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Effect of alteplase thrombolysis sequenced by low molecular heparin calcium antithrombosis on the neurological function and serum cytokines in patients with cerebral infarction

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ABSTRACT

Objective: To study the effect of alteplase thrombolysis sequenced by low molecular heparin calcium antithrombosis on the neurological function and serum cytokines in patients with cerebral infarction. **Methods:** Patients with acute cerebral infarction who received alteplase thrombolysis in Zigong Fourth People's Hospital between June 2014 and October 2016 were retrospectively analyzed and divided into the intervention group who received low molecular heparin calcium treatment and the control group who did not receive low molecular heparin calcium treatment. The serum was collected before and after treatment to determine the contents of platelet activation factors, nerve injury molecules, soluble apoptotic molecules and growth factors. **Results:** Serum CD62p, CD63, PAF, GMP-140, NSE, S100B, GFAP, sFas, sFasL, sTRAIL, IGF-1, VEGF, BDNF and bFGF levels of both groups of patients after treatment were lower than those before treatment, serum CD62p, CD63, PAF, GMP-140, NSE, S100B, GFAP, sFas, sFasL and sTRAIL levels of intervention group after treatment were lower than those of control group while IGF-1, VEGF, BDNF and bFGF levels were higher than those of control group. **Conclusion:** Alteplase thrombolysis sequenced by low molecular heparin calcium antithrombosis for acute cerebral infarction can inhibit platelet activation and cell apoptosis, alleviate nerve injury and improve neurotrophy status.

1. Introduction

Acute cerebral infarction is a clinical common type of stroke, atherosclerosis and thrombosis are the basic pathological characteristics of the disease, and early recanalization of intracranial artery and making ischemic brain tissue obtain blood reperfusion is the key to improve the prognosis[1,2]. Thrombolytic therapy is a common reperfusion treatment for acute cerebral infarction, and recombinant tissue-type plasminogen activator (rt-Pa) alteplase is the second generation of thrombolytic drug that can the specifically activate plasminogen, dissolve thrombus and restore brain tissue perfusion[3]. However, the broken emboli after thrombolytic therapy can move to the distal blood vessels with blood flow and increase the early reocclusion of the blood vessels. Therefore, anticoagulant or antiplatelet agents are required after thrombolytic therapy. Low molecular heparin is the drug with strong antithrombotic effects, low molecular heparin therapy was provided after alteplase thrombolysis in the study, and the effect of alteplase thrombolysis sequenced by low molecular heparin calcium antithrombosis on the neurological function and serum cytokines in patients with cerebral infarction was analyzed specifically.

2.Subjects and methods

2.1 Case information

Patients with acute cerebral infarction who received alteplase thrombolysis in Zigong Fourth People's Hospital between June 2014 and October 2016 were selected as the research subjects, all patients were with cerebral infarction for the first time and received thrombolytic therapy within 4.5 h, and the patients associated with

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cerebral hemorrhage and cerebral hernia were excluded. A total of 117 cases were included, the case data were retrospectively analyzed, and according to the use of low molecular heparin calcium after thrombolytic therapy, the patients were divided into the intervention group and the control group. Intervention group (n=48) were treated with low molecular heparin calcium combined with alteplase, including 28 male cases and 20 female cases that were 53-69 years old; control group (n=69) were treated with alteplase, including 44 male cases and 25 female cases that were 51-68 years old. There was no significant difference between the two groups of patients (P>0.05).

2.2 Therapy

The thrombolytic therapy of two groups of patients was as follows: 0.7-0.9 mg/kg was referred to calculate the alteplase dosage, the highest dose should not exceed 90 mg, saline was used to configure 1 mg/mL alteplase solution, 10% was by intravenous injection, and the rest 90% was by intravenous micro-pump injection within 1 h. Control group received aspirin combined with clopidogrel double-antibody therapy 24 h after thrombolysis. Intervention group were given continuous intravenous pumping of low molecular heparin calcium 500-1 000 U/h immediately after thrombolysis treatment, APTT was detected and maintained at 1.5 to 2.5 times of the baseline, and the treatment lasted for 3 d and was then replaced by aspirin combined with clopidogrel double-antibody therapy.

2.3 Serum index detection methods

Before treatment and 3 d after treatment, 5 mL of cubital venous blood was collected from two groups of patients, let stand at room temperature for coagulation and then centrifuged at 3 000 r/min for 10 min to separate supernatant, and the enzyme-linked immunosorbent assay kits were used to determine the levels of CD62p, CD63, PAF, GMP-140, NSE, S100B, GFAP, sFas, sFasL, sTRAIL, IGF-1, VEGF, BDNF and bFGF.

2.4 Statistical methods

SPSS 20.0 software was used to input and analyze data, the differences in serum indexes between two groups were analyzed by t test and P<0.05 indicated statistical significance in differences.

