-6 and TIMI in the coronary sinus of the experimental group ($p < 0.05$, $R^2 = 0.97$). However, the correlation between IL-6 and TIMI in the coronary sinus, distal coronary artery and all parts of coronary artery in the experimental group was unable to be determined ($p > 0.05$).

Discussion

A significant positive correlation was revealed between IL-6 and TIMI frame count after IR model. Moreover, IL-6 levels at the coronary sinus were positively correlated with coronary artery reflow only in Shenfu injection treated group, but not in control group, implying inflammation marker IL-6 may be a predictor for the myocardial injury. As known, Shenfu is a commonly used traditional Chinese medicine and has been widely regarded as an effective therapeutic approach in clinic for its protective effects on ischemia/reperfusion injury and therapeutic effects on acute myocardial infarction, shock, chronic congestive heart failure and ischemic cardiomyopathy with heart insufficiency[15-19]. The vast majority of people with heart disease in China take this medicine[20,21]. The function of SFI was partial inhibited the inflammatory response in heart disease. Our study confirmed IL-6 level in experiment group was significantly down-regulated than those in the control group after injecting Shenfu. On the other hand, our data implied the correlation between SFI and inflammation factor IL-6. Meanwhile, our data also showed TFC were significantly lower than that in control group after 30 min following blood flow opening. As known, TFC has been demonstrated to suggest disordered resistance vessel function or microvascular dysfunction in heart[22]. Hence, the correlation between IL-6 and TFC became important in animal ischemia-reperfusion model or in patients with myocardial disorder. The inflammation played a critical role in the process of pathophysiology of coronary heart disease, in which the inflammatory cascade is specifically crucial in the coronary artery disease. Prior researches revealed the underlying mechanism of the inflammation pertinent to the SCF phenomenon. Recent investigations[23-25] revealed that soluble adhesion molecules, vascular cell adhesion molecule-1 (VCAM-1), E-selectin and intercellular adhesion molecule-1 (ICAM-1), were potentially indicative of endothelial activation or inflammation in patients suffered from slow coronary flow. TFC record demonstrated the coronary flow rate from the patients[26]. The studies claimed that the levels of VCAM-1, ICAM-1, and E-selectin in the serum with SCF in these diseased people were dramatically upregulated than the control subjects with normal coronary flow. The conclusion from these works suggested that TFC record was significantly correlated with soluble ICAM-1, VCAM-1 and E-selectin concentrations in the patient plasma[27-30]. Similarly, with these studies, we found that increased inflammatory marker IL-6 existed in our study after successful myocardial IR injury model. Also, study showed that TIMI frame counts were significantly correlated with IL-6. This suggested that an inflammation might be a contributor to the development of coronary reflow after IR model. Collectively, these researches implied inflammation makers possibly shared a common mechanism in the blood after ischemia-reperfusion stress, which indicates endothelial activation and inflammation are likely to be in the causal pathway involving in coronary reflow.

Table III. Correlation analysis of IL-6 and TFC in control group.

	p	R^2
Coronary sinus	0.64	-0.44
Coronary artery sinus	0.64	-0.42
Coronary artery proximal	0.62	-0.36

Note: The correlation coefficient R represents the strength of the relationship, closer the coefficients are to 1 and 0. Only $p < 0.05$, correlation can be divided into strong and weak. If $p > 0.05$, no matter how much the R-value, still unable to determine the relevance.

Conclusions

IL-6 levels were significantly increased after reperfusion following myocardial infarction. The analysis between IL-6 levels and TFC suggested that IL-6 levels positively correlated with coronary re-flow. IL-6 levels may predict the inju-

ry grade from myocardial stress in the patients. Further research is needed to increase our understanding of the possible causality of these associations.

Ethical Approval

The research was conducted in accordance with the Declaration of Helsinki and the United National Institutes of Health.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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