Changes in Coagulation and Fibrinolytic System after Local Intra-arterial Thrombolysis for Acute Ischemic Stroke

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Abstract

Intracerebral hemorrhagic transformation is one of the most important complications of thrombolytic therapy for acute ischemic stroke. The relationship between changes in markers for the coagulation and fibrinolytic systems and occurrence of hemorrhagic transformation was determined after local intra-arterial thrombolytic therapy using urokinase (UK) (24 patients) or recombinant tissue plasminogen activator (t-PA) (10 patients) within 6 hours of onset. All 34 patients had no hypodensity areas on initial computed tomography scans. Plasma concentrations of fibrinogen-fibrin degradation products (FDP), fibrinogen, α_2 -plasmin inhibitor (α_2 -PI), plasmin- α_2 plasmin inhibitor complex (PIC), thrombin-antithrombin III complex (TAT), and D-dimer were measured. Hemorrhagic transformation occurred in seven patients (21%) with complete or partial recanalization; four in the UK group and three in the t-PA group. Doses of the thrombolytic agents did not correlate with the incidence of hemorrhagic transformation. The FDP levels in the hemorrhagic transformation group treated with UK significantly increased immediately and 1 hour after the therapy. The α_2 -PI activities decreased and PIC levels increased in both the hemorrhagic transformation and the nonhemorrhagic groups after the therapy. The TAT levels in both groups tended to be higher than the normal range, but there was no significant difference from the pretreatment levels. The D-dimer levels in the hemorrhagic transformation group were higher than those in the nonhemorrhagic group at 24 hours after the therapy. Furthermore, the D-dimer levels were significantly higher in patients with complete recanalization compared with those with none or partial recanalization. Activation of the fibrinolytic system after thrombolytic therapy may be a risk factor for intracerebral hemorrhagic transformation. D-dimer is useful as a marker for recanalization in thrombolytic therapy for acute ischemic stroke.

Key words: thrombolytic therapy, coagulation, fibrinolysis, urokinase, tissue-plasminogen activator

Introduction

Thrombolytic therapy using intravenous administration of streptokinase or urokinase (UK) in cases of acute ischemic stroke has been used since the 1960s, but the safety and efficacy are still questionable because serious complications of hemorrhagic infarction and intracerebral hemorrhages have occurred.⁴⁾ Modification of the therapy by local intra-arterial administration through a microcatheter under neuroradiographic guidance may supply a sufficient

concentration of thrombolytic agents to the occluded site with a lower incidence of systemic side effects of the drugs.¹²⁾ The development of new thrombus-selective thrombolytic agents such as recombinant tissue plasminogen activator (t-PA) have also increased the potential of this treatment.¹³⁾

Intracerebral hemorrhagic transformation is the most serious complication after recanalization by thrombolytic therapy. In particular, hemorrhagic transformation accompanied by extensive brain edema may cause a poor outcome for the patients. Hemorrhagic transformation develops more frequently with time after onset of ischemia, but may occur as little as 2 hours⁸⁾ or even within 90 minutes of

ischemic onset.¹⁾ Our previous study found that there is a good correlation between the occurrence of hemorrhagic transformation and reduction of the residual cerebral blood flow (CBF) in the ischemic region.¹⁵⁾ In brief, the risk of hemorrhagic transformation after recanalization of occluded vessels by local intra-arterial thrombolytic therapy in patients treated within 6 hours of onset was higher when the ratio of ischemic regional activity to ipsilateral cerebellar activity calculated by pretherapeutic technetium-99m-labeled hexamethylpropyleneamine-oxime single photon emission computed tomography (SPECT) was less than 0.35.

The present study examined several markers for the coagulation and fibrinolytic systems before and after local intra-arterial thrombolytic therapy to evaluate whether responses of the coagulation and fibrinolytic systems are another risk factor for hemorrhagic transformation.

Subjects and Methods

This study included 34 patients, 17 males and 17 females aged 35 to 83 years (mean $\pm$ SD 68 ± 8 yrs), with acute cerebral infarction admitted to our hospital between September, 1989 and May, 1992. Acute ischemic stroke was classified into cardioembolic infarction and atherothrombotic infarction according to the guidelines of the Cerebral Embolism Task Force²⁾ on the basis of the onset pattern, angiographic findings, and the results of cardiovascular examinations such as electrocardiography, echocardiography, and Holter electrocardiography. Local intra-arterial thrombolytic therapy was performed using UK (24 patients) or t-PA (10 patients) according to our three criteria: 1) no apparent hypodensity areas observed on computed tomography (CT) scans on admission; 2) the patient could be treated within 6 hours, in principle, of onset; and 3) affected arteries indicated by the symptoms were demonstrated to be occluded by cerebral angiography. Informed consent was obtained from the patient or relatives.

