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Blood rheology of angina pectoris patients with myocardial injury after ischemia reperfusion and its effect on thromboxane B_2 levels

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Abstract. This study investigated the changes in the blood rheology of patients with angina pectoris and ischemia reperfusion injury and their effect on thromboxane B_2 (TXB_2) levels to examine their relationship. Forty patients with unstable angina pectoris who underwent elective percutaneous coronary intervention (PCI) were selected for the unstable angina group (UA group) and forty patients deemed free of coronary heart disease by coronary angiography were selected for the control group. Venous blood samples were drawn from all participants; patients in the UA group had blood drawn 1 day before and 1 day after the PCI procedure. Blood samples were used to analyze blood rheology and examine hemodynamic parameters, at the same time radioimmunoassay was applied to measure the concentrations of serum endothelin-1 (ET-1) and TXB_2, and an automatic biochemical analyzer was used to detect the content of superoxide dismutase (SOD) and malondialdehyde (MDA). Our results showed the patients in the UA group all presented hyperviscosity; however the levels were higher for the patients in the UA group (after surgery) than for those in the UA group (before surgery). Patients in the control group exhibited normal levels, and the differences among groups were significant in pairwise comparisons ($P<0.05$). The levels of ET-1 and TXB_2 in the UA group were increased compared with those in control group and they were highest after surgery ($P<0.05$). For the patients in the UA group, the serum TXB_2 concentration increased gradually along with the increase in risk stratification. There were significant differences in comparisons between different strata and between UA patients and those in the control group ($P<0.05$). The serum SOD activity levels were lowest in the UA group (after surgery), higher in the UA group (before surgery) and highest in the control group. Conversely, the MDA content was highest in the UA group (after surgery), lower in the UA group (before surgery) and smallest in the control group; there were significant differences in pairwise comparisons. Based on our findings, a hyperviscosity syndrome was manifested in the blood rheology of patients with angina pectoris and ischemia reperfusion injury. The higher than normal TXB_2 levels can be used as a marker of platelet activation and a reference for clinical risk stratification, thus having great significance for the prevention and treatment of ischemia reperfusion injury and assessment of disease progression.

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Introduction

In the clinical practice, ischemia reperfusion injury (IRI) occurs after the coronary artery is recanalized by means of a medical intervention like a bypass graft. Myocardial injury is aggravated after blood perfusion is restored to the ischemic myocardium, leading to a series of ultrastructural injuries, and altered functional metabolism and electrophysiology (1,2). According to statistics of the World Health Organization (WHO), acute coronary artery infarction will become the major cause of human death from diseases by 2020 (3). The diagnosis and prevention of myocardial ischemia reperfusion injury (MIRI) have become a hot spot of clinical study. During ischemia reperfusion, myocardial tissues get exposed to large concentrations of oxygen free radicals, which damage the cell membranes via lipid peroxidation. Malondialdehyde (MDA) is a metabolic product of lipid peroxidation and it indirectly reflects the activity of oxygen free radicals on tissues. Levels of superoxide dismutase (SOD), an important antioxidant enzyme, can also be used to assess the oxidative state of tissues. The occurrences of coronary thrombosis and coronary artery spasm are the main reasons for IRI events. The strong vasoconstrictive effect of endothelin-1 (ET-1) is known to trigger myocardial ischemia, and thromboxane B_2 (TXB_2) is conducive to platelet aggregation and vasoconstriction. The characteristics of blood rheology and TXB_2 changes in angina pectoris patients with IRI were examined in this study, in order to investigate weather a relationship with MIRI exists.

Materials and methods

General information. Forty patients with angina pectoris, admitted to Beijing Shijitan Hospital from February 2014 to January 2015, and treated with elective percutaneous coronary

Table I. Comparisons of general information in the two groups (mean ± SD, n=40).

