Original article

Oral fibrinogen-depleting agent lumbrokinase for secondary ischemic stroke prevention: results from a multicenter, randomized, parallel-group and controlled clinical trial

CAO Yong-jun, ZHANG Xia, WANG Wan-hua, ZHAI Wan-qing, QIAN Ju-fen, WANG Jian-sheng, CHEN Jun, YOU Nian-xing, ZHAO Zhong, WU Qiu-yi, XU Yuan, YUAN Lei, LI Rui-xia and LIU Chun-feng

Keywords: *ischemic stroke; lumbrokinase enteric-coated capsules; fibrinogen; carotid atherosclerosis*

Background Elevated fibrinogen (Fg) level is a known risk factor for ischemic stroke. There are few clinical trials on oral fibrinogen-depleting therapies for secondary ischemic stroke prevention. We aimed to assess the effects of one-year therapy with oral lumbrokinase enteric-coated capsules on secondary ischemic stroke prevention.

Methods This is a multicenter, randomized, parallel group and controlled study that began treatment in hospitalized patients with ischemic stroke and continued for 12 months. Patients were randomized to either the control group that received the standard stroke treatment or the fibrinogen-depleting group that received the standard stroke treatment plus enteric-coated lumbrokinase capsules. The NIH Stroke Scale scores (NIHSSs) and plasma Fg level were recorded. The carotid artery intima-media thickness (IMT) and status of plaques were examined through carotid ultrasound examination. Primary outcomes included all-cause mortality, any event of recurrent ischemic stroke/transient ischemic attack (TIA), hemorrhagic stroke, myocardial infarction and angina, and other noncerebral ischemia or hemorrhage. Kaplan-Meier survival analysis and the Long-rank test were used to compare total vascular end point incidence between the two groups. Comparison of median values between two groups was done by the Student *t* test, one-way analysis of variance (ANOVA), or non-parametric rank sum test.

Results A total of 310 patients were enrolled, 192 patients in the treatment group and 118 patients in the control group. Compared to the control group, the treatment group showed favorable outcomes in the Fg level, carotid IMT, the detection rate of vulnerable plaques, the volume of carotid plaques, NIHSS scores, and incidence of total vascular (6.78% and 2.08%, respectively) and cerebral vascular events (5.93% and 1.04%, respectively) (P <0.05). In the treatment group, the volume of carotid plaques was significantly related to the carotid IMT, the plaque diameter, width and number (P=0.000, 0.000, 0.000, 0.022; F=13.51, 2.52, 11.33, –3.29, but there was a weak correlation with the Fg level (P=0.056). After 1-year therapy, the incidence of overall vascular end points was reduced by 4.7%.

Conclusion Long-term oral fibrinogen-depleting therapy may be beneficial for secondary ischemic stroke prevention.

Chin Med J 2013;126 (21): 4060-4065

Elevated fibrinogen level is a known risk factor for stroke.[1] It was also suggested that higher fibrinogen level measured in patients within 6 hours of stroke onset was associated with poor functional outcome.[2] However, venous fibrinogen-depleting therapy failed to show any benefit.[3,4] There are few clinical trials on oral fibrinogen-depleting therapy to treat ischemic stroke. Lumbrokinase is an effective enzyme extracted by a method modified from a Chinese traditional herb by Mihara in 1983. It has been marketed in China for more than 10 years mainly for the treatment of acute phase ischemic stroke. One study showed good functional outcome in patients with acute ischemic stroke when treated with lumbrokinase for 21days.[5] Our study was to observe the efficacy of one-year oral lumbrokinase enteric-coated capsules therapy for its effectiveness on secondary ischemic stroke prevention.

