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Effects of tumor necrosis factor and E-selectin on coronary artery flow

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Abstract. – OBJECTIVE: The aim of the study is to investigate the correlation between tumor necrosis factor (TNF-α), E-selectin and coronary artery flow following myocardial ischemia-reperfusion model (IR) in Yorkshire pigs.

MATERIALS AND METHODS: Establishment of IR model in pigs. Following the injury model, Experiment group was administrated intravenously Shenfu injection solution (SFI, 1 mL/kg). The control group received the same amount of saline. After 30 min of blood reflux, thrombolysis in myocardial infarction frame count (TFC) was recorded following surgery. TNF-α, E-selectin expression was determined by ELISA in the venous sheath, coronary sinus, artery sinus, and proximal segment of the coronary artery.

RESULTS: After the blood reflowing, TFC in both groups were upregulated, and TFC increased more than the control group. The difference is statistically significant ($p<0.05$) at the time of 30 min. TNF-α, E-selectin expression increased after IR. After reperfusion, TNF-α, E-selectin levels further increased and the myocardial injury was aggravated. SFI inhibited inflammation in the experimental group. TNF-α, E-selectin levels at coronary sinus, artery sinus, and distal segment of coronary artery after surgery was positively correlated with TIMI in the experimental group ($p<0.05$). TNF-α, E-selectin levels significantly increased after reperfusion ($p<0.05$).

CONCLUSIONS: The result demonstrated that TNF-α, E-selectin levels were positively correlated with coronary artery reflow only in the experimental group but not in the control group.

Key Words
TNF-α, E-selectin, Thrombolysis, Myocardial infarction, Shenfu injection solution, Reflow.

Introduction

The Thrombolysis in Myocardial Infarction (TIMI) frame count (TFC) is a validated risk-assessment tool allowing clinicians to predict myocardial infarction after reperfusion in patients[1,2]. That is a simple, reproducible, objective and quantitative index of coronary flow, which can predict myocardial infarction after reperfusion in patients with long-term and short-term prognosis[3-5]. As is known to all, there might occur inflammation reaction when tissue or organ was injuried. Inflammatory mediators may have dual roles. In the early stage, the inflammatory reaction is a protective response to infection or tissue injury. However, the prolonged inflammatory reaction is considered as a part of the pathogenesis of a variety of cardiac diseases[6]. Various populations were in poor health, suffering from myocardial injury[7]. After myocardial injury, some inflammation markers such as tumor necrosis factor (TNF-α) and interleukins are released by damaged cells[8,9]. In fact, elevated circulating levels of TNF-α and IL-6 have been reported as independent predictors of mortality in patients with heart failure[9,10]. E-selectin belongs to selectin family, which is composed of three Type-I cell-surface glycoproteins: E-, L- and P-selectin. E-selectin is not expressed under baseline conditions, except in skin microvessels[11], but is rapidly induced by inflammatory cytokines. Following injury, E-selectin binds to endothelial cells, allowing neutrophil attachment to the vessel wall and consequent aggregation; thus, promoting inflammation and immune injury[12-15].

The diagnosis of acute coronary syndrome remains challenging especially in patients without clear symptoms or electrocardiographic and/or biomarker features. A hallmark of ischemia/reperfusion is the activation of endothelial cells, leading to altered expression of molecular markers, including selectins[16]. These investigations suggested that E-selectin levels have been associated with various cardiovascular disease and

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unusually high expressed in patients with myocardial injury[16,17]. Transient (20 min) myocardial ischemia of heart in the rat was produced by ligation of the left anterior descending coronary artery ligation and followed by 2-, 5-, or 24-hour reperfusion. Imaging of the transient ischemic event was achieved by the use of selectin. The performance of this clinically translatable targeted ultrasound contrast agent was compared with that of the antibody-targeted streptavidin electins. The immunohistochemical findings revealed that both P- and E-selectin were expressed on the surface of the activated endothelium 2 and 5 hours after the acute ischemic event, whereas only E-selectin remained accessible after 24 hours. Therefore, the E-selectin expression in different time course can imply the resolution of the ischemic event. The current study demonstrated that the cTnI and CK-MB is not an ideal biological marker for the confirmation of diagnostic criteria after myocardial injury[18]. Thus, the seeking of alternative markers becomes more important.

