Plasma Fibrinolysis Inhibitor Levels in Acute Stroke Patients with Thrombolysis Failure

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Background and Purpose: Thrombolytics-induced recanalization fails in a significant portion of patients with ischemic stroke, which is partly due to the resistance of clots to lysis by thrombolytic agents. The pretreatment level of endogenous fibrinolysis inhibitors may affect such thrombolysis failure.

Methods: We studied 43 stroke patients whose arterial recanalization had been evaluated by angiography, and whose blood had been obtained prior to the administration of thrombolytic agents. Plasma samples from 34 healthy volunteers were used as normal controls. Plasminogen activator inhibitor type 1 (PAI-1) and thrombin-activatable fibrinolysis inhibitor (TAFI) levels were quantified using an enzyme-linked immunosorbent assay.

Results: Arteries were recanalized [Thrombolysis in Myocardial Infarction (TIMI) grade 2 or 3] in 30 patients, but not (TIMI grade 0 or 1) in the other 13. The plasma PAI-1 level was significantly higher in patients without recanalization (nonrecanalization) than in those with recanalization and in normal controls. The TAFI levels did not differ among the groups.

Conclusions: The pretreatment PAI-1 levels are increased in acute stroke patients with thrombolysis failure.

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Key Words: Fibrinolytic agents, Acute stroke, Plasminogen activator inhibitor type 1, Thrombin-activatable fibrinolysis inhibitor

INTRODUCTION

Early and complete recanalization of an occluded artery is probably the most effective way to reduce mortality and neurologic deficits in acute stroke patients. Plasminogen activators such as tissue-type plasminogen activator (t-PA) and urokinase have been widely used to restore the blood flow to the ischemic brain, and have shown that they are effective in acute stroke patients.1,2 However, many patients still remain disabled because of hemorrhagic transformation as well as thrombolysis failure or deterioration after recanalization. Actually, it is known that recanalization is achieved in only 30-70% of stroke patients with thrombolytic treatment.3

Few studies have examined the biomarkers that may be related to thrombolysis failure in stroke.4,5 However, it is important to rapidly detect subjects who might be unsuitable for conventional fibrinolytic therapy prior to thrombolytic therapy because they may be managed with
an alternative or additive strategy such as platelet glyco-
protein IIb/IIIa receptor antagonists or mechanical clot
removal.6,7

The action of endogenous fibrinolysis inhibitors may
influence the success or failure of clot lysis, and inter-
individual variation in the plasma levels of the fibrino-
lysis inhibitors may influence the individual suscepti-
bility to the fibrinolytic treatment. Although increased
endogenous fibrinolytic inhibitor levels such as plasmi-
nogen activator inhibitor type 1 (PAI-1) are associated
with thrombolysis failure and poor outcome in patients
with acute myocardial infarction,8 little is known about
PAI-1 as a biomarker of thrombolysis failure in stroke
patients. In this study, we examined the pretreatment
plasma levels of two well-known endogenous fibrinolysis
inhibitors, PAI-1 and thrombin-activatable fibrinolysis
inhibitor (TAFI), and investigated their potential
association with thrombolysis failure in acute stroke
patients who receive thrombolytic treatment.

MATERIALS AND METHODS

1. Patients

Among a total 106 stroke patients who received
thrombolytics over a 4-year period, 43 consecutive
patients whose arterial recanalization could be evaluated
by post-thrombolysis angiography (39 by catheter angi-
ography, 3 by MR angiography, and 1 by CT angi-
ography) and whose blood could be obtained before
administering the thrombolytic agents were enrolled in
this study. The exclusions were due to not performing
angiography in 8 patients and the inability to obtain
blood samples in 55 patients. The demographic charac-
teristics of sex and age, risk factors for stroke, laboratory
data, and the initial National Institutes of Health Stroke
Scale (NIHSS) score did not differ between the 43
included and 63 excluded patients (P<0.05). Seventeen
patients were treated with intravenous (IV) t-PA, 11
with intra-arterial (IA) urokinase, and 15 with combined
IV t-PA and IA urokinase. The indication and regimen
for IV, IA, or combined IV and IA treatment, and the
outcome measurements have been reported previously.9,10

Briefly, IV t-PA was indicated when the planned
infusion could be initiated within 3 hours after symptom
onset, and IA urokinase was administered to patients
showing no early clinical responses to IV t-PA at the
end of t-PA infusion or to those who could be treated
within 3-6 hours after symptom onset. The institutional
review board approved this study, and informed consent
was obtained from the patient or the patient's represent-
tative.