METHODS

Lumbrokinase was provided by the Institute of Biophysics,

DOI: 10.3760/cma.j.issn.0366-6999.20131332

Department of Neurology, Second Affiliated Hospital of Soochow University, Suzhou, Jiangsu 215004, China (Cao YJ, Zhang X and Liu CF)
Department of Neurology, Kunshan No.1 People's Hospital, Kunshan, Jiangsu 215300, China (Wang WH)
Department of Neurology, Taicang No.1 People's Hospital, Taicang, Jiangsu 215400, China (Zhai WQ and Li RX)
Department of Neurology, Zhangjiagang Traditional Chinese Medicine Hospital, Zhangjiagang, Jiangsu 215600, China (Qian JF)
Department of Neurology, Changshu No.1 People's Hospital, Changshu, Jiangsu 215500, China (Wang JS)
Department of Neurology, Wuxi Traditional Chinese Medicine Hospital, Wuxi, Jiangsu 214000, China (Chen J)
Department of Neurology, Changshu No.2 People's Hospital, Changshu, Jiangsu 215500, China (You NX)
Department of Neurology, Suzhou Municipal Hospital, Suzhou, Jiangsu 215000, China (Zhao Z)
Department of Neurology, Zhangjiagang No.1 People's Hospital, Zhangjiagang, Jiangsu 215600, China (Wu QY)
Department of Neurology, Wujiang No.1 People's Hospital, Wujiang, Jiangsu 215200, China (Xu Y)
Department of Neurology, Taicang Traditional Chinese Medicine Hospital, Taicang, Jiangsu 215400, China (Yuan L)
Correspondence to: Pro. LIU Chun-feng, Department of Neurology, Second Affiliated Hospital of Soochow University, Suzhou, Jiangsu 215004, China (Tel: 86-512-67783307. Fax: 86-512-68284303. Email: liucf20@hotmail.com)
Conflict of interest: none.

Chinese Academy of Sciences, Beijing, China. From May 2007 to June 2009, the second Affiliated Hospital of Soochow University and other 10 middle-sized hospitals in the southern Jiangsu Province conducted this multicenter clinical trial to explore the efficacy of long-term oral lumbrokinase for secondary ischemic stroke prevention.

Study characteristics and patient samples

Patients diagnosed with anterior circulation ischemic stroke or transient ischemic attack (TIA) within 6 months from the 11 participating hospitals were recruited into the study. Noncomatose subjects were eligible if they were between 40 and 80 years of age and had carotid circulation ischemic stroke confirmed by brain CT or MRI within 72 hours of symptom onset and the level of fibrinogen (Fg) is higher than 2.0 g/L. Carotid TIA patients were diagnosed when they complained with transient hemiparalysis, unilateral sensory disturbance, aphasia or monocular visual impairment lasting less than 24 hours. The research protocol was approved by the local Ethics Committee of Soochow University and all patients or family members signed informed consents. Patients with the following conditions were excluded: cardiogenic embolism and hemorrhagic transformation, on or need to be on anticoagulant therapy, severe hepatic, renal, hematopoietic and endocrine diseases, allergy to lumbrokinase, planning to have a major operation or carotid angioplasty, and pregnancy.

This was a multicenter, parallel group, open label, controlled trial with 2:1 simple randomization by random number table. Patients assigned with odd numbers were included in the treatment group and even numbers in the control group. Patients in the treatment group were given oral enteric-coated lumbrokinase capsules (600 000 units a time, three times a day, 30 minutes before meals) for one year. The control group was given capsules without lumbrokinase for one year. Both groups received standard stroke treatment otherwise. All patients had monthly followed-up by telephone and visits at the hospitals 6 and 12 months post discharge. At the two follow-up visits, data on patient's current medication, any disease history, the NIH Stroke Scale scores (NIHSSs), carotid ultrasonography, blood biochemical and coagulation tests, blood and plasma viscosity, the incidence of vascular endpoint events were collected.

End points and definitions

The primary endpoints included all-cause mortality, any event of recurrent ischemic stroke/TIA, hemorrhagic stroke, myocardial infarction and angina, and other noncerebral ischemia or hemorrhage. Other noncerebral ischemia or hemorrhage means hemorrhagic and ischemic events of other organs excluding brain, which was defined as a primary endpoint. In case of a suspected recurrent cerebrovascular or cardiovascular event, confirmation was sought from the treating doctor or hospital. If a patient had a noncerebral ischemia or hemorrhagic event, the treating doctor or hospital would need to confirm the diagnosis. When a patient died during the following-up period, the cause of death was recorded according to the medical records and death certificate. Safety end points included mortality, intracranial hemorrhage, major bleeding, and review of other physical and laboratory measurements. The safety committee received reports of all deaths and serious adverse events as planned for review.

