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Assessment of coagulation function and ultrasound features after reteplase and recombinant streptokinase thrombolysis of lower extremity deep venous thrombosis

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ABSTRACT

Objective: To assess coagulation function and ultrasound features after reteplase and recombinant streptokinase thrombolysis of lower extremity deep venous thrombosis. **Methods:** A total of 78 cases of patients with lower extremity deep venous thrombosis who were treated in our hospital were selected as research subjects and divided into observation group 39 cases and control group 39 cases according to different treatment regimens. Control group received recombinant streptokinase thrombolysis, observation group received reteplase thrombolysis, and then the effect of the two thrombolytic ways was evaluated by color Doppler ultrasonography and circulating blood test. **Results:** Ultrasound showed that complete recanalization rate of thrombus of observation group after treatment was higher than that of control group; plasma PT, APTT and TT values of observation group after thrombolysis were higher than those of control group, FIB, D-D, NO, ET, E-selectin, P-selectin, Hcy, CRP, IL-6, IL-8 and TNF-α values were lower than those of control group, and WBC, Nc and Mc cell number were less than those of control group. **Conclusion:** Reteplase for thrombolysis of lower extremity deep venous thrombosis has more distinguished effect on dissolving thrombus as well as optimizing body's coagulation, inflammatory system state and other aspects, and is a more ideal thrombolytic drug.

1. Introduction

Lower extremity deep venous thrombosis (DVT)is clinically more common, emboli fall off, move with blood circulation and finally embolize important viscera, pulmonary embolism is one of the most serious outcomes of DVT, and the mortality rate is extremely high. Patients clinically diagnosed with lower extremity DVT should select early intravenous thrombolysis if they do not receive surgical treatment[1]. Both reteplase and recombinant streptokinase are currently widely applied thrombolytic drugs, reteplase is the recombinant tissue-type plasminogen deletion mutant, it is mainly used for the treatment of myocardial infarction, and its application in the treatment of peripheral vascular obstructive disease is not for long. Recombinant streptokinase is more applied in DVT, it can activate plasminogen, catalyze fibrous protein hydrolysis and play to the role of thrombolysis and recanalization, but many reports have shown that at the same time of dissolving thrombus, recombinant streptokinase can lead to more hemorrhagic diseases, and the thrombolysis effect is unstable[2,3]. In the research, coagulation function and ultrasound features were mainly assessed after reteplase and recombinant streptokinase thrombolysis for lower extremity deep venous thrombosis, 78 cases of patients with lower extremity deep venous thrombosis who received treatment in our hospital from April 2013 to December 2015 were selected as research subjects, and the specific report was as follows.

2. Research subjects and methods

2.1 Subjects

A total of 78 cases of patients with lower extremity deep venous thrombosis were divided into observation group 39 cases and control group 39 cases according to different treatment regimens. Control group included 18 male cases and 21 female cases, they were 23-61

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years old, the average was (42.84±8.15) years, the course of disease was 2-11 d, the average was (5.39±0.76) d, 24 cases involved left lower extremity, 11 cases involved right lower extremity, and 4 cases involved both lower extremities; observation group included 17 male cases and 22 female cases, they were 21-63 years old, the average was (41.76±8.63) years, the course of disease was 3-12 d, the average was (5.76±0.71) d, 23 cases involved left lower extremity, 11 cases involved right lower extremity, and 5 cases involved both lower extremities. The research was approved by the hospital ethics committee, differences in baseline information were not significant between two groups, $P>0.05$ and they could be further compared.

2.2 Clinical features and diagnosis

All included patients had obvious affected-side lower extremity swelling and pain, superficial vein distention, high skin temperature and features, and those with past varicosis of great saphenous vein were locally manifested as mass scleroma. Diameter in 15 cm above affected-side superior border of patella was 3-10 cm larger than that of the normal side, and diameter in 15 cm below lower segment of patella was 2-7 cm larger than that of the normal side. Extremity vein anterograde angiography was conducted to clear clinical diagnosis.

2.3 Treatment methods

Control group were treated with recombinant streptokinase, specifically as follows: recombinant streptokinase 500 000 U/d was dissolved in 5% glucose solution for intravenous drip that was finished within 8 h, for consecutive 7 d. Observation group of patients accepted reteplase thrombolysis treatment, specifically as follows: reteplase 10 U was dissolved in 250 mL saline for intravenous injection. A tourniquet was used to block affected-ankle superficial vein, reteplase was infused from the affected-side dorsal vein and finished within 3 h, for consecutive 2 d.