The patency of the occluded arteries was assessed
using the Thrombolysis in Myocardial Infarction (TIMI)
grading system,11 and the patients were grouped into
nonrecanalization (TIMI grade 0 or 1) and recanalization
(TIMI grade 2 or 3).

2. Blood sampling

On their arrival at hospital, blood was drawn from the
patients into a heparinized tube at the time of the initial
blood sampling for the emergent laboratory workup.
Control blood samples were obtained from volunteers
aged >40 years at the time of their annual institutional
health examinations. The examinations included routine
history taking, a physical examination, blood pressure
measurements, chest x-ray, electrocardiography, and
blood tests including hemoglobin, fasting sugar, and total
cholesterol. Those with a previous history of hyper-
tension, diabetes, stroke, coronary artery diseases, inflam-
matorv diseases, or malignancies were excluded. Those
with a systolic blood pressure >140 mmHg, a diastolic
blood pressure >90 mmHg, a fasting blood sugar >140
mg/dl, or a total cholesterol >240 mg/dl were also
excluded. The control blood samples were obtained from
34 volunteers (17 men and 17 women) with a mean age
of 48 years.

The control blood samples obtained from the patients
and volunteers were immediately refrigerated and centrifuged
at 900Xg for 15 minutes at 4°C, and the plasma were
then stored at -80°C until analysis.

3. Measurement of plasma PAI-1 and TAFI levels

The PAI-1 and TAFI antigen levels were determined
using a commercially available enzyme-linked immuno-
sorbent assay (ELISA) kit (Zymutest PAI-1 Ag and Zymutest TAFI Ag, Hyphen BioMed, Andresy, France) according to the manufacturer’s instructions.

4. Statistical analysis

The levels of plasma PAI-1 and TAFI were compared among the groups for significant differences using the Kruskal-Wallis test. The Mann-Whitney U test and Fisher’s exact test were used to compare the variables between the nonrecanalization and recanalization groups.

Table 1. Demographic characteristics of the patient groups

|                      | Nonrecanalization (N=13) | Recanalization (N=30) | P value |
|----------------------|--------------------------|-----------------------|---------|
| Median age (range), years | 66 (50-76)               | 67 (25-85)            | 0.99    |
| Sex (men : women)     | 4:9                      | 17 : 13               | 0.19    |
| Risk factors, number (%) |                         |                       |         |
| Hypertension          | 8 (62)                   | 18 (60)               | 1.00    |
| Diabetes mellitus     | 3 (23)                   | 10 (33)               | 0.72    |
| Smoking               | 1 (8)                    | 9 (30)                | 0.24    |
| Previous stroke       | 3 (23)                   | 9 (30)                | 0.73    |
| Cardiac embolic sources | 8 (62)                   | 17 (57)               | 1.00    |
| Laboratory data, median (range) |                 |                       |         |
| Hemoglobin, g/l       | 128 (93-171)             | 139 (107-175)         | 0.08    |
| White blood cells, ×10^9/l | 8640 (4590-14270)       | 8440 (5550-19050)     | 0.43    |
| Blood sugar, mmol/l   | 7.16 (5.44-12.04)        | 7.05 (2.55-18.54)     | 0.57    |
| Cholesterol, mmol/l   | 4.53 (2.69-6.06)         | 4.71 (3.16-6.79)      | 0.59    |
| Involved arteries, number |                          |                       | <0.01   |
| Middle cerebral artery | 5                       | 22                    |         |
| Internal carotid artery | 6                       | 2                     |         |
| Basilar artery        | 1                       | 6                     |         |
| Posterior cerebral artery | 1                     | 0                     |         |
| Initial NIHSS score, median (range) |     | 20 (13-33)            | 17 (4-30) | 0.47    |
| Thrombolysis methods, number |                 |                       |         |
| IV t-PA               | 8                       | 9                     | 0.25    |
| IA Urokinase          | 1                       | 10                    |         |
| IV t-PA+IA Urokinase  | 4                       | 11                    |         |
| HT, number (%)        | 4 (31)                  | 8 (27)                | 1.00    |

HT: hemorrhagic transformation, NIHSS: national institutes of health stroke scale, IV t-PA: intravenous tissue-type plasminogen activator, IA: intra-arterial.