Carotid ultrasound examination

Ultrasonography was performed by using a GEVivid 7D (GE Company, USA) system with a 7.5-MHz transducer, including carotid intima-media thickness (IMT), diameter, the number and distribution of plaques of both carotid arteries. All examinations were performed by one trained radiologist in each hospital who had no knowledge of the clinical history and profile of the subjects. The IMT of the distal wall of the carotid artery was measured at 2.0, 2.5 and 3.0 cm proximal to the carotid bifurcation in each of the right and left common carotid arteries. Measurements were made on longitudinal scans obtained in the anterior oblique, lateral and posterior oblique views. The IMT was defined as the distance between two echogenic lines separated by a hypoechoic or anechoic space, with the outer line corresponding to the media-adventitia border and the inner line representing the lumen-intima border. The mean IMT was calculated as the average value of the IMT measurements for the six sites in the carotid arteries. Plaque demonstrates a thickness of IMT >1.2 mm.[6] The vulnerable plaques were defined to show hypoechoic, or both hypoechoic and hyperechoic signals. Those showing hyperechoic with a smooth surface were defined as stable plaques.[7-9] The plaque volume was calculated as "the average plaque diameter × the average plaque width × IMT".

Statistical analysis

SPSS 13.0 software (IBM Company, USA) was used for statistical analysis. Sample size was calculated based on the incidence of vascular events of 3% in the trial group and 12% in the placebo group, with a probability of type I error of 0.05 and type II error of 0.10. Results were expressed as a percentage or mean ± standard deviation (SD). Differences in frequencies were compared using the chi-square test. Comparison of median values between two groups was done by the Student's *t* test, one-way analysis of variance (ANOVA), or non-parametric rank sum test. Forward stepwise multiple linear regression analysis was performed to detect factors that influenced the plaque volume. Comparison of total vascular end point incidence between the two groups was evaluated by Kaplan-Meier survival analysis and the Long-rank test. All statistical analyses were 2-tailed. A P value <0.05 was considered statistically significant in all analyses.

RESULTS

A total of 310 patients were enrolled: 192 patients in the treatment group and 118 patients in the control group. All were included in the analysis. About 88.0% patients in the treatment group and 89.0% in the placebo group completed

the therapy (Figure 1). Their average age was (67.10±9.27) years. There was no statistical difference in age, sex, blood pressure, combined medications, and past history between two groups (P >0.05). The usage rate of antihypertensive, antiplatelet and statin drugs of both groups had no significant difference (Table 1).

Fg Changes (Table 2)

The baseline Fg levels of the treatment and control group were (3.47±1.07) g/L, (3.53±1.19) g/L, respectively. After 1 year, in the treatment group, Fg level decreased to (2.68±0.95) g/L but unchanged in control group ((3.56±0.86) g/L, P <0.05); CRP level decreased (P=0.013); D-dimer level significantly deceased (P=0.013); t-PA activity was elevated (P=0.018); blood and plasma viscosity apparently improved (both P <0.05). In both groups, the content of PT and APTT were unchanged (P >0.05).

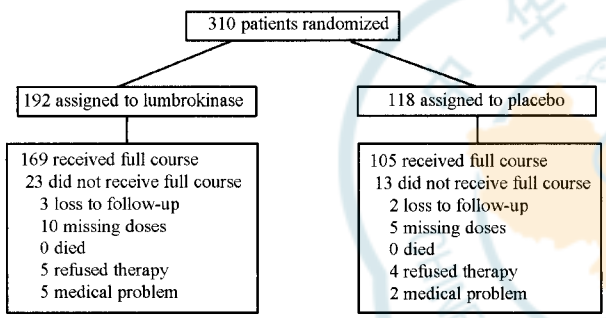

Figure 1. Trial profile. Missing doses indicates patients who have missed more than 100 doses in total or 30 consecutive doses. Medical problem indicates patients with other concurrent diseases not suitable for continuation during the follow-up period.

Table 1. Baseline characteristics of the study groups

Variables	Treatment (n=192)	Control (n=118)
Gender (n (%))		
Male	143 (74)	74 (63)
Female	49 (26)	44 (37)
Age (years)	66.61±8.87	67.89±9.88
Blood pressure (mmHg)		
Systolic blood pressure	148.97±23.73	148.14±22.71
Diastolic blood pressure	85.68±12.91	89.88±16.06
Concomitant medications (n (%))		
Baseline usage of antiplatelet drugs	154 (80.21)	98 (83.05)
Baseline usage of statin drugs	56 (29.17)	38 (33.20)
Baseline usage of antihypertensive drugs	56 (29.17)	37 (31.36)
1-year usage of antiplatelet drugs	104 (54.17)	62 (52.54)
1-year usage of statin drugs	41 (21.35)	24 (20.34)
1-year usage of antihypertensive drugs	53 (27.60)	36 (30.51)
Past history (n (%))		
None	58 (30.21)	29 (24.58)
Hypertension	86 (44.79)	62 (52.54)
Diabetes mellitus	8 (4.17)	4 (3.39)
Hyperlipidemia	1 (0.52)	2 (1.70)
Hypertension+diabetes	26 (13.54)	20 (16.95)
Hypertension+hyperlipidemia	4 (2.08)	1 (0.85)
Hypertension+CHD	2 (1.04)	4 (3.39)
Hypertension+diabetes+CHD	2 (1.04)	1 (0.85)

