Effects of aspirin combined with cilostazol on thromboangiitis obliterans in diabetic patients

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Abstract. The present study explored the effects of aspirin combined with cilostazolin in the treatment of diabetic patients with thromboangiitis obliterans and the effects on the related inflammatory factors. A total of 90 diabetic patients with thromboangiitis obliterans admitted to Weifang People's Hospital from August 2015 to June 2017 were selected and divided into the control group (n=45) and the combination group (n=45). Patients in the control group were given aspirin, and those in the combination group were given aspirin combined with cilostazol. Before treatment and 6 weeks after treatment, the clinical data including ankle-brachial index (ABI), 6-min walk test (6MWT) and test data including serum inflammatory factors interleukin (IL)-8, IL-6 and matrix metalloprotease (MMP)-2 and MMP-9 of the two groups were collected for quantitative and statistical analysis. Compared with those in the control group, the ABI and 6MWT in the combination group could be effectively reduced, and the differences were statistically significant (P<0.05). At the same time, cilostazol combined with aspirin could effectively reduce the levels of serum inflammatory factors MMP-2 and MMP-9 in patients, except for nitric oxide (NO), and the differences were statistically significant (P<0.05). Compared with that before treatment, the control and the combination group can significantly improve the clinical symptoms of the patients, and aspirin combined with cilostazol can effectively improve the clinical curative effect of diabetic patients with thromboangitis obliterans and delay the progression of the disease.

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Introduction

As a non-steroidal anti-inflammatory drug, aspirin blocks the production of thromboxane A2 (TXA2) pathway by arachidonic acid though inhibiting the production of platelet cyclooxygenase-1 (COX-1), thereby inhibiting the function of platelets and reducing the formation of thrombosis (1). However, increasing numer of studies have shown that aspirin alone cannot effectively inhibit platelets and reduce the incidence rate of cardiovascular-related diseases, which is also known as aspirin tolerance (2,3). Its mechanism is unclear and current research suggests that it may be related to a variety of factors such as accelerated platelet renewal, genetic factors, and the non-COX-1 activation pathway (4). Cilostazol, as a quinoline derivative, is used in combination with aspirin to reduce the incidence of cardiovascular and cerebrovascular diseases. It has been reported in most literature that cilostazol can inhibit cyclic adenosine monophosphate (cAMP) degradation and increase cAMP in platelets and vascular endothelial cells. Increased cAMP can reduce the release of adenosine diphosphate (ADP), thereby inhibiting platelet aggregation and preventing thrombosis (5). At the same time, cilostazol can well dilate peripheral artery vessels and relieve vascular obstruction, thereby reducing the incidence rate of atherosclerosis and thromboangiitis obliterans (5). Based on other literature, the clinical effects of cilostazol combined with aspirin on diabetic patients with thromboangiitis obliterans were explored in this study, so as to provide preclinical research data for a more effective cure of patients.

Patients and methods

General data. A total of 90 diabetic patients with thromboangiitis obliterans admitted to Weifang People's Hospital (Weifang, China) from August 2015 to June 2017 were selected and divided into the control (n=45) and the combination group (n=45). The control group included 28 males and 17 females, aged 48-79 years. The combination group included 26 males and 19 females, aged 50-82 years. This study was approved by the Ethics Committee of Weifang People's Hospital. Signed informed consents were obtained from all participants before the study. Inclusion criteria of diabetic patients were in line

Table I. Comparison of the basic data of the two groups of patients.

Items	Groups	
	Control (n=45)	Combination group (n=45)
Sex		
Male	28	26
Female	17	19
Age (years)	56.3±6.8	58.1±4.7
Complication		
Hypertension	14	16
Hyperlipidemia	17	19
Myocardial infarction	7	5
Coronary heart disease	9	11

with the World Health Organization (WHO) standards in 1999. Thromboangiitis obliterans was diagnosed with symptoms such as numbness of the limbs, redness and swelling, and heat pain along the vessel shape and other inflammatory reaction, and color Doppler ultrasound showed vascular lesions. The two groups of patients were comparable in complications, age and sex (P>0.05) (Table I).

Research methods. The two groups of patients were treated with corresponding drugs according to their own complications under the basic treatment of diet and exercise. Patients with hyperglycemia were given oral antidiabetic drugs or subcutaneous injection of insulin. Patients with hypertension were given antihypertensive drugs or intravenous drip diuretics to reduce blood pressure. Patients with hyperlipidemia were given lipid-lowering drugs chosen according to the patient's own condition. Based on the basic treatment, the control group was treated with aspirin at 0.1 g/day, the combination group was treated with 0.1 g aspirin and cilostazol at 0.1 g/day at the same time. After 6 weeks of administration, the clinical data collected from the two groups of patients were analyzed and compared.