Item	Control group	UA group	t/χ^2	P-value
Age (years)	59.9±9.8	61.3±8.9	0.041	0.260
Male/female/(cases)	25/15	23/17	0.032	0.222
Hypertension/(cases)	17	20	0.055	0.296
Diabetes mellitus/(cases)	9	12	0.064	0.318
BMI (kg/m^2)	25.01±2.28	26.86±3.09	1.382	0.320
TC (mmol/l)	4.35±1.02	4.41±0.92	1.483	0.431
TG (mmol/l)	1.60±0.96	1.48±0.64	1.568	0.443
HDL (mmol/l)	1.14±0.32	1.01±0.25	1.720	0.798
LDL (mmol/l)	2.54±0.82	2.54±0.62	1.711	0.713

Comparisons with control group, P>0.05. BMI, body mass index; TC, total cholesterol; TG, triglyeride; HDL, high density lipoprotein; LDL, low density lipoprotein.

Table II. Comparisons of blood rheology in the two groups (mean ± SD, n=40).

Group	Viscosity at high shear rate (mPa/sec)	Viscosity at medium shear rate (mPa/sec)	Viscosity at low shear rate (mPa/sec)	Plasma viscosity (mPa/sec)	Erythrocyte aggregation index
Control group	5.61±0.31	7.68±0.39	10.99±0.78	1.70±0.05	7.13±0.73
UA group (before surgery)	9.84±0.21[a]	9.78±0.37[a]	15.25±0.41[a]	2.18±0.16[a]	8.13±0.63[a]
UA group (after surgery)	11.13±0.11[a,b]	11.78±0.35[a,b]	20.08±0.33[a,b]	3.28±0.25[a,b]	10.22±0.42[a,b]
F-value	3.88	9.38	10.64	2.92	9.03
P-value	0.027	0.022	0.014	0.036	0.028

Comparisons with control group, [a]p<0.05; comparisons with those before surgery, [b]P<0.05. UA, unstable angina group.

intervention (PCI), were selected for the unstable angina (UA) group. All UA patients had their diagnosis confirmed and had not had any related symptoms for 48 h prior to the procedure. Forty patients deemed free from coronary heart disease by coronary angiography during the same period of time, were selected for the control group. Patients with PCI or coronary angiography contraindications were excluded from the study. The Ethics Committee of Beijing Shijitan Hospital approved the study and the participants signed the informed consent form. The patients with angina pectoris were divided into low-, intermediate- and high-risk groups based on their medical history, pain characteristics, clinical manifestations, electrocardiograms and cardiac biomarkers taking into account the Guidelines for Diagnosis and Treatment of Patients with Unstable Angina and Non-ST-Segment Elevation Myocardial Infarction (2007 edition).

Test methods. Peripheral venous blood samples (10 ml) were withdrawn from the patients in the UA group 1 day before and 1 day after the PCI. The samples were divided into a 7 ml sample treated with anticoagulant and a 3 ml sample without anticoagulant. All samples were centrifuged at 3,000 rpm for 10 min at 4˚C; then plasma and serum were collected and stored at -80˚C for later examination.

Detection indexes. A MVIS2035 blood rheology analyzer (Chongqing Tianhai Medical Equipment, Shandong, China) was used to detect hemodynamic parameters (viscosities at high, medium and low shear rates, plasma viscosity, as well as erythrocyte aggregation index). Radioimmunoassay using the FM2000 γ-immunoassay counter (Hybribio, Xi'an, China) was performed to measure the concentrations of serum ET-1 and TXB_2. The 3100 type automatic biochemical analyzer (Hitachi, Tokyo, Japan) was used to detect the content of SOD and MDA in plasma samples.

Statistical analysis. SPSS 18.0 software (IBM Corp., Armonk, NY, USA) was used for statistical analysis of data. Measurement data are presented as mean ± SD, and Chi-square test was applied for analysis; analysis of variance was used for comparison between groups and t-test for pairwise comparisons. A P<0.05 indicates a statistically significant difference.

Results

Comparisons of clinical characteristics in the two groups. Patients' characteristics such as age, sex, history of hypertension and diabetes mellitus, body mass index (BMI) and four items of blood-lipid tests [total cholesterol (TC), triglyeride (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL)] were balanced and comparable between the two groups (P>0.05) (Table I).

