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Changes in coagulation and tissue plasminogen activator after the treatment of cerebral infarction with lumbrokinase

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Abstract. This paper aimed to investigate the effect of lumbrokinase on the anticoagulation and fibrinolysis in treating cerebral infarction. Lumbrokinase was used in patients with cerebral infarction. Patients were randomly divided into treatment group ($n = 31$) and control group ($n = 20$). Single blind method was used in this investigation. The Chinese stroke score was used to evaluate the results of treatment before and after administration of lumbrokinase. Kaolin partial thromboplastin time (KPTT), prothrombin time (PT), fibrinogen content, vWF content were analyzed, and tissue plasminogen activator (t-PA) activity, plasminogen activator inhibitor (PAI) activity, D-dimer level were assayed. In both groups, the stroke score decreased after administration, but in the treatment group, it was more obvious. In the treatment group, KPTT was prolonged, t-PA activity and D-dimer level increased, while the content of fibrinogen decreased significantly. There were no significant changes of PT and PAI activity in both groups. It is concluded that lumbrokinase is beneficial to the treatment of cerebral infarction. The effect of lumbrokinase is related to the inhibition of intrinsic coagulation pathway and the activation of fibrinolysis via an increase of t-PA activity.

1. Introduction

Lumbrokinase is an effective enzyme extracted by a method modified from Mihara in 1983 from a Chinese traditional herb. This enzyme is heat-stable and displays a very broad optimal pH range. It has six fractions and acts as plasminogen activator. It dissolves fibrin clot by converting plasminogen to plasmin [1]. Nowadays, lumbrokinase is widely used for cerebral vascular diseases. To investigate the changes of anticoagulation and fibrinolysis in the treatment of cerebral infarction with lumbrokinase, we randomly divided 51 patients into treatment group ($n = 31$) and control group ($n = 20$). The capsule was administered orally to the treatment group three times before meals everyday for 28 days. The lumbrokinase capsule was provided by Shuanglong Pharmaceutical Company, Qingdao, China. Each capsule contains 400 mg lumbrokinase. The dosage was 2 capsules. The placebo capsule used in control group was made of the same matrix as lumbrokinase capsule and appeared in the same color, shape and package. Blood samples were taken before treatment and at 1st, 2nd, 3rd, 4th week after administration. The neurological status was evaluated and kaolin partial thromboplastin time (KPTT), prothrombin time (PT), fibrinogen content (FIB), tissue plasminogen activator (t-PA) activity, plasminogen activator inhibitor (PAI) activity, D-dimer level was assayed.

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Table 1
Baseline demographic and clinical characteristics of patients

	Treatment group ($n = 31$)	Control group ($n = 20$)	p
Onset before entry (day)	3.0 ± 1.5	2.8 ± 1.4	$p > 0.05$
Age	66.9 ± 8.3	65.5 ± 9.8	$p > 0.05$
(range)	(47∼83)	(48∼88)	
Sex (male)	15 (48.4%)	10 (50%)	$p > 0.05$
Hypertension	22 (70.9%)	13 (65%)	$p > 0.05$
Diabetes mellitus	10 (35.5%)	8 (40%)	$p > 0.05$
Cardiac disorders	7 (22.6%)	5 (25%)	$p > 0.05$
Hyperlipidemia	10 (32.2%)	6 (30%)	$p > 0.05$
Recurrent transient ischemic attack	2 (6.4%)	1 (5%)	$p > 0.05$

2. Patients and methods

The diagnosis of acute cerebral infarction was based on the criteria that was determined in the second Chinese cerebral vascular disease conference [2]. Computed tomographic head scans were used to exclude patients with intracerebral hemorrhage, tumor or other mass lesion. 51 patients meeting entry criteria were randomly divided into treatment group ($n = 31$) and control group ($n = 20$).

The patients in the treatment and placebo groups did not differ for age, sex, past history, the time onset before entry (Table 1). The neurological status was evaluated according to the Chinese stroke scale which expresses the severity of neurological impairment numerically from 0 to 50 (normal). The capsule (400 mg), three times a day for 28 days in combination with dextran (500 ml/day) were given to the treatment group, while dextran (500 ml/day) alone was given to the control group. We evaluated the convalescence of the patients according to the Chinese stroke score, before study entry at 1st, 2nd, 3rd, 4th week after randomization. The KPTT, PT, FIB, t-PA, PAI, D-dimer were also assayed at the same time. The KPTT, PT, FIB, were assayed by ACT-3000 machine automatically. The activity of t-PA and PAI were determined by chromogenic assay and D-dimer level were measured by enzyme-linked immunoadsorbent assay.

The results were expressed as the mean $\pm$ SD for statistical analysis. The Student "t" test and simple linear regression correlation method were used.