3.Results

3.1 Serum platelet activation factor levels

Before treatment and 3 d after treatment, analysis of serum platelet activation factors CD62p (pg/mL), CD63 (pg/mL), PAF (ng/mL) and GMP-140 (ng/mL) levels between two groups of patients was as follows: serum CD62p, CD63, PAF and GMP-140 levels were not significantly different between two groups of patients before treatment (P>0.05), serum CD62p, CD63, PAF and GMP-140 levels of both groups of patients after treatment were lower than those before treatment (P<0.05), and serum CD62p, CD63, PAF and GMP-140 levels of intervention group after treatment were lower than those of control group (P<0.05).

3.2 Serum nerve injury molecule levels

Before treatment and 3 d after treatment, analysis of serum nerve injury molecules NSE (pg/L), S100B (pg/L) and GFAP (ng/L) levels between two groups of patients was as follows: serum NSE, S100B and GFAP levels were not significantly different between two groups of patients before treatment (P>0.05), serum NSE, S100B and GFAP levels of both groups of patients after treatment were lower than those before treatment (P<0.05), and serum NSE, S100B and GFAP levels of intervention group after treatment were lower than those of control group (P<0.05).

Table 1.

Serum platelet activation factor levels in two groups of patients before and after treatment.

Groups	n	Time	CD62p	CD63	PFA	GMP-140
Intervention group	48	Before treatment	9.39±1.15	7.41±0.89	26.48±3.72	50.94±8.75
		3 d after treatment	3.25±0.45$^{*\&}$	2.28±0.36$^{*\&}$	10.21±1.52$^{*\&}$	26.68±3.52$^{*\&}$
Control group	69	Before treatment	9.52±1.07	7.62±0.93	26.13±3.47	51.25±7.92
		3 d after treatment	5.86±0.78*	4.74±0.74*	17.98±2.56*	35.41±5.42*

*: comparison within group before and after treatment, P<0.05; $^{\&}$: comparison between intervention group and control group after treatment, P<0.05.

Table 2.

Serum nerve injury molecule levels in two groups of patients before and after treatment.

Groups	n	Time	NSE	S100B	GFAP
Intervention group	48	Before treatment	22.58±3.85	1.89±0.25	35.58±5.48
		3 d after treatment	9.24±1.18$^{*\&}$	0.77±0.09$^{*\&}$	13.25±1.85$^{*\&}$
Control group	69	Before treatment	23.03±3.47	1.93±0.23	36.02±5.81
		3 d after treatment	14.52±1.98*	1.25±0.18*	19.39±2.42*

*: comparison within group before and after treatment, P<0.05; $^{\&}$: comparison between intervention group and control group after treatment, P<0.05.

3.3 Serum soluble apoptotic molecule levels

Before treatment and 3 d after treatment, analysis of serum soluble apoptotic molecules levels between two groups of patients was as follows: serum sFas, sFasL and sTRAIL levels were not significantly different between two groups of patients before treatment ($P>0.05$), serum sFas, sFasL and sTRAIL levels of both groups of patients after treatment were lower than those before treatment ($P<0.05$), and serum sFas, sFasL and sTRAIL levels of intervention group after treatment were lower than those of control group ($P<0.05$).

3.4 Serum growth factor levels

Before treatment and 3 d after treatment, analysis of serum growth factors IGF-1 (μmol/L), VEGF (pg/mL), BDNF (ng/mL) and bFGF (pg/mL) levels between two groups of patients was as follows: serum IGF-1, VEGF, BDNF and bFGF levels were not significantly different between two groups of patients before treatment ($P>0.05$), serum IGF-1, VEGF, BDNF and bFGF levels of both groups of patients after treatment were lower than those before treatment ($P<0.05$), and serum IGF-1, VEGF, BDNF and bFGF levels of intervention group after treatment were higher than those of control group ($P<0.05$).

4. Discussion

Alteplase is a new thrombolytic drug, thrombolytic effect is exact for thrombolytic treatment of acute cerebral infarction, but intracranial arterial thrombi can form new emboli after thrombolysis, which will move to the distal vessels along with blood circulation to cause luminal stenosis and even re-occlusion, activate platelets and for thrombi again in local area[4,5]. Re-occlusion after thrombolysis can seriously affect the blood perfusion of the brain tissue, so antithrombolytic therapy is needed after the thrombolytic therapy[6,7]. Low molecular heparin calcium is the most widely used clinical anti-thrombosis drug, and low molecular heparin calcium was provided immediately after thrombolysis in this study to achieve the goal of resisting thrombosis and preventing vascular re-occlusion. The activation of platelets is a key pathological link causing vascular reocclusion after thrombolysis, and CD62p, CD63, PAF, GMP-140 and other molecules play an important role in the activation of platelets[8]. CD62p and CD63 are the glycoproteins in the platelets, which can be shifted and shed during platelet activation, and become the markers to reflect the activation of the platelets[9]; PAF is the most powerful platelet activating-stimulating factor, which can significantly induce platelet activation by combining the PAF receptor on the platelet surface[10]; GMP-140 can also mediate the damage of endothelium and the activation of platelets[11]. In the study, analysis of the serum levels of platelet activating factors showed that serum CD62p, CD63, PAF and GMP-140 levels of intervention group were significantly lower than those of control group. It means that low molecular heparin calcium therapy after alteplase thrombolysis can significantly inhibit the platelet activation and prevent the vascular re-occlusion.