Comparisons of hemodynamic parameters in the two groups. The blood rheology in the UA group was manifested

Table III. Comparisons of risk stratifications and TXB_2 levels in angina pectoris patients with reperfusion injury (mean ± SD).

Group	Cases	TXB_2 (pg/ml)
Control group	40	82±17
Low-risk group	10	177±27[a]
Intermediate-risk group	14	219±34[a-c]
High-risk group	16	260±38[a,b]
F-value		4.062
P-value		0.008

Comparisons with control group, [a]P<0.01; comparisons with low-risk group, [b]P<0.01; comparisons with high-risk group, [c]P<0.01. TXB_2, thromboxane B_2.

as hyperviscosity. All the parameters compared, such as viscosity at high, medium and low shear rates, and the erythrocyte aggregation index, were highest in the UA group (after surgery), lower in the UA group (before surgery), and lowest in the control group. There were significant differences in pairwise comparisons (P<0.05) (Table II).

Comparisons of ET-1 and TXB_2 concentrations in the two groups. The levels of ET-1 and TXB_2 in the UA group were higher than those in the control group and they increased further after surgery (P<0.05) (Figs. 1 and 2).

Comparisons of risk stratifications and TXB_2 levels in angina pectoris patients with reperfusion injury. In the UA group, the serum TXB_2 concentrations increased gradually along with the increase of risk stratification, and the levels were significantly higher than those in the patients in the control group (P<0.05) (Table III).

Comparisons of SOD and MDA content between the two groups. The results for the activity of serum SOD showed the UA group (after surgery) had the lowest levels, the UA group (before surgery) had higher levels and then the control group displayed the highest levels of all. On the other hand, the MDA levels in the UA group (after surgery) were higher than those in the UA group (before surgery), and those levels in turn were higher than the levels in the control group; there were significant differences in pairwise comparisons (P<0.05) (Table IV).

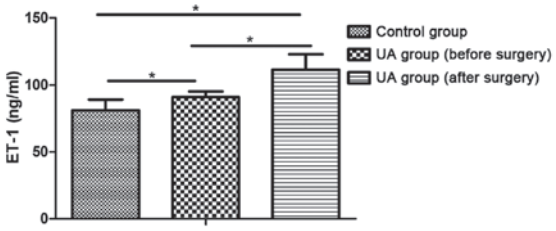

Figure 1. Comparison of radioimmunoassay results for endothelin-1 (ET-1) content. Radioimmunoassay shows that the ET-1 level in unstable angina (UA) group is increased compared with that in control group and it is increased more obviously after surgery (*P<0.05).

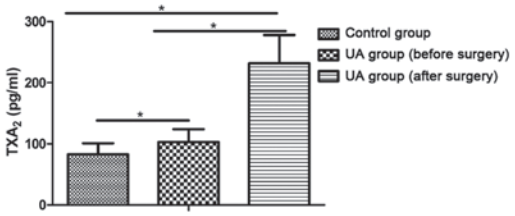

Figure 2. Comparison of radioimmunoassay results for thromboxane A_2 (TXA_2) content. Radioimmunoassay shows that the TXA_2 level in unstable angina (UA) group is increased compared with that in control group and it is increased more obviously after surgery (*P<0.05).

Discussion

MIRI refers to a syndrome caused by inflammatory responses, damage to endothelial cells, blood stream obstruction and reperfusion arrhythmia as well as other injuries due to free radical damage, calcium ion and leucocyte injuries (4).

Study has shown that the occurrence of hypercoagulability in ischemic angina pectoris is closely related to the adhesion and aggregation of platelets (5). TXA_2 is a vasoconstrictive factor with unstable activity, mainly synthesized and released by platelet micro-particles. It can further activate the platelets on the basis of inflammation, thus promoting the occurrence of coronary artery spasm and formation of intravascular thrombosis (6). TXB_2 is a stable metabolic product of TXA_2 in plasma, and it can be used to get a reflection of the actual level of TXA_2 (7). For patients with unstable angina pectoris, the content of TXB_2 in plasma increases because of the platelet adhesion caused by damage to the vascular endothelium (8). The results of our experiments showed that, in the UA group, the serum TXB_2 concentration increased along with the rise in the risk stratification; and the differences in comparisons were all statistically significant (P<0.05). ET-1 is a bioactive peptide with strong

Table IV. Comparisons of SOD and MDA content in the two groups (mean ± SD, n=40).