2.4 Ultrasonography

Lower-extremity color Doppler ultrasonography adopted GE Logiq9 and Acuson 128XP/10, Sequoia ultrasonic diagnostic instrument for detection of lower extremity vein thrombus. The patients took supine position with the thighs abducted and externally rotated and the knees flexed, and the position of femoral vein below the groin was determined, scanning up for external iliac vein and scanning down for deep femoral superficial vein. Patients were told to take prone position for popliteal, posterior tibial and fibular veins inspection, a pillow was put under the ankle, and knee was slightly flexed and relaxed, avoiding spontaneous collapse of popliteal vein.

2.5 Detection indexes

Blood coagulation function: after two groups of patients received a course of treatment, 2 mL of fasting peripheral venous blood was collected in the morning, anticoagulated with sodium citrate and centrifuged for 15 min with 3 000 r/min to get plasma, and coagulation function indexes were detected within 2 h, specifically including prothrombin time (PT), activated partial thromboplastin time (APTT), plasma thrombin time (TT), plasma fibrinogen (FIB) content and D-dimer (D-D).

Thrombosis-related factors: 2 mL of fasting peripheral venous blood was collected from patients after treatment during the same period, nitrate reduction colorimetric method was used to determine nitric oxide (NO) level, immunoradiometric assay was used to determine levels of endothelin (ET), and enzyme-linked immunosorbent assay was used to determine E-selectin, P-selectin and homocysteine (Hcy) levels.

Inflammatory factors and inflammatory cells: peripheral venous blood was collected from patients during the same period, enzyme-linked immunosorbent assay was used to determine C-reactive protein (CRP), interleukin-6 (IL-6), interleukin-8 (IL-8) and tumor necrosis factor- α (TNF- α) levels, and the number of inflammatory cells was further determined, including white blood cells (WBC), neutrophil (Nc), mononuclear cells (Mc), etc.

2.6 Statistical methods

SPSS 23.0 software was used to statistically analyze the data in the research, measurement data was in terms of Mean ± SD, comparison between two groups was by t test, count data comparison was by *chi*-square test, and $P<0.05$ was set as the standard of statistical significance in differences.

3. Results

3.1 Color Doppler ultrasongraphy results

After observation group received treatment, 30 cases (76.92%) were manifested as endovenous thrombus disappearance as well as full and unobstructed blood flow signal; 9 cases (23.08%) were manifested as endovenous thrombus length reduction by more than 50%. After control group received treatment, 21 cases (53.85%) were manifested as endovenous thrombus disappearance as well as full and unobstructed blood flow signal; 13 cases (33.33%) were manifested as endovenous thrombus length reduction by more than 50%. 5 cases (12.82%) were without significantly changed blood flow signal before and after thrombolysis.

3.2 Blood coagulation function

Hypercoagulable state caused by different factors is the main cause of lower extremity deep vein thrombosis in patients, antagonism of hypercoagulable state and targeted thrombolysis is the main principle of current treatment of lower extremity deep vein thrombosis, and after two groups of patients with lower extremity deep venous thrombosis received different thrombolysis regimens in the study, the changes in peripheral blood coagulation function were as follows: plasma PT, APTT and TT values of observation group after treatment were higher than those of control group while FIB and D-D values were lower than those of control group ($P<0.05$), shown in Table 1.

Table 1.
Comparison of plasma coagulation function index values between two groups after one course of treatment.

Groups	PT (s)	APTT (s)	TT (s)	FIB (g/L)	D-D (ng/mL)
Observation	12.37±0.58	32.54±3.79	15.78±1.84	2.97±0.34	214.28±35.49
Control	11.02±0.53	29.37±3.13	14.12±1.32	3.31±0.48	371.03±45.82
t	5.293	6.281	5.893	5.823	11.293
P	<0.05	<0.05	<0.05	<0.05	<0.05

3.3 Thrombosis–related factors

In lower extremity deep venous thrombosis process, hypercoagulable state promotes the eventual thrombus generation, and in the gradual formation process of thrombus, nitric oxide (NO), endothelin (ET), E-selectin, P-selectin and homocysteine (Hcy) are involved and become one of the core factors that affect the thrombotic state. In the research, detection of the levels of above factors in circulating blood of two groups after treatment showed that NO, ET, E-selectin, P-selectin and Hcy values in circulating blood of observation group after treatment were lower than those of control group (P<0.05), shown in Table 2.