To indicate the severance and progress of myocardial injury after ischemia/reperfusion, the efforts were made by carrying out many different trials. There is no research combined the reliable TFC with TNF-α, E-selectin to predict the recovering of the ischemic event. In our study, we aimed to investigate the TFC, TNF-α, E-selectin change after ischemia/reperfusion. Further, we analyzed the correlation between TFC and TNF-α, E-selectin after IR injury to predict the coronary flow following ischemia/reperfusion, which may pave the way for a large clinical diagnostic window.

Materials and Methods

Ethics Statement

This study was conducted in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Ethics Committee. All surgeries were performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

Animals

12 healthy 4-month-old Yorkshire pigs (Yantai Longda breeding Co., Ltd. Shandong, China), 30-40 kg, male or female, was administrated orally with 300 mg minced aspirin (Bayer HealthCare, Shanghai, China) dissolved in water 1 hour before the surgery.

Establishment of Myocardial Ischemia-Reperfusion Model in Yorkshire Pigs

All the necessary components are 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 iodophor disinfection of bilateral inguinal area, 1% lidocaine (International Medication Systems Limited, South El Monte, CA, USA) was applied to the puncture area under inguinal ligament. 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 HighWire Medical Devices Co. Ltd, Jiangsu, China) was carefully inserted into the arterial sheath. 7000 Unit intravenous heparin (Kunming Jida Pharmaceutical Co., Ltd., Yunnan, China) was intrathecally administrated. 6F was pushed along the arterial sheath to guide the catheter to the left coronary ostia and BMW guidewire (BMW, Suzhou High Wire Medical Devices Co. Ltd, Jiangsu, China) was sent to the distal segment of the left anterior descending artery (LAD). Then, the angiogram procedure was conducted. The middle part of the anterior descending branch, nearly 1/3 to the middle of the vessel was designated as target vessel. The appropriate size of the balloon was determined according to the LAD diameter results measured by DSA. 2.0-3.0×15 mm balloon was selected and dilated at 6-10 atm of pressure until the LAD artery was completely occlusive, which was assessed by angiography. The process was holding for 30 min. Then, the multi-purpose arterial catheter (5F, Arrow International Inc., Cleveland, OH, USA) was delivered to the coronary sinus ostium along the venous sheath. After an angiogram, the balloon was deflated and retracted. The blood perfusion was recovered, and the blood flow recovery was confirmed by angiography. LAD TIMI frame counts were evaluated.

Detection of TNF-α, E-selectin

After the IR injury 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 solution (Ya'an 39 Pharmaceutical Co., Ltd., Chengdu, Sichuan, China) intravenously within 2 minutes

Table I. TFC in control and experimental group.

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

Compared with the control group, *$p<0.05$.

after the model. The control group was given the same amount of saline. LAD TIMI frame count (TFC) was examined within 1 minute after the model and applied to evaluate coronary blood flow and myocardial perfusion. TNF-α, E-selectin level was determined from the samples collected from venous sheath, coronary sinus, artery sinus, and distal segment of coronary artery by ELISA (BioSource International, Camarillo, CA, USA).

Statistical Analysis

SAS9.12 software (SAS Inc., Chicago, IL, USA) was applied for statistical analysis. Measurement data were presented as mean ± standard deviation (SD). Correlation analysis was utilized to analyze the correlation between TNF-α, E-selectin and the reperfusion of slow blood flow. The comparison of measurement data between the 2 groups was performed by using the *t*-test. A value of $p<0.05$ was considered statistically significant.