There was no statistically significant difference in age, sex, blood pressure, combined medications and past history between two groups (P >0.05). CHD:

Table 2. Blood indexes

Items	Treatment group		Control group	
	Baseline	1 year	Baseline	1 year
Fg (g/L)	3.47±1.07	2.68±0.95	3.53±1.19	3.56±0.86
CRP (mg/L)	5.91±1.33	3.22±1.67	5.13±1.62	3.84±1.59
t-PA (IU/ml)	116.21±10.68	121.37±13.34	118.76±14.10	105.85±4.36
PAI-1 (AU/ml)	83.26±9.48	81.67±6.74	75.55±8.24	72.48±10.11
D-Dimer (mg/L)	0.82±0.30	0.45±0.25	0.37±0.28	0.49±0.29
PT (seconds)	12.12±1.62	12.32±1.84	12.30±1.60	12.23±1.41
APTT (seconds)	30.04±5.76	30.30±5.12	30.45±4.66	30.73±4.32
INR	1.01±0.24	1.09±0.25	1.03±0.18	1.02±0.31
Blood viscosity (low cut)	13.50±10.06	9.97±1.75	11.80±6.16	10.96±1.53
Blood viscosity (medium cut)	9.48±8.36	5.97±0.97	8.70±5.89	6.73±0.90
Blood viscosity (high cut)	7.49±6.22	4.49±0.80	6.78±4.84	5.23±0.67
Plasma viscosity (mPa/s)	3.69±2.01	1.46±0.23	4.24±3.67	1.67±0.22

Plaque number and echo intensity changes

Baseline plaque number of both carotid arteries in treatment group was 1.12±0.66 and 1.08±0.89 per patient and in control group 0.93±0.45 and 1.06±0.70, with no significant difference. After 1 year of therapy, there was a significant reduction of the plaque number in treatment group (P=0.043) but a significant elevation in the control group (P=0.023).

There were mainly hyperechoic plaques in both groups. In the treatment group, the baseline vulnerable plaque rates consisting of hypoechoic and mixed echo plaques were 28% in the left side and 17.50% in the right side. After 1 year, the rates were reduced to 7.90% and 9.50%, respectively (left side, χ^2=16.49, P <0.001; right side, χ^2=3.05, P=0.080). Detection rate of vulnerable plaques (%) = (number of low echo plaques + number of mix-echo plaques) × 100% / total number of echo plaques.

IMT and plaque volume changes

In treatment group, bilateral carotid IMT was reduced significantly after 1 year (left side, (1.26±0.49) mm vs. (1.09±0.40) mm, right side (1.25±0.51) mm vs. (1.06±0.35) mm, both sides P <0.001). In control group, the IMT thickness increased after 1 year (right side, (1.40±0.79) mm vs. (1.59±0.69) mm, P=0.055), especially on the left side ((1.38±0.52) mm vs. (1.62±0.56) mm, P=0.001).

There was no significant difference in the baseline plaque volume between two groups. After 1 year of therapy, the carotid plaque volume reduced, falling by 41.84% in the left side and 51.30% in the right side (both sides P=0.02) in the treatment group. However, the carotid plaque volume increased by 103.38% in the left side and 80.04% in the right side in the control group (left side P=0.008, right side P=0.038).

Multiple linear regression analysis of the carotid plaque volume

Forward stepwise multiple linear regression analysis was performed to assess the factors influencing the carotid

plaque volume. NIHSS scores, Fg level, carotid IMT and diameter, the plaque number, plaque diameter and width were regarded as independent variables. Fg level, carotid IMT, the plaque number, the plaque diameter and width were entered into the regression equation (R^2=0.85; F=51.88; P=0.000). Carotid IMT, the plaque diameter, the plaque width and the plaque number showed a significant association with average carotid plaque volume (t=4.25, 7.18, 10.63, –2.36; P=0.000, 0.000, 0.000, 0.022; F=13.51, 2.52, 11.33, –3.29). However, the Fg level was found to be weakly associated with average carotid plaque volume (t=1.95, P=0.056, F=3.53).

