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Effects and significance of lumbrokinase on vasoactive substances in patients with coronary artery disease

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[ABSTRACT] AIM: To evaluate the effects of lumbrokinase on patients with coronary heart disease (CHD) and its effects on plasma levels of alpha-granule membrane protein-140 (GMP-140), thromboxane B_2 (TXB_2), 5-hydroxytryptamine (5-HT), parameters of blood rheology. **METHODS:** Forty-eight patients with angina pectoris were randomly divided into control group (n = 23, isosorbidedinitrate 10mg, tid) and treatment group (n= 25, isosorbidedinitrate and lumbrokinase capsules 400 mg, tid) for 4 weeks. Before and after treatment, attack of angina pectoris, nitroglycerin consumption, and change of electrocardiogram ST-T were performed, including plasma GMP-140, TXB_2, 5-HT and blood rheology. **RESULTS:** The plasma levels of GMP-140, TXB_2, 5-HT were higher in CHD group than that of healthy control group ($P< 0.01$). After the treatment of attacks of angina pectoris, nitroglycerin consumption, plasma levels of GMP-140, TXB_2, 5-HT and parameters of blood rheology were reduced in 2 groups, which was more remarkable in treatment groups than in control group ($P< 0.01$). **CONCLUSION:**Plasma levels of GMP-140, TXB_2, 5-HT may play important roles in sustaining and developing of CHD. Combined isosorbidedinitrate with lumbrokinase treatment have a vital role in improving clinic symptoms of CHD by regulating plasma levels of vasoactive substances.

[KEY WORDS] lumbrokinase; isosorbidedinitrate; nitroglycerin; coronary heartdisease; vasoactive substance; blood rheology

INTRODUCTION

Coronary heart disease (CHD) is a multifactorial disease with its cause, development, and incidence highly correlated to arterial linings and platelets. Thromboxane A_2 (TXA_2) and 5-hydroxytryptamine (5-HT) are potent vasoconstrictors that not only damage the linings of the arteries, but also facilitate the activation and aggregation of platelets, thus play significant roles in the development of CHD[1]. This study aimed to investigate the antiplatelet effects of lumbrokinase in patients with CHD by measuring plasma levels of alpha-granule membrane protein-140 (GMP-140), thromboxane B_2 (TXB_2), 5-hydroxytryptamine (5-HT), and parameters of blood rheology. Researching and understanding the mechanism of lumbrokinase in the treatment of angina pectoris would provide new insights for preventing and treating CHD.

1. MATERIAL AND METHODS

1.1 Selection of Participants: According to the 1979 WHO diagnostic criteria of ischemic heart disease, 48 patients with angina pectoris (28 males and 20 females) with mean age of (57.2 + 12.4) years were selected from outpatient specialty clinic and inpatient departments between January and June of 2000. Among the patients, 36 cases had stable angina and 12 cases had aged myocardial infarction. Healthy control group: 30 healthy participants were selected during routine health checkup (18 males and 12 females) with mean age of (56.2 + 10.5) years.

1.2 Methods: Selected patients had to stop all heart disease medications, includingantiplatelets and calcium-channel blockers 2 weeks prior to study. Patients with angina pectoris were randomly divided into 2 groups: 23 patients in the control group were given isosorbidedinitrate 10 mg tid, and the 25 patients in the treatment group were given both isosorbide dinitrate10 mg tid and lumbrokinase 400 mg tid (Trade name: Xui Tong by Peking University College of Life Science and Zhuhai Bocom Medicine Industry Limited Company; Batch number 950417, 200 mg/capsule). The study period was 4 weeks. Both groups have similar and compatible clinical background information; hence, the results can be compared.