For comparison between groups, the Mann-Whitney U test was used for age, laboratory data, and NIHSS score, and Fisher's exact test was used for sex, risk factors, involved arteries, and HT.
angiography in 39 of the 43 enrolled patients. Among 17 patients treated with IV t-PA, catheter angiography was performed for IA thrombolysis immediately after infusion of t-PA in 11 patients, but IA urokinase was not administered because the artery was occluded at the proximal carotid level (3 patients) or the artery was opened or occluded at the distal branch (8 patients). The remaining six patients received diagnostic investigations within 48 hours after symptom onset (two by catheter angiography, three by MR angiography, and one by CT angiography). The median time from symptom onset to angiography was 4 hours and 10 minutes. Recanalization was achieved in 30 of the 43 patients. An occlusion at the middle cerebral artery or the basilar artery was recanalized more often (P<0.01). Otherwise, there were no significant differences between the two groups in the risk factors for stroke, the initial laboratory data, the severity of the neurological deficits based on the initial NIHSS score, the thrombolysis method, and the frequency of hemorrhagic transformation (Table 1).

The plasma PAI-1 and TAFI levels, which were measured by ELISA, were compared between the two thrombolytics-treated groups and the controls. The time interval from symptom onset to blood sampling did not differ between the two thrombolytics-treated groups (medians of 98 minutes and 80 minutes in the non-recanalization and recanalization groups, respectively; P=0.84). The PAI-1 levels differed significantly among the three groups, and was highest in the nonrecanalization group: medians of 45.2 ng/ml (interquartile range [IQR], 32.5-50.0 ng/ml), 33.1 ng/ml (IQR, 24.9-38.1 ng/ml), and 23.6 ng/ml (IQR, 17.5-37.5 ng/ml) in the nonrecanalization, recanalization, and control groups, respectively (P=0.007) (Fig. 1-A). However, the TAFI levels did not differ significantly among the groups (P=0.097) (Fig. 1-B).

Logistic regression showed that only the plasma PAI-1 levels were independently associated with recanalization (odds ratio, 0.916; 95% CI, 0.844-0.994).

**DISCUSSION**

In this study, the plasma PAI-1 levels were higher in patients with acute stroke, and higher pretreatment plasma PAI-1 levels were associated with thrombolysis failure. But the plasma TAFI levels did not differ between the control and patient groups. One study showed that increased plasma PAI-1 levels predicted clot lysis resistance in stroke patients treated with t-PA.12 In that study, evidence of successful thrombolysis was based on transcranial Doppler investigations, which is consistent with our determination of the success or failure of thrombolysis in a more definitive and direct manner by angiography.

PAI-1 is reportedly increased in blood obtained within 1-7 days after symptom onset in acute stroke patients.13-15 The findings in the present study further demonstrate...
that plasma PAI-1 levels are increased during the very early stage of stroke, because all samples were obtained from patients who were eligible for thrombolytic therapy. T-PA is rapidly neutralized by PAI-1, which binds to the active site of t-PA and forms inactive complexes.\textsuperscript{16,17} Therefore, the presence of a pretreatment plasma PAI-1 in excess of endogenous and/or exogenous t-PA or urokinase may depress their fibrinolytic activity.\textsuperscript{17}

The thrombus composition has been suggested as one of the important causes of thrombolytic resistance, and platelet-rich thrombi are disposed to resistance to thrombolysis.\textsuperscript{18,19} Possible sources of increased PAI-1 in acute stroke are the endothelium, atherosclerotic plaques, and platelets, which are major sources of circulating PAI-1.\textsuperscript{16} The latent PAI-1 in alpha-granules of platelets is converted into the active form and is released upon platelet activation and aggregation. Therefore, large amounts of active PAI-1 are released from fresh platelet-rich thrombi, which contribute to the thrombolytic resistance.\textsuperscript{17,20} In a murine carotid injury model that induced platelet-rich thrombi in wild-type and PAI-1-deficient mice, PAI-1 was found to be a major determinant of the resistance of platelet-rich arterial thrombi to lysis by t-PA.\textsuperscript{21} In contrast, the inactivation of PAI-1 using a monoclonal antibody against PAI-1 accelerated the lysis of the platelet-rich thrombi in the mesenteric arteriole of rats.\textsuperscript{12} All these findings indicate an important role of PAI-1 in inducing resistance to thrombolytics via platelet-mediated mechanisms, and suggest that an increased plasma PAI-1 level can be used as a biomarker for the prediction of thrombolysis failure in stroke patients.

Although TAFI acts as a fibrinolytic inhibitor, the plasma TAFI levels were neither increased in stroke patients nor associated with thrombolysis failure. Previous studies on coronary artery disease are also controversial.\textsuperscript{22} It has been suggested that, in contrast to PAI-1, genetic factors are much more important determinants of the plasma TAFI levels than environmental factors,\textsuperscript{23} which may explain the absence of an elevated plasma TAFI in the present study.

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