Table 2.
Thrombosis-related factor levels in circulating blood of two groups after treatment.

Groups	NO (μmol/L)	ET (ng/L)	E-selectin (μg/L)	P-selectin (ng/mL)	Hcy (μmol/L)
Observation	63.28±7.12	35.72±4.11	21.28±3.04	22.38±3.05	10.09±1.76
Control	91.56±9.43	52.38±6.39	38.76±4.51	33.07±4.26	14.28±1.94
t	9.382	7.283	7.021	6.482	6.832
P	<0.05	<0.05	<0.05	<0.05	<0.05

2.4 Inflammatory factor and inflammatory cell levels

Inflammatory process is involved in thrombosis and eventual embolism events, and detecting the levels of inflammatory factors and inflammatory cells can directly reflect the body's inflammatory state and indirectly reflect the degree of thrombolysis. In the study, it was found after two groups received different thrombolysis that inflammatory factors such as CRP, IL-6, IL-8 and TNF-α values in circulating blood of observation group were lower than those of control group, and the number of inflammatory cells such as WBC, Nc and Mc were less than those of control group (P<0.05), shown in Table 3.

4. Discussion

Lower extremity deep venous thrombosis (DVT) is one of the most common clinical peripheral vascular diseases, the incidence of cardiovascular diseases ranks third in the United States, and about 0.1%-0.5% of the DVT patients die from secondary pulmonary embolism each year. The occurrence of lower extremity DVT is closely related to tumor, long-tem bed rest for surgery and so on, patients with mild disease are with hidden onset and self-limiting course of disease, and the currently clinically diagnosed are mostly critical patients[4,5]. In view of the serious consequences of lower extremity DVT, positive treatment measures must be taken after clinical diagnosis, among which thrombolysis therapy is the main way of treatment. Reteplase belongs to the third generation of thrombolytic drug wtih longer half-life (around 15 min), and because its affinity to fibrous protein on thrombus surface is not strong, it can make the drug diffuse to blood clot and play a better role of thrombolysis. Reteplase is not generally combined with the plasminogen in circulation, so it will not cause systemic fibrinolytic state, and has good clinical application security[6]. Recombinant streptokinase is the highly purified gene product synthesized in non-pathogenic Escherichia coli with genetic engineering method, and can activate plasminogen into plasmin and catalyze fibrous protein levels and thrombolysis. Thrombolysis effect of recombinant streptokinase is enhanced with the existence of fibrinogen, and therefore, it can be used for the diseases that are with thrombosis as the main pathological changes.

Recombinant streptokinase is more applied in patients with lower extremity DVT and can clearly achieve thrombolytic effect, but many cases have reported that the thrombolytic method can lead to sharply increased incidence rate of hemorrhagic events in patients[7,8]. Reteplase has been successfully applied in the treatment of patients with acute myocardial infarction, but is less reported in treatment of lower extremity DVT. In order to find the most reasonable thrombolytic means for lower extremity DVT, reteplase and recombinant streptokinase thrombolysis were applied respectively in patients with lower extremity DVT who received treatment during the same period in our hospital in the research, and were specifically studied from ultrasound results, blood coagulation function, inflammatory factor levels and other aspects[9]. Venography is the gold standard in the diagnosis of DVT and the determination of the illness severity, but its invasiveness and contrast allergy restrict the clinical application and popularization, and color Doppler ultrasongraphy becomes the currently most common noninvasive method for DVT. Color Doppler ultrasound can clearly show the venous anatomic structure and hemodynamic characteristics, two groups of patients received ultrasongraphy after treatment in the research, and the results showed that complete recanalization rate of thrombus of observation group was higher after treatment, indicating that reteplase was with better thrombolytic effect, and could effectively dissolve venous emboli and restore blood supply, avoiding embolism events in other important organs and necrotizing events of lower extremities.