Clinical observation indexes. The clinical data of this study were mainly collected by the research nurses who were in charge of this study. Among them, clinical observation indexes included the subjective symptoms of the patients before and after treatment and whether there was pain, such as numbness, redness and swelling, heat pain and other clinical symptoms in the affected limbs of patients with thromboangiitis. Besides, symptom score criteria (6) were adopted for scoring according to the degree of severity described by the patients. It was significantly effective if it declined by >1 point at the end of treatment, effective if it declined by 1 point at the end of treatment, and ineffective if it increased by ≥1 point at the end of the treatment (6). If the affected limbs were lower, the patients were asked if they had intermittent claudication and lame distance, and the results were recorded. Symptom score criteria are shown in Table II. The arterial blood flow, ankle-brachial index (ABI; contractile pressure ratio of ankle artery to brachial artery) and 6 min walk test (6MWT) were detected once a day before and after treatment.

Experimental test indicators. Before treatment and after 6 weeks of treatment, the fasting venous blood was extracted from patients by the research nurses in the early morning, and the specimens were then sent to the Clinical Laboratory to be detected by a specialized laboratory technician. Red blood cell lysate was added into the blood samples to remove red blood cells, the serum was separated after coagulation, the supernatant was taken by centrifugation at 2,500 x g at 4˚C for 10 min, and then the concentrations of various inflammatory factors, such as interleukin (IL)-6, IL-8, matrix metalloprotease (MMP)-2 and MMP-9, were determined by double-antibody sandwich enzyme-linked immunosorbent assay (ELISA). The detection procedures were performed according to the manufacturer's instructions.

Statistical analysis. The data in this study were statistically analyzed by GraphPad Prism 6.0 (GraphPad Software, Inc., La Jolla, CA, USA). The quantitative data were analyzed by t-test, and the data were expressed as mean ± SD. P<0.05 indicates that the difference is statistically significant.

Results

Comparison of clinical scores of the two groups of patients before and after treatment. There was no statistical difference in clinical scores between the two groups of patients before treatment, and the scores were comparable. After treatment, the clinical symptoms such as numbness, intermittent claudication, pain, and sense of coldness or redness and swelling of patients in the combination group were significantly improved compared with those in the control group, and the differences were statistically significant (P=0.01, 0.02, <0.001 and 0.02, respectively) (Table III), indicating that the effects of cilostazol combined with aspirin in improving the clinical symptoms of diabetic patients with thromboangiitis obliterans are better than those of aspirin alone.

Comparison of the changes in ABI and 6MWT between the two groups. There were no significant differences in the ABI and 6MWT of the patients between the control and the combination group before treatment (P>0.05). After treatment, the ABI and 6MWT of the patients in the control and the combination group were significantly improved. Among them, the ABI and 6MWT in the combination were significantly higher than those in the control group (P<0.001 and <0.001, respectively, P<0.05), indicating that cilostazol combined with aspirin has a better effect than aspirin alone (Table IV).

Comparison of the changes in serum inflammatory factors and vasoactive substances between the two groups of patients. Before treatment, there were no significant differences in the levels of serum inflammatory factors IL-8, IL-6, C-reactive protein (CRP) and vasoactive substances nitric oxide (NO) and endothelin-1 (ET-1) of patients between the control and the combination group, and the levels were comparable. After treatment, the levels of serum inflammatory factors IL-6, IL-8

Table II. Symptom score criteria.

Items	0 point	1 point	2 points	3 points	4 points
Intermittent claudication (M)	>450	350-450	250-349	100-249	<100
Numbness	No or slight	Occasional	Tolerable	Intolerable	Intolerant
Keen feel	No	Occasional	Tolerable	Intolerable	Continuous pain
Sense of coldness or red and swollen	No	Occasional	Often	With other inflammatory reactions	Continuous, unbearable

P<0.05 was considered to indicate a statistically significant difference.

Table III. Comparison of clinical scores between the two groups before and after treatment (mean ± SD).

	Intermittent claudication		Numbness		Keen feel		Sense of coldness or red and swollen	
Groups	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Control (n=45)	2.62±0.19	2.09±0.18	2.49±0.19	2.38±0.18	2.77±0.14	2.47±0.16	2.50±0.15	2.29±0.16
Combination (n=45)	2.09±0.19	1.53±0.14	2.67±0.18	1.89±0.11	2.53±0.15	1.73±0.16	2.36±0.18	1.67±0.21
t	1.90	2.51	0.67	2.31	1.20	3.2	0.57	2.34
P-value	0.06	0.01	0.51	0.02	0.23	<0.001	0.57	0.02

P<0.05 was considered to indicate a statistically significant difference.

