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Tissue Plasminogen Activator Mass Concentration in Ischemic Acute Stroke Patients

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Abstract: The role of endogenous fibrinolysis in cerebral infarction has been investigated, but with conflicting results. In this study, variations of tissue-type plasminogen activator (t-PA) antigen concentrations over time after stroke were examined at the acute and subacute phases of ischemic stroke in a group of patients and in a control group. The relationships between t-PA levels and stroke severity, lesion volume and clinical status were also investigated.

This study was carried out in 45 patients with acute ischemic stroke and 21 control subjects who had similar characteristics. Levels of t-PA mass concentration were measured via the ELISA, while Student's t, Chi-square and Pearson correlation methods were used.

Age ratio (57.42 ± 15.36 , 53.19 ± 10.45 , $P = 0.2$) and sex distribution (F/M: 24/21, 9/12, $P = 0.42$) of the patients and the control group were the same. The average t-PA values of the patients were considerably higher than those of the control group during acute and subacute periods [$(17.9 \pm 8.1$ ng/ml, 26.3 ± 17.9 ng/ml) vs. $(9.9 \pm 3.8$ ng/ml), ($P < 0.001$)]. No significant relation was found between t-PA either with infarct size nor with clinical neurologic status.

These results suggest that increased t-PA levels exist in ischemic stroke and the endogenous fibrinolytic system is activated during acute and subacute periods of ischemic stroke.

Key Words: ischemic stroke, risk factors, tissue plasminogen activator, t-PA

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Introduction

The majority of acute ischemic strokes in humans are due to thrombotic or thromboembolic occlusions (1,2). Several individual risk factors for ischemic stroke such as hypertension, smoking, hypercholesterolemia and obesity are also well known (1-4).

T-PA, 70,000 D mass, containing 517 amino acids composed of single serine protease, is synthesized by endothelial cells and levels which are related to the fibrinolytic system. Increased levels reflect increased fibrinolytic activity. It is a poor enzyme in the absence of fibrin, but in the presence of fibrin it strikingly enhances the activation rates of plasminogenesis. Its concentration is high in small vessels and renal vasculature. In normal conditions t-PA is low in plasma (5-10 ng/ml) and shows circadian rhythm (5). T-PA has been reported as an independent predictor for an atherothrombotic event, mostly in patients with cardiovascular disease. In addition, plasma levels of t-PA have emerged as a strong predictor of myocardial infarction (6-9). The neuroprotective effects of t-PA were demonstrated in

initial experimental stroke studies in animal models (10,11). Some studies strongly suggest that t-PA protects the brain from ischemic insult through its desirable thrombolytic action (12,13). The cerebral protective effects of t-PA were also shown in earlier stroke studies, which led to clinical trials with t-PA (14,15). The role of t-PA in cerebrovascular diseases have received less attention, and only a few studies have considered aspects of hemostasis in ischemic stroke patients (16-20). High levels of t-PA have been observed in patients with a history of stroke, while in prospective studies high levels of t-PA predicted an increased risk of stroke (14,21,22). In this study, we aimed to evaluate t-PA mass concentration and its possible relationship with other vascular risk factors in a group of patients with a first ischemic stroke.

Materials and methods

This study was based on 45 consecutive patients, aged 20 to 80 years, admitted to our neurology department as

a result of their first ever stroke. The control group comprised 21 subjects without stroke, randomly selected from the outpatient clinic. The study design was approved by our local university ethical committee and informed consent was obtained from all subjects or their relatives if the patient was unable to communicate.

The diagnostic investigation included CT or MR of the brain, duplex ultrasonography of the cervical arteries, echocardiography and detailed laboratory studies. The infarct size was calculated based on the maximum length and width of the hypodense area on the CT films. To calculate irregular volumes we used the following formula: $A \times B \times C / 2$ (23). We measured the largest diameter (A) and the perpendicular diameter (B) of the ischemic lesion and the third diameter (C) by summing up the thicknesses of the slices where the lesion was visible. Clinical neurologic status was evaluated according to a modified Matthew Scale (MMS). The patients were subclassified into a mild neurologic deficit group (MMS \geq 50) a and severe neurologic deficit group (MMS score $<$ 50).

Blood samples were taken from all patients within 3 d of acute stroke onset. Only 28 out of the 45 patients were re-examined during the subacute (2-3 weeks) period because 10 patients died and we lost contact with five. Antecubital venipuncture with a 21-gauge butterfly needle was performed by the same physician between 07.30 and 09.00 hours to minimize circadian variability in fibrinolytic measures. Venipuncture was performed on a reclining subject without tourniquet to avoid augmenting of t-PA release, which is known to accompany venous stasis, physical exertion and orthostatic changes. (24). Blood was drawn after a minimum of 10 min rest in the recumbent position. The first 5 ml of blood was discarded and next 9 ml was collected directly into pre-cooled tubes that contained sodium citrate. The tubes were gently inverted, then immediately centrifugated at 2000 g for 30 min. The recovered plasma was frozen at -80 °C until analysis.

The mass concentration of t-PA in plasma (in previous studies often termed t-PA antigen) was determined with an enzyme-linked immunosorbent assay (Biopool TintElizet-PA) that detects t-PA in complex. For t-PA mass concentration, the coefficient of variation at 8 μ g/l was 9.5% according to the manufacturer (25).

Comparisons between patients and controls were made with Student's t test or the Mann-Whitney U test

for continuous variables. The Chi-square or Fisher's exact test were used for proportions. Spearman correlation coefficients (rs) were applied to test for correlations between continuous variables.