The value of thrombolysis for patients with acute cerebral infarction is to allow the ischemic brain tissue to get a timely blood perfusion and reduce the ischemia hypoxia damage to the brain tissue. Providing low molecular heparin calcium immediately after alteplase therapy can prevent vascular re-occlusion through the antithrombotic effects of heparin, which helps alleviate neural function injury and promote neural functional recovery. During ischemic hypoxic injury of brain tissue, a variety of metabolic enzymes and functional proteins in neurons and glial cells will be released from the broken cells to the outside, which enter blood circulation and become the marker molecules to reflect neural function damage. The NSE is a molecule located in the neuron, which is involved in regulating glycolysis and provides energy for neuron metabolism; S100B and GFAP are the molecules located in glial cells and they are mainly involved in the regulation of cytoskeleton morphology and calcium ion homeostasis in cells[12,13]. In the study, analysis of the changes in serum nerve function injury marker molecules before and after acute cerebral infarction treatment showed that serum NSE, S100B and GFAP levels of both groups of patients decreased significantly after treatment, and serum NSE, S100B and GFAP of intervention group after treatment were significantly lower than those of control group. This means that alteplase thrombolysis can reduce neural function

Table 3.

Serum soluble apoptotic molecule levels in two groups of patients before and after treatment (pg/mL).

Groups	n	Time	sFas	sFasL	sTRAIL
Intervention group	48	Before treatment	127.64±22.32	178.59±23.10	75.59±9.35
		3 d after treatment	53.59±8.65*&	74.48±9.35*&	32.47±5.51*&
Control group	69	Before treatment	128.03±15.62	180.11±21.38	77.12±9.83
		3 d after treatment	89.42±11.25*	124.49±17.85*	47.58±6.72*

*: comparison within group before and after treatment, $P<0.05$; &: comparison between intervention group and control group after treatment, $P<0.05$.

Table 4.

Serum growth factor levels in two groups of patients before and after treatment.

Groups	n	Time	IGF-1	VEGF	BDNF	bFGF
Intervention group	48	Before treatment	103.52±15.62	315.96±42.62	2.95±0.44	22.31±3.52
		3 d after treatment	83.21±9.35*&	267.54±33.49*&	2.31±0.37*&	17.79±2.44*&
Control group	69	Before treatment	105.12±14.48	312.49±45.68	3.02±0.47	22.92±3.77
		3 d after treatment	62.38±9.61*	198.32±22.52*	1.77±0.22*	11.32±1.96*

*: comparison within group before and after treatment, $P<0.05$; &: comparison between intervention group and control group after treatment, $P<0.05$.

damage caused by ischemia hypoxia, and the combination of low molecular heparin can further reduce the nerve function injury caused by re-occlusion.

During brain tissue injury caused by ischemia hypoxia, the lesion of neurons and glial cells in ischemic lesions is closely related to the activation of apoptosis. Fas/FasL is the important pathway to regulate neuron and glial cell apoptosis, it is significantly activated in the process of cerebral infarction, splits into soluble form and enters into the blood circulation[14]; sTRAIL is a newly discovered apoptotic inducer, and the homotrimer formed by it is with extremely strong apoptotic activity[15]. In the study, analysis of serum levels of above soluble apoptotic molecules showed that serum sFas, sFasL and sTRAIL levels of intervention group after treatment were significantly lower than those of control group. This suggests that low molecular heparin calcium therapy after alteplase thrombolysis can significantly inhibit the apoptosis caused by ischemia. Excessive apoptosis in cerebral infarction lesions will not only directly cause cellular damage, but can also affect the endocrine function of neurons and glial cells, reduce the secretion of the IGF-1, VEGF, BDNF, bFGF and other neurotrophy-related cytokines and indirectly cause cellular damage. IGF-1 and bFGF are the cytokines with extensive growth promotion activity, and they can promote the regeneration of neurons, glial and vascular endothelium; VEGF is a cytokine that promotes angiogenesis, which can promote the establishment of collateral circulation within cerebral infarction[16]; BDNF is a necessary cytokine for neuronal regeneration and synaptic repair, which can directly facilitate the repair of nerve function[17,18]. In the study, analysis of serum levels of these cytokines showed that serum IGF-1, VEGF, BDNF and bFGF levels of intervention group after treatment were significantly higher than those of control group. This means that low molecular heparin calcium therapy after alteplase thrombolysis can significantly improve the nutritional status of neurological function after cerebral infarction, and is beneficial to the recovery of neural function.

Immediate low molecular heparin calcium therapy after alteplase thrombolysis for acute cerebral infarction can inhibit platelet activation and apoptosis, also significantly reduce neural function damage and improve neural nutritional status.

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