Table IV. Comparison of the changes in ABI and 6MWT in the two groups of diabetic patients with thromboangiitis obliterans.

	ABI		6MWT (m)	
Groups	Before treatment	After treatment	Before treatment	After treatment
Control (n=45)	0.73±0.05	1.07±0.07	245.1±4.20	284.1±3.34
Combination (n=45)	0.73±0.05	0.91±0.01	244.5±5.51	316.6±5.70
t	0.84	12.62	0.08	4.89
P-value	0.40	<0.001	0.93	<0.001

P<0.05 was considered to indicate a statistically significant difference. ABI, ankle-brachial index; 6MWT, 6-min walk test.

and CRP in the two groups were decreased, while that of vasoactive substance ET-1 was increased, and the differences were statistically significant (data not given). Among them, compared with the control group after treatment, the levels of serum inflammatory factors of the patients in the combination group were decreased, and the differences were statistically significant (P<0.05), indicating that cilostazol combined with aspirin has a better clinical effect than aspirin alone in reducing serum inflammatory factors. The levels of NO in the two groups were increased after treatment, but the difference was not statistically significant (P>0.05) (Table V). The mechanism still needs to be further studied.

Comparison of the changes in MMP levels between the two groups of patients. Compared with that before treatment, the levels of MMP-2 and-9 in the two groups of patients were significantly decreased (P<0.05), and the effects in the combination group were better than those in the control group (Fig. 1).

Pearson's correlation analyses of serum inflammatory factors CRP and MMP-9 with patient's symptom score. There were positive correlations of the patient's symptom score with CRP and MMP-9 (P<0.05) (Fig. 2).

Discussion

Thromboangiitis obliterans often occur independently, and the main manifestation is an occlusive disease caused by arteriole thrombosis in the limbs (6). Its incidence is not related to age, sex and vascular location, and the etiology is not clear at present. It is considered to be an autoimmune disease, which is mainly related to the overexpression of anti-endothelial cell antibodies and the increased expressions of anti-neutrophil antibodies and leukocyte compatible antigens in the body itself (5,7). Immunological studies have suggested that thromboangiitis obliterans is associated with the deposition of

Table V. Comparison of the changes of serum inflammatory factors IL-6, IL-8, CRP, NO and ET-1 between the two groups (mean ± SD).

	IL-8 (μg/l)		IL-6 (pg/ml)		NO (μM/l)		ET-1 (μM/l)		CRP (mg/l)	
Groups	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Control (n=45)	114.1±2.76	106.5±2.12	14.2±0.32	11.6±0.36	102.6±1.06	107.6±1.28	196.7±2.93	177±2.99	7.01±0.17	6.26±0.14
Combination (n=45)	110.9±2.91	93.8±2.29	13.4±0.31	9.58±0.26	100.6±3.52	105.6±2.27	188.9±3.79	140.9±3.56	7.23±0.29	4.17±0.18
t	1.90	4.07	1.83	4.58	0.55	0.55	1.63	7.77	0.37	9.29
P-value	0.06	<0.001	0.07	<0.001	0.58	0.58	0.11	<0.001	0.71	<0.001

P<0.05 was considered to indicate a statistically significant difference. IL, interleukin; CRP, C-reactive protein; NO, nitric oxide; ET-1, endothelin-1.

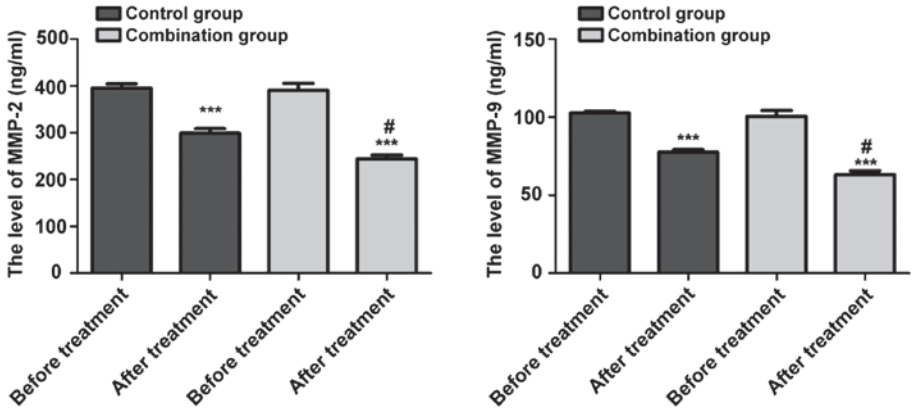

Figure 1. Comparison of the levels of MMP-2 and MMP-9 between the two groups of patients before and after treatment. ***Comparison in the same group before and after treatment, P<0.05; #comparison of the effects after treatment between the control and the combination group, P<0.05. MMP, metalloprotease.