1.3 Parameters and Testing Methods: Each patient was visited by study investigators twice a week to evaluate the number of angina attacks, heart rate, blood pressure, nitroglycerin consumption, and possible side effects. Both groups had 4 ml of venous blood drawn from the antecubital vein before and after the 4-week treatment in plastic test tubes mixed well with 20% EDTA-Na_2 at a ratio of 9:1. Serum TXB_2 and GMP-140 [3]were measured by radioimmunoassay (RIA) method (testing kits provided by Thrombosisand Hemostasis Research Office, Suzhou Medical School), and serum 5-HT was measured by fluorescence [4]; all samples were assayed via two-tube method done at the same time. Other parameters measured include blood rheology, cardiac enzymes, liver and kidney functions, and blood lipids.

1.4 Data Analysis: All calculations were represented as $\bar{x} \pm s$, and the differences evaluated by *t* test.

2. RESULTS

2.1 Changes of plasma levels of GMP-140, TXB_2, and 5-HT: During attacks of angina pectoris, plasma level of GMP-140 was 36.6 + 6.78 $\mu g \cdot L^{-1}$, TXB_2 was 168 + 26.4 $ng \cdot L^{-1}$,and 5-HT was 1.78 + 0.62 μmol^{-1}. When the attacks had subsided, the levels were significantly lowered to 25.6 + 3.77 $\mu g \cdot L^{-1}$, 121 + 22.5 $ng \cdot L^{-1}$, and 0.97 + 0.28 μmol^{-1}($P< 0.01$) respectively. However, the numbers post-attacks were still relatively higher than those found in the healthy control group. Levels found in healthy control group were: 17.8 + 4.37 $\mu g \cdot L^{-1}$, 110 + 21.3 $ng \cdot L^{-1}$, and 0.71 + 0.30 μmol^{-1}($P< 0.05 \sim 0.01$) respectively.

2.2 Changes in angina pectoris attacks: In the treatment group, angina attack frequency and nitroglycerin usage were significantly lowered after 4 weeks of treatment compared to those of 1 week prior to treatment ($P< 0.01$). Even though control group also showed a significant reduction in angina attack frequency ($P<0.05$), the attack frequency and nitroglycerin usage were still higher than those of the treatment group ($P< 0.01$). See Table 1.

Table 1.Changes in Angina Attacks and Nitroglycerin Usage Before and After Treatment ($\bar{x} \pm s$)

Group	Angina Attacks		Nitroglycerin Usage	
	Before Tx	After Tx	Before Tx	After Tx
Control (n=23)	13 ± 5.6	9.9 ± 3.8^{b}	8.2 ± 4.8	6.1 ± 2.2
Treatment (n=25)	14 ± 5.2	5.1 ± 0.9^{cf}	8.8 ± 4.5	2.3 ± 0.8^{cf}

Compared with before Tx, $^{b}P<0.05$, $^{c}P<0.01$; Compared with control group, $^{f}P<0.01$

2.3 Changes in blood rheology: At the end of study period, plasma levels of GMP-140, TXB_2, and 5-HT were significantly lowered in both groups ($P< 0.05 \sim 0.01$), with greater reduction in the treatment group ($P< 0.01$) as all numbers were lowered to within the normal healthy range ($P> 0.05$). The control group's numbers were still above normal healthy values ($P< 0.01$). All blood rheology parameters such as blood viscosity, plasma viscosity, packed red blood cell volume, and platelet aggregation rate were significantly reduced in the treatment group ($P< 0.01$); whereas only plasma viscosity was observed to be significantly reduced in the control group ($P> 0.05$). See Table 2. Adverse effects: Neither group observed changes in blood and urine analysis includingblood lipids, glucose, liver, and kidney functions.

Table 2.Changes in GMP-140, TXB_2, 5-HT, and Blood Rheology Before and After Treatment ($\bar{x} \pm s$)