Results

The Records of TIMI Frame Count

First, the TFC was recorded in different stage, blood reflowing immediately and 30 min after surgery. The TFC results were shown in Table I. There was no significant difference of TFC between the two groups at the moment of blood opening immediately ($p>0.05$). After 30 minutes of the blood flow opening, there was significant difference between the experimental group and the control group ($p<0.05$). TFC was significantly higher in the experimental group than that in the control group ($p<0.05$).

TNF-α expression

The levels of TNF-α were detected at different time courses. The results showed that there was no significant difference of TNF-α level between the two groups in femoral vein before surgery ($p>0.05$) (Figure 1). After surgery, TNF-α levels in the experimental group were significantly lower than that in the control group ($p<0.05$) (Figure 1A). In the coronary sinus, there is no significant difference of TNF-α level when the blood was blocked. However, TNF-α level was significantly lower in the experiment group than those in the control group in immediately and 30 minutes after opening ($p<0.05$) (Figure 1B). There is no significant difference of TNF-α level in the coronary artery sinus between the two groups at the moment of the blood blockage and immediately reflowing. The TNF-α level was significantly decreased in the experimental group at time of 30 minutes after blood opening ($p<0.05$) (Figure 1C). In the distal part of coronary artery, there was no significant difference of TNF-α level between the two groups. But TNF-α level in the experimental group decreased markedly, at the moment of immediately and 30 minutes after opening ($p<0.05$) (Figure 1D). The results suggested that TNF-α elevated after the IR model and its level went up further gradually (Figure 2).

E-selectin Expression

E-selectin was measured at different time courses (Figure 2). The results showed that there was no significant difference of E-selectin between the two groups in femoral vein before surgery ($p>0.05$). After surgery, E-selectin levels in the experimental group were significantly lower than that in the control group ($p<0.05$) (Figure 2A). In the coronary sinus, coronary artery sinus or the distal part of coronary artery, E-selectin level all significantly deceased in the experimental group than in the control group at the time point of blood blockage, immediately or 30 minutes after blood opening (Figure 2B, 2C, 2D).

Correlation Analysis

The levels of TNF-α and E-selectin significantly increased after reperfusion in the coronary arteries, that indicated there was a close correlation between their expression level and the coronary reflow. Further study needs to confirm the results in future.