The pretherapeutic CBF was evaluated in the territory of occluded vessels by SPECT. Cerebral digital subtraction angiography was performed by the Seldinger method using a 5-F catheter by placing a 6-F sheath in the right or left femoral artery. The occluded vessel was identified, and the tip of a Tracker-18 catheter (Target Therapeutics, Los Angeles, Cal., U.S.A.) was advanced into the thrombus, or upstream or downstream from the occlusion site. UK (240,000 U; Wakamoto Pharmaceutical Co., Ltd., Tokyo) or t-PA (8 mg; Silteplase; Dai-ichi

Pharmaceutical Co., Ltd., Tokyo) was dissolved in physiological saline (20 ml) and injected manually over about 10 minutes. Angiography was performed immediately after each infusion, which was repeated until recanalization of the occluded vessel was confirmed. The maximum dose of the thrombolytic agents was 1,200,000 U UK or 32 mg t-PA. t-PA was mostly used after April, 1991. Recanalization of the vessel was considered "complete" when the blood flow was clearly re-established regardless of atheromatous plaque left at the occluded site, "partial" when the blood flow was partially restored but dispersed embolus occluded more distal vessels or when a major artery with multiple occlusions was partly recanalized, and "none" when no reperfusion was observed. The patients were given 10% glycerol (200 ml) or 20% mannitol (300 ml) before the therapy, and intravenous heparin (5000 U) and drip infusion of micromolecular dextran (500 ml) during the therapy.

Blood coagulation and fibrinolytic system indicators were measured in arterial blood obtained through a short 6-F sheath placed in the right or left femoral artery before and immediately after the thrombolytic therapy, and in venous blood from the arm at 1, 24, and 48 hours after the therapy. Fibrinogen-fibrin degradation products (FDP) (normal range <10 mg/dl), fibrinogen (normal range 200–400 mg/dl), and α_2 -plasmin inhibitor (α_2 -PI) (normal range 80–130%) were assayed by latex agglutination, sodium sulfite precipitation, and chromogenic substrate S-2251 (Kabi Diagnostica, Stockholm, Sweden), respectively. Plasmin- α_2 plasmin inhibitor complex (PIC) (normal range <0.8 mg/dl), thrombin-antithrombin III complex (TAT) (normal range <3.0 μ g/l), and D-dimer (normal range <150 ng/ml) were measured by enzyme-linked immunosorbent assay.

CT was performed immediately after the therapy, on the next day, and at 1 week, 2 weeks, and 1 month after the therapy in all patients. The patients were classified based on the CT findings into nonhemorrhagic and hemorrhagic transformation groups. The patients treated with UK in the nonhemorrhagic group were further classified into three subgroups: 1) patients treated with 480,000 U or less, 2) those treated with 720,000 U, and 3) those treated with 960,000 U or more. The small number of patients treated with t-PA were not subdivided. Recanalization in the nonhemorrhagic group patients treated with UK was classified as complete, partial, or none, and in those treated with t-PA as complete or partial, to evaluate D-dimer as an indicator for recanalization.

All values were expressed as the mean $\pm$ SD. Differences between the two groups were examined by Student's *t*-test, the Mann-Whitney U test, or Fisher's exact probability test and statistical analysis of coagulation and fibrinolytic systems used Scheffé's *F* test.

Results

I. Recanalization and hemorrhagic transformation

Table 1 summarizes the clinical details of the UK and t-PA groups. No significant difference was observed in age, sex, disease type, or the interval from onset to treatment. Complete or partial recanalization was observed in 92% of the patients in the UK group and 100% of those in the t-PA group. Hemorrhagic transformation occurred in a total of seven patients (21%) with complete or partial recanalization; four in the UK group and three in the t-PA group. All these patients had cardioembolic infarction. The neurological symptoms were exacerbated in one UK group patient with massive parenchymal hematoma and in one UK group patient with localized hematoma in the territory of the lenticulostriate

arteries. One patient in both groups demonstrated only mild brain edema on CT scans immediately after the treatment, but hemorrhage with extensive brain edema occurred 6–7 hours after the treatment. None of the patients showed a tendency to systemic bleeding. There was no significant difference in the doses of the thrombolytic agents between the hemorrhagic transformation group ($84 \pm 27 \times 10^4$ U UK or 24 ± 6.5 mg t-PA) and the nonhemorrhagic group ($71 \pm 25 \times 10^4$ U UK or 25 ± 5.5 mg t-PA).