3. Results

To investigate neurological status, we used the criteria according to the Chinese stroke scale [3]. Before administration, the mean score of treatment group was 19.22 ± 7.46, in control group the score was 18.34 ± 10.47. There was no significant change in two groups ($p > 0.05$). After administration in both groups, the Chinese stroke scores decreased at 1st, 2nd, 3rd, 4th week, but in the treatment group it was more obvious (Table 2). The result indicated that lumbrokinase may significantly improve patient's signs and symptoms.

CT scan was performed on 20 patients in each of the treatment group and placebo group. On admission and 4 weeks thereafter, infarction size was calculated based on the maximum length and width of the hypodense area on the horizontal CT films in which the area appeared largest in treatment group heal

Table 2
Changes of the Chinese stroke score before and after administration

	Before administration	One week	Two weeks	Three weeks	Four weeks
Treatment group	19.22 ± 7.46	$14.87 \pm 8.00^{**}$	$12.12 \pm 9.02^{**}$	$12.04 \pm 10.10^{**}$	$11.25 \pm 9.46^{**}$
Control group	18.34 ± 10.47	$15.26 \pm 9.80^{*}$	$15.04 \pm 10.88^{*}$	$13.00 \pm 9.03^{*}$	$12.20 \pm 9.60^{*}$

$^{*}p < 0.05$, $^{**}p < 0.01$.

larger infarct. In the treatment group, it was observed that the KPTT was prolonged in all the time after administration (Fig. 1, $P < 0.05$) and the concentration of plasma fibrinogen were decreased significantly (Fig. 2, $p < 0.05$) in Chinese stroke scores mostly decreased. The increase of t-PA activity was positively correlated control group, there was no significant change. In both groups, the content of PT kept the same value (Table 3, $p > 0.05$). At the same time, in treatment group the t-PA activity was slightly elevated at 1st, 2nd week and it was significantly elevated at 3rd week. In the treatment group, t-PA activity was decreased at 4th week after administration (Fig. 3, $p < 0.05$). There was no significant change of PAI activity in both groups (Table 4, $p > 0.05$). The plasma level of D-dimer was significantly increased at 1st, 2nd, and 3rd week after administration and decreased to normal at 4th week (Table 5, $p < 0.05$). In Fig. 3, the activity of t-PA reached maximum at 2nd week after administration in treatment group, while with decrease of the Chinese stroke scores (Fig. 4, $r = 0.8047$, $p < 0.05$).

4. Discussion

As previously reported, several studies showed that enhanced coagulation, low endogenous fibrinolytic activity appeared in the acute, subacute and chronic patients with cerebral thrombosis [4]. In our study, we observed that the KPTT were significantly prolonged after administration ($p < 0.05$), while there was no change in control group ($p > 0.05$). The KPTT is a screening test for intrinsic coagulation pathway. The increase of KPTT reflected the decrease of factors that participates to the cascade reaction of intrinsic coagulation system. It also indicated that lumbrokinase may inhibit intrinsic coagulation pathway [5].

Eduin et al. reported that t-PA serine protease consists of a single polypeptide chain of 52 amino acids. Plasminogen is converted by t-PA into plasmin via cleavage of the Arg 560–Val 561 bond. t-PA is secreted from the vascular endothelial cells [6]. Guo-ping Zhang et al. have already reported that the t-PA activity in rat pulmonary microvascular endothelial cell culture media was significantly elevated after cultivation with lumbrokinase (50–200 mg/l). There was a dose-dependent effect. PAI activity showed no such changes. Lumbrokinase per se had certain t-PA activity, which was positively correlated with its concentration [7]. Our study showed that after administration the activity of t-PA was significantly increased in 4 weeks. No such changes were observed in control group. There were no significant changes of PAI activity in both groups. Our results indicated that lumbrokinase has t-PA activity and it can stimulate the vascular endothelial cells to secrete t-PA. So the results tend to show lumbrokinase activates fibrinolysis via the increase of t-PA activity.

It is already known that an elevated serum fibrinogen concentration is a risk factor for stroke. Thus high levels of fibrinogen may predispose to thrombosis by producing a hypercoagulable and hyperviscosity state. It may reduce blood flow perfusion through hemorheological effects [8]. After administration, the fibrinogen content decreased significantly in treatment group. We suggest that the decrease of fibrinogen content is beneficial to the prevention and treatment of stroke.

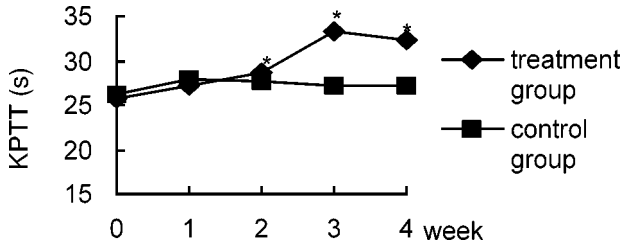

Fig. 1. Changes in kaolin partial thromboplastin time before and after administration.