NIHSS scores (Table 3) and the incidence of vascular end-point events (Table 4)

After 6 months of therapy, NIHSS scores were significantly improved in both groups (both P <0.001). After a year, there was a further reduction of NIHSS scores in both groups. Compared to control group, NIHSS scores in the treatment group showed a significant reduction after one year (both P <0.001).

At the 12-month follow-up, in the control group, 7 patients (5.93%) had cerebrovascular events: 2 TIA, 4 cerebral infarction, and 1 cerebral hemorrhage. There was also 1 other organ ischemia event in the control group. In the treatment group there were 2 (1.04%) cerebrovascular events (1 TIA and 1 cerebral infaction), 1 angina pectoris, and 1 lower gastrointestinal bleeding. There were significant differences in total vascular events and cerebrovascular events between two groups (χ^2=3.97, P=0.046; χ^2=5.79, P=0.016). Kaplan-Meier survival analysis showed a significant reduction of total vascular events, falling by 4.7% in treatment group (Log-rank test, χ^2=4.33, P=0.038, Figure 2).

Safety assessment

There were 5 patients with adverse events in treatment group (excluding vascular endpoints), 7 patients in the control group. Three and two patients complained with dizziness in the treatment and control group, respectively.

Table 3. The changes of NIHSS scores between two groups

NIHSS scores	Treatment group	Control group	P values
NIHSS (baseline)	5.71±3.78*,‡	6.18±5.49$^{§,‖}$	0.369
NIHSS (a half year)	3.72±2.76*,†	3.78±3.63§	0.178
NIHSS (one year)	2.35±2.16†,‡	3.62±3.53$^{‖}$	<0.001

$^{*,‡,§,‖}P$ <0.001, $^{†}P$=0.001.

Table 4. Vascular endpoints (n (%))

Vascular events	Treatment group	Control group	χ^2 values	P values
Cerebrovascular events	2 (1.04)	7 (5.93)	5.79	0.016
TIA	1 (0.52)	2 (1.69)	1.03	0.311
Cerebral infarction	1 (0.52)	4 (3.39)	3.45	0.056
Cerebral hemorrhage	0	1 (0.85)	1.62	0.203
Cardiovascular events				
Angina pectoris	1 (0.52)	0	1.62	0.203
Myocardial infarct	0	0	-	-
Other organ vascular events				
Ischemia	0	1 (0.85)	1.62	0.203
Hemorrhage	1 (0.52)	0	1.62	0.203
Total vascular events	4 (2.08)	8 (6.78)	3.97	0.046

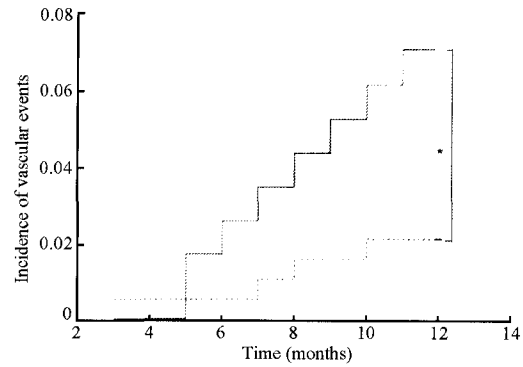

Figure 2. Kaplan-Meier survival analysis. Solid line = control group; broken line = lumbrokinase group. There was a significant difference between the control group and lumbrokinase group ($^{*}P$ <0.05 by the log-rank test).

Three and four patients complained with nauseating and vomiting in each group. All patients were not treated and continued the treatment, with disappearance of symptoms themselves. There was no significant difference in the adverse event incidence between two groups (χ^2=1.998, P=0.157).

DISCUSSION

Plasma fibrinogen level is strongly associated with risks of developing CHD, stroke, vascular mortality, and nonvascular mortality in healthy middle-aged adults. The age- and sex- adjusted hazard ratio per 1-g/L increase in usual fibrinogen level for all the events is around 2.0.[1] Our study has provided new information on the use of fibrinogen-depleting therapy for ischemic stroke. The present trial is a relatively early study to demonstrate the benefit of long-term oral fibrinogen-depleting therapy on the secondary prevention of ischemic stroke.