Index	Control group	UA group (before surgery)	UA group (after surgery)	F-value	P-value
SOD (U/ml)	119.8±23.9	90.5±20.3[a]	61.9±7.5[b,c]	3.08	0.041
MDA (nmol/ml)	1.7±0.4	2.8±0.8[a]	4.9±1.5[b,c]	8.03	0.033

Comparisons with control group, [a]P<0.05 and [b]P<0.01; comparisons with those before surgery, [c]P<0.01. SOD, superoxide dismutase; MDA, malondialdehyde; UA, unstable angina group.

myocardial toxicity, which can reflect the secretory function of the vessel's endothelium. It has intense vasoconstrictive effects and can promote myocardial ischemia, ventricular and vascular remodeling by activating relevant hormones and accelerating the proliferation of vascular smooth muscle cells (9-11). This study proved the ET-1 levels in UA groups was higher than those in the control group, and the ET-1 level after the postoperative reperfusion injury was statistically different from that before surgery ($P<0.05$). Our findings suggest TXB_2 may stimulate the interaction between platelet activation and local inflammatory factors like ET-1 as well as other endothelial secretory factors, thus creating a vicious cycle after reperfusion injury.

Animal experiments have proven that severe coronary stenosis can lead to massive production of free radicals and aggregation of platelets (12). Relevant studies have confirmed that when reperfusion injury occurs in the ischemic myocardium, the production of oxygen free radicals bursts in the body, proteins and lipids are oxidized and disabled, and the activity of lysosomes is decreased, resulting in cell death (13,14). SOD is an important antioxidant enzyme in the myocardium, and oxygen free radicals can enhance the lipid peroxidation by inhibiting the activity of SOD, thus causing myocardial injury. When the blood supply is restored in the ischemic myocardium, a large quantity of oxygen free radicals produced in the tissues can damage the cell membranes through lipid peroxidation, thus increasing membrane permeability, causing transduction abnormalities of lipid signaling molecules and inducing neutrophil accumulation and formation of microthrombi, which can lead to no-reflow phenomenon and aggravated myocardial injury (15-17). MDA is a metabolic product of lipid peroxidation triggered by oxygen free radicals in myocardial cells, which can lead to degeneration, senescence, mutation and death of myocardial cells by promoting the cross-linking of nucleic acids, proteins and phospholipid (18). Our data showed that the activity of serum SOD was smallest in the UA group (after surgery), higher in the UA group (before surgery) and highest in the control group. Conversely, the MDA content was highest in the UA group (after surgery), lower in the UA group (before surgery) and lowest in the control group, with significant differences in pairwise comparisons ($P<0.05$). After the postoperative reperfusion injury, MDA and SOD levels changed significantly compared with those before the surgery ($P<0.01$). The reperfusion injury occurs in the ischemic myocardium as the blood and oxygen supplies are restored. On the one hand, the activity of xanthine oxidase in the body is strengthened (19); on the other, when the atheromatous plaques are desquamated from the tunica intima, inflammatory factors and the complement system are activated, and a large number of oxygen free radicals are released (20). A large amount of SOD molecules are required to eliminate the oxygen free radicals in the body; therefore, the active enzyme sites available decline. Moreover, excessive oxygen free radicals can induce extremely strong lipid peroxidation, and consequently, the MDA content is increased accordingly.

In conclusion, a hyperviscosity syndrome is present in the blood rheology of patients with angina pectoris and IRI, and the increasing TXB_2 levels can be used as markers of platelet activation and reference for clinical risk stratification, providing great help in the prevention and assessment of disease progression during treatment of IRI.

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