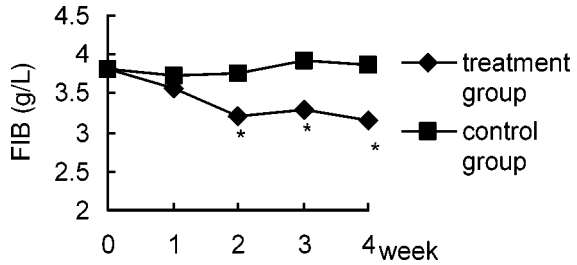

Fig. 2. Changes in fibrinogen before and after administration.

Table 3
Changes of prothrombin time before and after administration (unit: sec)

	Before administration	One week	Two weeks	Three weeks	Four weeks
Treatment group	11.90 ± 0.65	12.70 ± 1.73	12.39 ± 2.13	12.40 ± 1.43	12.33 ± 2.31
Control group	11.62 ± 0.84	11.92 ± 1.81	12.06 ± 0.85	12.72 ± 0.82	13.24 ± 2.10

$p < 0.05$.

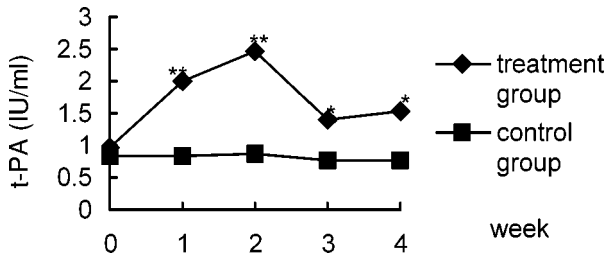

Fig. 3. Changes in plasma t-PA activity before and after administration.

Table 4
Changes in plasma PAI activity before and after administration (unit: IU/ml)

	Before administration	One week	Two weeks	Three weeks	Four weeks
Treatment group	7.84 ± 4.48	9.21 ± 4.92	7.39 ± 4.36	6.89 ± 2.86	6.42 ± 3.49
Control group	8.41 ± 4.08	10.04 ± 4.64	8.43 ± 3.80	7.50 ± 2.7	7.82 ± 1.78

$p > 0.05$.

Table 5
Changes in D-dimer level before and after administration (unit: μg/l)

	Before administration	One week	Two weeks	Three weeks	Four weeks
Treatment group	0.06 ± 0.05	$0.16 \pm 0.22^{**}$	$0.14 \pm 0.10^{**}$	$0.09 \pm 0.04^{*}$	0.07 ± 0.03
Control group	0.06 ± 0.04	0.07 ± 0.09	0.07 ± 0.05	0.08 ± 0.02	0.07 ± 0.02

$^{*}p < 0.05$, $^{**}p < 0.01$.

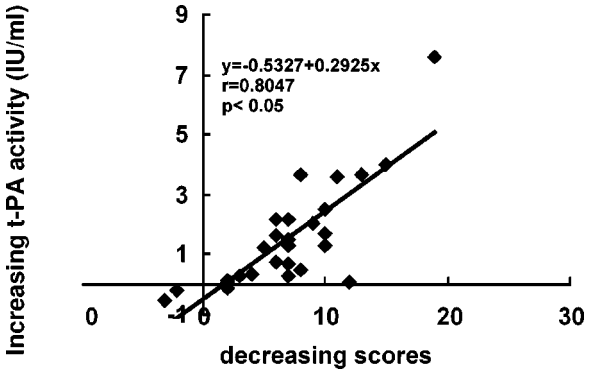

Fig. 4. Correlation of t-PA activity and Chinese scores of stroke.

D-dimer is one of the principal degradation fragments of fibrin. Significant increase in D-dimer level indicated that the production of fibrin degradation fragments was enhanced. As for coagulation and fibrinolytic activation in thrombolytic therapy, the D-dimer level was markedly high in some patients of the hemorrhagic transformation group compared with the non hemorrhagic group. So it also may indicate fibrinolytic activation and bleeding tendency [9]. In our study, D-dimer levels were significantly elevated in the treatment group, indicating increased fibrin formation and dissolution via the increase of t-PA activity.

After administration, the patients in treatment group had a good or excellent outcome. The Chinese stroke score was reduced significantly from the baseline score. Our study also indicated the decrease of Chinese stroke scale score were positively correlated with the increase of t-PA activity.

5. Conclusion

Lumbrokinase is beneficial for the treatment of cerebral infarction. The effects of lumbrokinase is related to the inhibition of intrinsic coagulation pathway and activation of fibrinolysis via the increase of t-PA activity. Meanwhile the fibrinogen level declines significantly. We suggest lumbrokinase may be used in the prevention of cerebral infarction and prevention of the second cerebral infarction in patient with a previous cerebrovascular ischemic event.

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