Parameter	Control Group (n=23)		Treatment Group (n=25	
	Before Tx	After Tx	Before Tx	After Tx
GMP-140/ μg·L	36.8 ± 6.62	27.4 ± 3.44[b]	37.3 ± 6.77	20.3 ± 3.25[cf]
TXB_2/ng·L^{-1}	167 ± 25.9	141 ± 22.7[b]	169 ± 26.2	111 ± 21.9
5-HT/μmol^{-1}	1.70 ± 0.57	1.28 ± 0.60[b]	1.64 ± 0.59	0.78 ± 0.26[cf]
High Shear Rate Blood Viscosity /150·S^{-1}	7.52 ± 2.61	6.81 ± 1.88	8.61 ± 2.04	6.95 ± 1.67[b]
Low Shear Rate Blood Viscosity /10·S^{-1}	12.9 ± 3.52	11.7 ± 3.35	13.8 ± 3.12	10.2 ± 2.10[c]
Plasma Viscosity/mPa	2.27 ± 0.86	1.39 ± 0.81[b]	2.59 ± 0.73	1.48 ± 0.69[b]
Packed Cell Volume/ %	44.2 ± 4.11	43.5 ± 3.84	44.7 ± 3.95	42.1 ± 3.67[c]
Platelet Aggregation Rate/ %	46.5 ± 6.24	44.2 ± 6.32	47.3 ± 7.71	31.8 ± 6.35

Compared with before Tx, [b]$P<0.05$, [c]$P<0.01$; Compared with control group, [f]$P<0.01$

DISCUSSION

GMP-140 is a platelet alpha granule membrane protein which is not expressed on normal platelet membrane, but only on activated platelets. It is the most characteristic expression of activated platelets and thus, has a significant role in the activation and promotion of thrombus formation[4]. Both TXA_2 and 5-HT are important vasoactive chemicals within platelets. Because TXA_2 has a short half-life, radioimmunoassay (RIA) for its metabolite, TXB_2, was employed in this study in order to assess the levels of TXA_2. By measuring plasma GMP-140, TXB_2, and 5-HT levels, we can gain insights into the activation and general states of peripheral platelets. This study showed that patients with coronary heart disease (CHD) had elevated plasma levels of GMP-140, TXA_2, and 5-HT during angina attacks compared to healthy controls. As the attacks subsided the levels also dropped correspondingly, suggesting activation platelets and the release of both $TXB_2$and 5-HT during angina attacks.

Studies have shown that CHD patients have above-normal platelet activation and release of vasoactive chemicals. The release of TXB_2 and 5-HT can constrict blood vessels, damage the arterial lining, and induce platelet aggregation, thus contributing to the formation of arterial plaque and eventual atherosclerosis. Therefore, these vasoactive chemical are central to the pathogenesis of CHD[6]. Recent studies suggest that damaged coronary artery linings not only have the internal collagen exposed, but also have increased concentrations of thrombin, ADP and TXA_2, which all induce platelet activation and aggregation, thus increasing the risk of thrombus formation[7]. The research of Lacoste et al. [8] indicates that reducing platelet thrombus formation can effectively reduce the chance of coronary artery events. This study shows that combining lumbrokinase and isosorbidedinitrate for 4 weeks can significantly reduce angina attacks of CHD patients and the use of nitroglycerin. The reduction in plasma levels of GMP-140, TXA_2, 5-HT and blood rheology improvement are much more significant than using isosorbidedinitrate alone. These results indicate that lumbrokinase can effectively control and prevent the angina attacks, and its antiplatelet activation function could be one of the underlying mechanisms.

Lumbrokinase is a protease derived from saline extract of fresh earthworms. It has a molecular weight of about 30 kDa with t-PA-, plasmin-, and antiplatelet-like actions. Animal studies have shown that lumbrokinase'sfibrinolytic activity can prevent thrombus formation and dissolve thrombus ex vivo. It has been utilized in the treatment of ischemic stroke with encouraging results and no known adverse effects[9]. Hence, lumbrokinase should be promoted to as a potential treatment in ischemic conditions.

Conventionally nitrates are considered the first line of treatment for angina pectoris. The studied combination therapy of lumbrokinase and isosorbidedinitrate can prevent and treat angina pectoris by dilating the coronary arteries, improving oxygen supply to cardiac muscles, inhibiting platelet activation, inhibiting fibrin and thrombus formation, thus may be beneficial in the prevention and treatment of angina pectoris.

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