II. Changes in coagulation and fibrinolytic systems

In the UK group, the FDP levels in patients with hemorrhagic transformation significantly increased

Table 1 Clinical characteristics of the UK and t-PA groups

	Group		Difference
	UK	t-PA	
No. of patients	24	10	
Age (yr)	69 ± 11	68 ± 9.4	NS*
Sex ratio (M/F)	12/12	5/5	NS*
Cause			NS**
cardioembolic	20	9	
atherothrombotic	4	1	
Interval (hr)	5.2 ± 0.8	4.7 ± 1.4	NS*
Dose	$80 \pm 30 (\times 10^4 \text{ U})$	$24 \pm 6.1 \text{ (mg)}$	
Site of occlusion			$p < 0.05^{***}$
ICA	2	3	
MCA M ₁	9	3	
MCA M ₂	9	3	
ACA	1	0	
BA	3	1	
Recanalization			$p < 0.05^{***}$
complete	15	5	
partial	7	5	
none	2	0	
Hemorrhagic transformation	4	3	NS*

*Student's *t*-test, **Fisher's exact probability test, ***Mann-Whitney U test. ACA: anterior cerebral artery, BA: basilar artery, ICA: internal carotid artery, MCA: middle cerebral artery.

Fig. 1 FDP levels in patients with (closed circle) and without hemorrhagic transformation (open symbols) after thrombolytic therapy (upper: UK group, lower: t-PA group). $\circ$: $\leq 48 \times 10^4$ U UK ($n = 9$) or t-PA ($n = 7$), $\triangle$: 72×10^4 U UK ($n = 5$), $\square$: $\geq 96 \times 10^4$ U UK ($n = 6$), $\bullet$: UK ($n = 4$) or t-PA ($n = 3$). *Significant difference from the pretreatment value ($p < 0.05$). #Significant difference between two groups ($p < 0.05$).

Fig. 2 Fibrinogen levels in patients with (closed circle) and without hemorrhagic transformation (open symbols) after thrombolytic therapy (upper: UK group, lower: t-PA group). $\circ$: $\leq 48 \times 10^4$ U UK (n = 9) or t-PA (n = 7), $\triangle$: 72×10^4 U UK (n = 5), $\square$: $\geq 96 \times 10^4$ U UK (n = 6), $\bullet$: UK (n = 4) or t-PA (n = 3). *Significant difference from the pretreatment value ($p < 0.05$).

immediately and 1 hour after therapy compared to each dose subgroup of patients without hemorrhagic transformation. The FDP levels in patients treated with 960,000 U or more significantly increased immediately and 1 hour after the therapy, although not as much as in patients with hemorrhagic transformation, compared with the pretreatment levels. The values in each dose subgroup returned to the pretreatment levels within 24 hours of the therapy (Fig. 1 upper). In the t-PA group, the FDP levels in all patients rose slightly above the pretreatment levels, then returned to near the pretreatment levels 1 hour after the therapy and were within the normal range at 48 hours (Fig. 1 lower).

In the UK group, the fibrinogen levels decreased in the patients treated with 960,000 U or more until 48 hours after the therapy. However, there were no

Fig. 3 α_2 -PI activities in patients with (closed circle) and without hemorrhagic transformation (open symbols) after thrombolytic therapy (upper: UK group, lower: t-PA group). $\circ$: $\leq 48 \times 10^4$ U UK (n = 9) or t-PA (n = 7), $\triangle$: 72×10^4 U UK (n = 5), $\square$: $\geq 96 \times 10^4$ U UK (n = 6), $\bullet$: UK (n = 4) or t-PA (n = 3). *Significant difference from the pretreatment value ($p < 0.05$).

significant differences between the hemorrhagic transformation group and the various nonhemorrhagic subgroups (Fig. 2 upper). In the t-PA group, no significant reduction of fibrinogen level was seen in either hemorrhagic transformation or nonhemorrhagic group (Fig. 2 lower).

In all patients in the UK group, the α_2 -PI activities significantly decreased immediately and 1 hour after the therapy. The values returned to 50% or 60% of pretreatment activities 24 hours later, and were still lower than the normal range at 48 hours. There were no differences in the values between the hemorrhagic transformation group and the nonhemorrhagic group (Fig. 3 upper). In the t-PA group, the α_2 -PI activities significantly decreased immediately after the therapy. However, the values began to rise at 1 hour, and returned to the pretreatment levels within 24–48

Fig. 4 PIC levels in patients with (closed circle) and without hemorrhagic transformation (open symbols) after thrombolytic therapy (upper: UK group, lower: t-PA group). $\circ$: $\leq 48 \times 10^4$ U UK (n = 8) or t-PA (n = 7), $\triangle$: 72×10^4 U UK (n = 4), $\square$: $\geq 96 \times 10^4$ U UK (n = 5), $\bullet$: UK (n = 2) or t-PA (n = 3). *Significant difference from the pretreatment value ($p < 0.05$). #Significant difference between two groups ($p < 0.05$).

hours of the therapy (Fig. 3 lower).