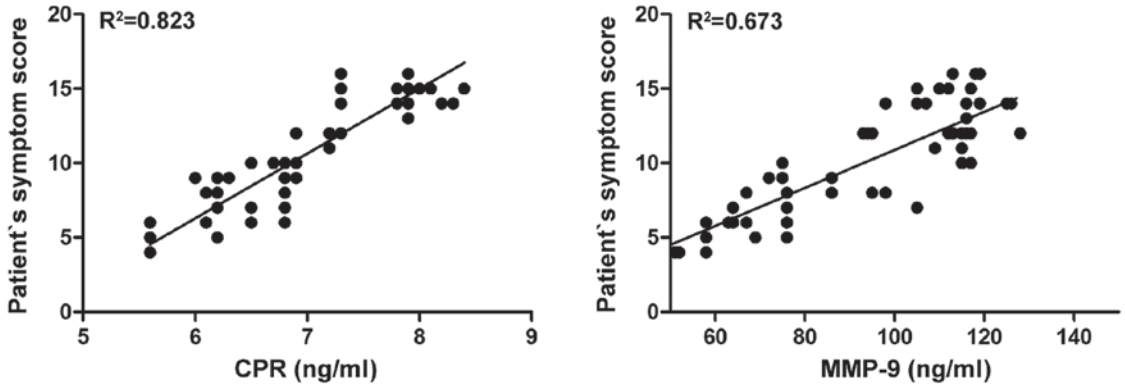

Figure 2. Pearson's correlation analysis of serum inflammatory factors CRP and MMP-9 with patient's symptom score. CRP, C-reactive protein; MMP, metalloprotease.

overactivated cluster of differentiation 3 $(CD3)^+$ T, $CD20^+$ B cells, and immune proteins such as immunoglobulin A (IgA), IgG, and IgM on the internal elastic lamina (8). Diabetes mellitus is mainly classified into type 1 and 2. Type 2 often occurs in obese elderly patients and often accompanied by other complications. Increased plasminogen activator inhibitors and von Willebrand factors in the blood circulation of diabetic patients lead to the increased activity of fibrin protease and the decreased prostaglandin I2 (PGI2), and consequently cause systolic/diastolic dysfunction in the vessel and the increased platelet adhesion and aggregation, thus triggering the occurrence of thrombus (9). In addition, hyperglycemia

in the blood circulation will lead to the increase of terminal glycosylation products, which are easy to bind to specific receptors on the vascular endothelium and further lead to endothelial dysfunction (10). In addition, vascular endothelial cells can synthesize and secrete vasoactive substances such as ET-1 and NO to regulate the systolic/diastolic function of vascular smooth muscle. The combination of terminal glycosylation products and vascular endothelial cells breaks the disorder of endothelial cell function and causes secretion dysfunction of endothelial cells, and the imbalance of NO and ET-1 secretion inhibits the active factors in blood vessel wall and circulating blood to put the circulatory system in a state of hypercoagulability (10), causing vascular occlusive embolism, and myocardial infarction, which pose threats to the life of patients (11,12). Patients with hyperglycemia are often accompanied by increased stress, endothelial dysfunction and high expression of IL, which are caused by insulin resistance (13-15).

Aspirin is often used as an antiplatelet agent in clinical practice to treat acute myocardial infarction and vascular embolism (16). However, aspirin will also produce clinical resistance, and excessive consumption will lead to gastrointestinal hemorrhage, so its application has some limitations (17,18). Cilostazol, as a phosphodiesterase inhibitor, can increase the level of adenosine monophosphate and inhibit the activity of phosphodiesterase in platelets and smooth muscle endothelial cells, which in turn can counteract platelet aggregation (19,20). This study aimed to explore the clinical advantages of cilostazol combined with aspirin and provide a certain data support for clinical application. In this study, compared with the control group, cilostazol combined with aspirin could reduce the duration of intermittent claudication and the patient's clinical symptoms such as redness and numbness. At the same time, in laboratory data, cilostazol combined with aspirin can decrease the serum levels of IL-8, IL-6 and MM-9 and other inflammatory response factors in patients. These data revealed that cilostazol combined with aspirin has a better clinical effect than aspirin alone in the treatment of diabetic patients with thromboangiitis obliterans, which is worth of further clinical confirmation and promotion. However, cilostazol combined with aspirin does not seem to have a significant effect on the optimization of NO level, which remains to be further verified.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

JY and QZ designed the study and performed the experiments, JY, SZ, YG and WG collected the data, SZ and PS analyzed the data, JY and QZ prepared the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Weifang People's Hospital (Weifang, China). Signed informed consents were obtained from all participants before the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare they have no competing interests.

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