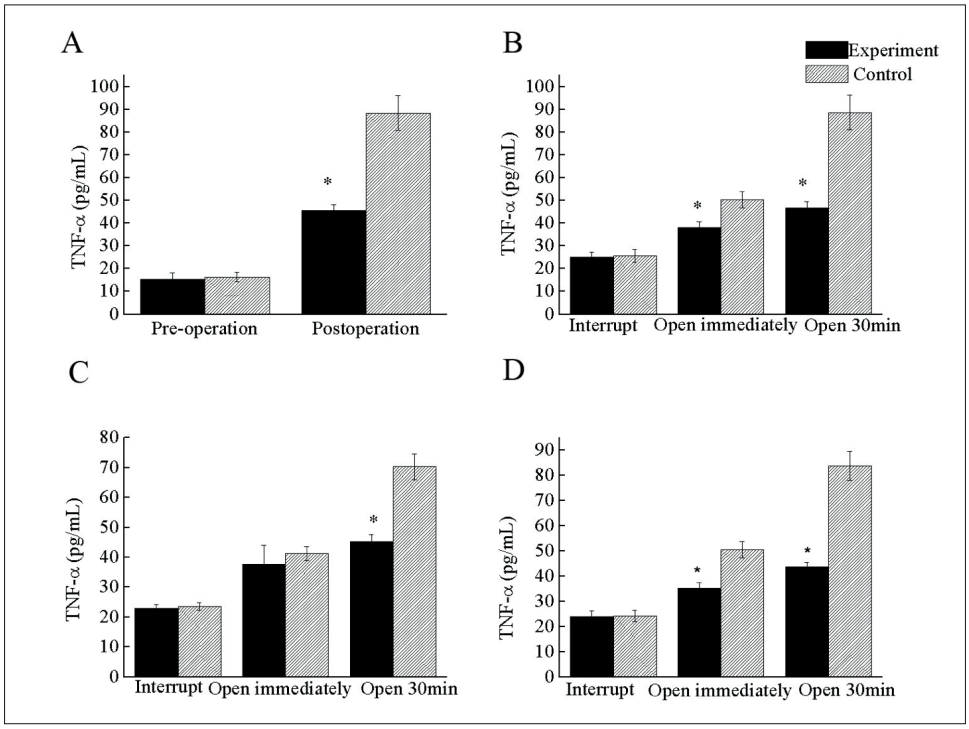

Figure 1. TNF-α expression in different time courses. **A**, TNF-α change in femoral vein. **B**, TNF-α change in coronary sinus. **C**, TNF-α change in coronary artery sinus. **D**, TNF-α change in the distal segments of coronary artery. Compared with the control group, *p<0.05

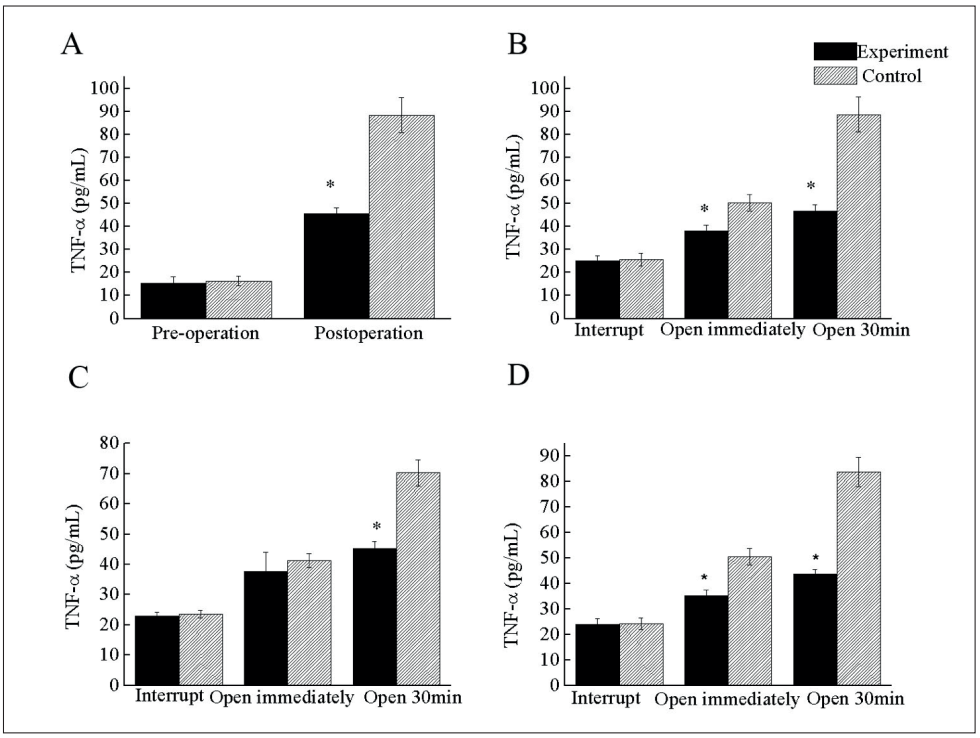

Figure 2. E-selectin expression in different time courses. **A**, E-selectin level in femoral vein. **B**, E-selectin levels in coronary sinus. **C**, E-selectin levels in coronary artery sinus. **D**, E-selectin levels in the distal segments of coronary artery. Compared with the control group, *p<0.05

Table II. Correlation analysis of TNF-α and TFC.