In all patients in the UK group, the PIC levels significantly increased immediately after the therapy and returned to the pretreatment levels at 24 hours. There were no differences between the hemorrhagic transformation group and the various nonhemorrhagic subgroups (Fig. 4 upper). In the t-PA group, the PIC levels in the hemorrhagic transformation group and the nonhemorrhagic group also significantly increased immediately and 1 hour after the therapy. Furthermore, there were significant differences between these groups. The values in both groups returned to the pretreatment levels by 24 hours after the therapy (Fig. 4 lower).

The UK and t-PA groups showed no significant elevation of the TAT level after the therapy, com-

Fig. 5 TAT levels in patients with (closed circle) and without hemorrhagic transformation (open symbols) after thrombolytic therapy (upper: UK group, lower: t-PA group). $\circ$: $\leq 48 \times 10^4$ U UK (n = 7) or t-PA (n = 7), $\triangle$: 72×10^4 U UK (n = 4), $\square$: $\geq 96 \times 10^4$ U UK (n = 4), $\bullet$: UK (n = 2) or t-PA (n = 3).

pared with the pretreatment levels. However, the values in most patients were higher than the normal range before and after the therapy (Fig. 5).

The D-dimer levels in both the UK and t-PA groups showed a tendency to increase until 1 or 24 hours after the therapy. There was a significant difference in the values between the hemorrhagic transformation group and the nonhemorrhagic group, at 24 hours after the therapy in the UK group, and at 24 and 48 hours after the therapy in the t-PA group (Fig. 6A, B). In the nonhemorrhagic group treated with UK, the D-dimer levels in patients with complete recanalization was higher than that in patients with partial and no recanalization 1 hour after the therapy. In the nonhemorrhagic group treated with t-PA, the values in the patients with complete recanalization was also high, immediately and 1 hour after the therapy, compared with that in the patients

with myocardial infarction, pulmonary embolism, and deep vein thrombosis causes a marked increase in D-dimer level when recanalization occurs and is useful for monitoring and predicting the outcome.^{6,9)} However, the extensive increase in D-dimer level is considered to include lysable fibrin from other systemic pools. In this study, the FDP levels in patients with hemorrhagic transformation were significantly higher than in patients without hemorrhagic transformation regardless of the UK dose. The D-dimer levels in patients with hemorrhagic transformation were also significantly higher than in patients without hemorrhagic transformation. A striking finding in this study was that the D-dimer levels in the nonhemorrhagic group were significantly higher in the patients with complete recanalization than in those with partial or no recanalization, suggesting that recanalization of occluded vessels may be an important cause of increased D-dimer level. PIC, an irreversible complex of plasmin and α_2 -PI, is an indicator of generation of plasma *in vivo* because the half-value period of plasmin is very short and it combines rapidly with α_2 -PI in plasma. Therefore, PIC increases and α_2 -PI decreases in activated fibrinolysis. We found the fibrinolytic system was activated by changes in PIC levels and α_2 -PI activities in all patients, but there was no significant difference between the hemorrhagic and nonhemorrhagic groups.

Thrombin generated *in vivo* is rapidly inactivated by antithrombin to form TAT. An increase in TAT level is a specific marker of activation of the coagulation system. Both the hemorrhagic transformation group and the nonhemorrhagic group had a high TAT level before and up to 48 hours after therapy suggesting activation of the coagulation system. Activation of the coagulation and fibrinolytic systems in patients with acute ischemic stroke has previously been described.^{3,14)} Takano *et al.*¹⁰⁾ have suggested that TAT or D-dimer could be used as markers for the differentiation of cardioembolic stroke from atherothrombotic stroke or lacunar stroke in the acute phase. The differences in the responses of the coagulation and fibrinolytic systems to cardioembolic stroke and atherothrombotic stroke may be caused by the different compositions of platelets and fibrin or sizes of the thrombus. Cardioembolic stroke causes mainly fibrin-rich thrombi and atherothrombotic stroke results in mainly platelet-rich thrombi. TAT and D-dimer could also be useful indicators of acute cardioembolic stroke patients that are prone to recurrent embolization during the acute stage.¹¹⁾

The present study showed no definite correlation between the incidence of hemorrhagic transforma-

tion and dose of the thrombolytic agents, which suggests that other factors such as residual CBF in the ischemic region are responsible. However, activation of the fibrinolytic system may support the promotion of hemorrhagic transformation, since the FDP and the D-dimer levels increased significantly in the hemorrhagic transformation group.

Activation of the fibrinolytic system after thrombolytic therapy may be a risk factor for intracerebral hemorrhagic transformation. D-dimer is useful as a marker for recanalization in thrombolytic therapy for acute ischemic stroke.

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