Lumbrokinase is extracted from Lumbricus rubellus, which 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.[10] It has selective affinity for Fg and directly hydrolyzes Fg to soluble fibrinogen degradation products. Lumbrokinase can also reduce platelet aggregation rate and ameliorate blood and plasma viscosity.[11,12] t-PA and PAI are both key enzymes of Fibrinolytic system. T-PA is an activator, however, PAI is an inhibitor for fibrinolysis through integration with t-PA. Decrease of ratio of t-PA/PAI increases the risk of ischemic stroke.[13,14] Our study showed that after treatment, the activity of t-PA was significantly increased, which supported the findings in the animal study.[15] Therefore, lumbrokinase has indirectly increased t-PA activity and directly stimulated vascular endothelial cells to secrete t-PA. However, PAI-1 (Plasminogen activator inhibitor-1) did not change. A high serum Fg concentration produces a high hypercoagulable and hyperviscosity state. Our study showed that Fg concentration was decreased but not too low after 1 year treatment (Fg level >0.7 g/L after 1 year). Meanwhile,

blood and plasma viscosity improved. Such changes may have the benefit of stopping the progression of arterial atherosclerosis. However, the content of PT and APTT did not change, which means lumbrokinase does not affect coagulation system. It may reduce the risk of hemorrhage. There were no obvious adverse events in the treatment group with any difference in adverse events incidence compared to the control group. So it is a safe drug.

As a principal degradation fragments of fibrin, D-dimer level is an important predictive factor for the diagnosis and prognosis of arterial atherosclerosis. Our study showed that the D-dimer level was decreased, which was different from previous reports.[5] This may be explained by the possible ceiling effect of lumbrokinase activity. After reaching a certain point, Fg level would not drop further. Consequently Fg level was not too low after 12 months of treatment. The other explanation is that Lumbrokinase may degrade both fibrinogen and fibrin deposits, which leads to the decrease of D-dimer level. CRP, a major inflammatory index, is notably inhibited by lumbrokinase.

Fg is an independent risk factor for atherosclerosis and related to the severity of disease.[16] Our study showed that defibrinogenation therapy improved carotid atherosclerosis. After 1 year of treatment, we found that in the treatment group, IMT notably was thinned, average plaque number and vulnerable plaque rate were decreased, and plaque volume was significantly reduced. These benefits may be related to the anti-platelet effect of Lumbrokinase through the elevation of cAMP level and Ca^{2+} release. Lumbrokinase can also inhibit ICAM-1 expression that may have an antithrombotic effect. Furthermore, lumbrokinase reduces cellular apoptosis activity through activation of JAK1/STAT1 pathways.[17] The key point is that lumbrokinase can significantly reduce the Fg level, which plays an important role in the progression of atherosclerosis. There are several ways in which Fg participates in atherosclerosis progression. Firstly, *in vivo* Fg can be converted to fibrin and insoluble degradation products, which deposit in the vascular wall and promote the aggregation and adhesion of white blood cells to accelerate inflammatory process of atherosclerosis.[18] Secondly, increasing Fg level affects the endothelial cell function to stimulate its synthesis and secretion of plasminogen inactivators, which leads to delayed remove of local microembolism. Endothelium can be injured and cholesterol is easy to infiltrate.[19,20] Thirdly, Fg and its degradation products stimulate smooth muscle cells and can link to platelets to activate them.[21] Fourthly, high Fg level can alter the permeability of microphages cap to make plaques vulnerable and thrombus formation. Fifthly, high Fg level increases blood viscosity and substrates, which can be risk factors of small vessel obstruction. Additionally, we found that Fb, Fg, and FDPs are involved in the progression of the instability of atherosclerotic plaques via increasing the expression of MMPs and VEGF. This effect might be mediated by the NF-κB pathway.[22]

Our study is a multicenter, randomized, parallel-group, open label and controlled clinical trial, but not a double-blinded study. For the practical reason, serum Fg, d-dimer and t-PA levels were not checked on a schedule time interval and therefore it was difficult to detect how and when exactly these change of indexes took place.

Our trial has found that after 1 year of lumbrokinase therapy, the incidence of overall vascular end points was reduced by 4.7%. There is clearly a close relationship between the plasma Fg level and atherosclerosis or stroke.[23] And the trial may provide new evidences for clinical use of fibrinogen-depleting therapy for the prevention of ischemic stroke. However, more prospective randomized large clinical trials are needed to provide better evidence of the role of lumbrokinase in reducing ischemic strokes.

Acknowledgement: The authors are grateful to FENG Ping, the chief of Department of Clinical Laboratories and PENG Chun-lei, the chief of Blood Laboratory in the Second Affiliated Hospital of Soochow University for the help in detection of blood biochemical parameters. We also thank Prof. David Wang, the Professor of Neurology Department in the College of Medicine, at the University of Illinois in the USA for the revision of the manuscript.

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(Received May 20, 2013)
Edited by JI Yuan-yuan