	Experimental		Control	
	p	R	*p*	R
Coronary sinus	0.02	0.997	0.64	-0.435
Coronary artery sinus	0.02	0.998	0.65	-0.460
Distal coronary artery	0.04	0.988	0.61	-0.327

As shown in Table II, there was a positive correlation between TNF-α and TIMI in coronary sinus, coronary artery sinus and distal coronary artery in the experimental group ($p<0.05$, R=0.997, R=0.998, R=0.988). However, the correlation between TNF-α and TIMI in the control group can be determined ($p>0.05$). Similarly, there was a positive correlation between E-selectin and TIMI in coronary sinus, coronary artery sinus and distal coronary artery in the experimental group ($p<0.05$, R=0.996, R=0.973, R=0.999) (Table III). The correlation between TNF-α and TIMI in the control group could not be determined ($p>0.05$).

Discussion

The main finding of this study was that the levels of TNF-α, E-selectin significantly increased after myocardial reperfusion, which aggravated myocardial injury after reperfusion. TNF-α, E-selectin level was positively correlated with coronary artery re-flow only in experimental group treated with SFI but not in control group.

Shenfu is a commonly used traditional Chinese medicine. The main active components of Shenfu is Ginsenoside and Aconitine, which treat cardiac diseases for a long time in China[19,20]. The majority of people with cardiac diseases in China take this medicine[21,22]. Shenfu solution injection (SFI) has been widely regarded as an effective therapeutic approach in clinic for its protective effects on ischemia/reperfusion injury. There were therapeutic effects on acute myocardial infarction, shock, chronic congestive heart failure and ischemic cardiomyopathy with heart insufficiency[23-26]. Our study showed that TFC were significantly lower in the experimental group than the control group after 30 minutes of the blood flow opening. The results suggested that SFI benefited to the TFC in the experimental group. The underlying mechanism remains unknown yet.

As known, TNF-α and interleukins were released by damaged cells following injury. Our data showed that TNF-α level was significantly lower in the experiment group than the control group in 30 minutes after opening in femoral vein. The results suggested that SIF could ameliorate the inflammation reaction, which could protect the myocardial damage. These findings are in line with the results from the prior study, which demonstrated SIF inhibited NF-kappa B activity in I/R myocardium[27]. SIF attenuated myocardial injury and regulated myocardial immune disorders by reduction of TNF-α. SIF protected post-resuscitation myocardial injury by modulating the expression imbalance of transcription factors GATA-3 and T-bet[28]. Also, more experiments are needed to elucidate the underlying mechanism in our study. The common factors and mechanism involved in I/R will be a presumable consideration.

Table III. Correlation analysis of E-selectin and TFC.

	Experimental		Control	
	p	R	*p*	R
Coronary sinus	0.03	0.996	0.31	0.572
Coronary artery sinus	0.07	0.973	0.67	-0.499
Distal coronary artery	0.01	0.999	0.52	-0.053

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.

The previous investigation showed that E-selectin level could be detected in the early stage and could persistent express when myocardial ischemia injury occured. The immunohistochemical assay revealed that both P- and E-selectin were expressed on the surface of the activated endothelium after the acute ischemic event, whereas only E-selectin remained accessible after 24 hours[16,29]. In this work, E-selectin levels in femoral vein in the experimental group were significantly lower than that in the control group after surgery. In the coronary sinus, coronary artery sinus or distal part of the coronary artery, the E-selectin levels all significantly decreased in the experimental group. This suggested that E-selectin was going down due to the SIF treatment. These findings confirmed the previous results and indicated that E-selectin is a reliable marker for the myocardial IR injury[30].

Conclusions

We showed that TNF-α and E-selectin significantly increased after reperfusion in the coronary arteries and closely correlated with the TFC record. Also, the correlation study explored the underlying biological mechanism of the myocardial injury, which will be useful for the precision therapy in the future. More rigorously designed trails with high methodological quality studies are necessary to confirm the results due to the insufficient small-sampled size in the present investigation.

Conflict of Interest

The authors declare no conflicts of interest.

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