Coagulant dysfunction and the resulting hypercoagulable state are the direct causes of the lower extremity DVT, thrombolytic therapy dissolves emboli and also affects systemic coagulation function in patients, and real-time detection of prothrombin time (PT), activated partial thromboplastin time (APTT), plasma thrombin time (TT), plasma fibrinogen (FIB) content, D-dimer (D-D) and other blood coagulation function-related parameters can directly reflect thrombolytic effect[10,11]. The research results showed that PT, APTT and TT values in circulating blood of observation group were higher after thrombolysis while FIB and D-D values were lower, indicating that reteplase could significantly reverse the

Table 3.
Comparison of inflammatory factor levels and inflammatory cell number in circulating blood of two groups after treatment.

Groups	Inflammatory factors			Inflammatory cells			
	CRP (mg/L)	IL-6 (ng/L)	IL-8 (μg/L)	TNF-α (μg/L)	WBC ($\times10^9$/L)	Nc ($\times10^9$/L)	Mc ($\times10^9$/L)
Observation	6.39±0.75	112.83±15.39	0.23±0.04	1.34±0.18	5.48±0.83	3.82±0.53	1.42±0.23
Control	9.47±0.89	139.26±15.39	0.39±0.05	1.59±0.21	7.12±0.95	5.29±0.62	1.85±0.28
t	7.123	9.392	5.182	5.893	6.721	6.124	5.893
P	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

hypercoagulable state in the body, dissolve the thrombus, extend related clotting time and reduce the production of coagulation-promoting factors. Current study shows that a variety of cytokines are involved in venous thrombosis, and their levels can also indirectly reflect the thrombolytic effect[12]. Endothelin (ET) is a vasoconstrictive active substance, and local vascular injury can cause stress and ET release by cells. There may be the increase of local ET levels in plasma and infarct location after vascular injury, thrombosis or infarction. Nitric oxide (NO) is an endothelial-derived relaxing factor that can be synthesized and released by vascular endothelial cells, macrophages, etc., in the process of ischemia-reperfusion injury. E-selectin is mainly expressed on cell membrane surface, and research has confirmed that its level is positively correlated with DVT severity. P-selectin is a molecular marker of early thrombosis, which mediates and participates in platelet/endothelial cell adhesion and inflammatory state of vessel wall. Some scholars put forward that high homocysteine (Hcy) is an independent risk factor for venous thrombosis diseases, and it was also found in many thrombosis diseases that Hcy expression increases in the circulating blood[13]. In this study, detection of the levels of above thrombosis-related factors showed that NO, ET, E-selectin, P-selectin and Hcy values in circulating blood of observation group were lower after thrombolysis, indicating that reteplase thrombolysis might block thrombosis-related processes, exert complete thrombolytic activity and prevent the recurrence of thrombosis.

When veins are injured or with internal environment disorder, they will show the early period of thrombus, produce many tissue factors, and cause gradual increase of venous endothelial permeability and leukocyte adhesion to each other. Many researches believe that inflammatory factors are closely related to thrombosis, and in the process of thrombosis, inflammatory cells can secrete a variety of inflammatory factors and act on intrinsic and extrinsic coagulation system, further inducing the expression of tissue factors and activating blood coagulation pathway[14,15]. C-reactive protein is the first discovered protein that is closely associated with inflammation, has high pro-coagulation activity and can accelerate intima injury and promote thrombosis. Interleukin-6 (IL-6), interleukin-8 (IL-8) and tumor necrosis factor- α (TNF- α) are currently the most commonly studied thrombosis-related pro-inflammatory factors, their levels increase in patients with DVT and they can activate blood coagulation system and promote adhesion molecule and platelet release. Inflammatory cell number can macroscopically reflect the body's inflammatory state, the inflammatory cells with the most clinical reference value include white blood cells (WBC), neutrophil (Nc) and mononuclear cells (Mc), and their levels are positively correlated with the body′s systemic inflammatory state[16,17]. In the study, the inflammatory state of two groups were analyzed after treatment, and the results showed that CRP, IL-6, IL-8, TNF- α, WBC, Nc and Mc values of observation group were lower after treatment, indicating that in the process of thrombolysis treatment, reteplase decreased patients' systemic inflammatory state, and further promoted the realization of thrombolytic effect.

To sum up, it is concluded as follows: reteplase for thrombolysis of lower extremity deep venous thrombosis has more distinguished effect on dissolving thrombus as well as optimizing body′s coagulation, inflammatory system state and other aspects, is a more ideal thrombolytic drug, and is worth popularization and application in clinical practice in the future.

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