Results

We found no statistically significant differences between patients and control subjects regarding age, sex distribution, body mass index, diabetes mellitus and current smoking or alcohol intake. The rate of hypertension and hypercholesterolemia were higher in the patient group than in the control group. For both patients and control subjects, the basic clinical features, demographic characteristics, and t-PA levels according to stage, clinical neurological status and prognosis are shown in the Table and Figure. There was no significant difference between males and females regarding t-PA mass concentration.

In patients with ischemic stroke, t-PA mass concentration levels were significantly increased in all periods compared with those of the controls ($P < 0.001$), and showed no substantial changes between periods, although t-PA mass concentration was slightly higher in the subacute period than in the acute period.

Table. The basic clinical features, demographic characteristics, and t-PA levels of the control subjects and patients according to stage, clinical neurological status and prognosis.

	Patients N = 45	Controls N = 21	P
Age	57.42 \pm 15.36	53.19 \pm 10.45	0.2
Sex			
Male/Female	24/21	9/12	0.42
Body mass index, kg/m ²			
Current Smokers	19 (42.2%)	7 (33.3%)	0.59
Alcohol	4 (8.9%)	2 (9.5%)	0.93
Obesity	25 (56.6%)	8 (38.1%)	0.29
Hypercholesterolemia	21 (46.7%)	5 (23.8%)	0.07
Diabetes mellitus	12(26.7%)	3 (14.3%)	0.26
Hypertension	31(68.9%)	7 (33.3%)	0.006
T-PA mass concentrations			
Acute stage n = 45	17.9 \pm 8.1	9.38 \pm 3.4	0.001
Subacute stage n = 31	18.7 \pm 17.9		
MMS score \geq 50 n = 24	18.5 \pm 8.4		
MMS score $<$ 50 n = 21	17.2 \pm 7.9		
Surviving patients 30	19.8 \pm 9.7		
Deceased patients 10	16.9 \pm 7.1		

Values are numbers of subjects or mean \pm SD, as appropriate
T test or Mann-Whitney U test for continuous variables; Chi-square or Fisher's exact test for proportions.

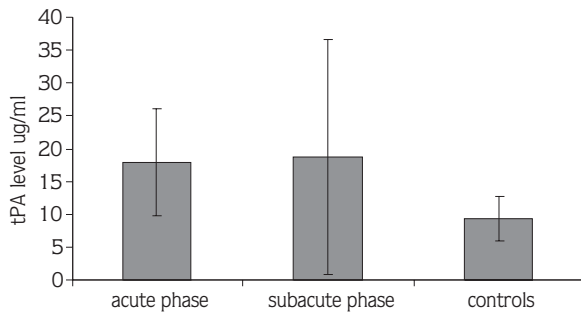


Figure. Bar graph shows the distributions of plasma t-PA mass concentrations in patients and controls.

No significant relations were found at the $P < 0.05$ significance level between t-PA levels and stroke risk factors including body mass index, smoking, cholesterol levels or blood pressure.

Regarding infarct size, which ranged from 0.30 ml to 600.00 ml (112.2 ± 192.3), no correlation was found with t-PA mass concentration at the acute and subacute phase of stroke (acute $P = 0.68$, $r = 0.064$; subacute $r = 0.09$, $P = 0.65$).

Patients who died did not differ from those who survived regarding t-PA mass concentration in the acute phase (Table). Regarding t-PA mass concentration levels, no significant difference was determined between stroke patients having $MMS > 50$ from those with $MMS < 50$, and between patients who died and survived.

Discussion

We found that t-PA concentrations in the acute and subacute period of acute ischemic were significantly increased compared with controls, in agreement with the findings of most previous studies (9,21,26), though not all (14). Although the t-PA mass concentration of our patients before stroke is unknown, the sustained elevation of t-PA levels in the subacute period may indicate increased baseline t-PA levels in patients with ischemic stroke. Although the clinical importance of increased fibrinolytic activity in acute stroke is unclear, previous reports show that fibrin formation may greatly exceed endogenous fibrinolysis during acute ischemic stroke and may indicate that the acute stroke phase is an acute hypercoagulable state (9,21). Altered fibrinolytic activity is considered as an acute phase reaction in many studies. It can be thought that a high level of t-PA is a

vascular risk factor already present before the stroke (9). The reported finding that fibrin formation may greatly exceed endogenous fibrinolysis during the acute phase of ischemic stroke may indicate that the acute stroke phase is an acute hypercoagulable state (12,13,27). T-PA has been suggested to be an acute phase reactant. In patients with angina pectoris, t-PA antigen levels have been found to correlate with levels of acute phase protein fibrinogen, C reactive protein (28).

However, it is possible that the acute-phase increases of t-PA are very short. Thus, in previous studies, t-PA levels increased immediately after surgery but returned to baseline values within 24 h (29,30).

Because stroke is also an acute event followed by a convalescent phase, the findings of these surgical studies may have an implication for the interpretation of our findings.

In our study, the early rapid increases in the t-PA antigen were probably undetected because most of the blood samples were collected 1 to 3 d after the onset of stroke. Although t-PA antigen levels may increase as an acute-phase reaction, this increase is often of very short duration, and our follow-up results show that increases in t-PA antigen levels among stroke patients cannot be explained solely by an acute-phase reaction. Thus, it is conceivable that impaired fibrinolysis preexisted in our stroke patients and less likely that it represents a phenomenon secondary to the stroke event. Accumulated evidence suggests that elevated t-PA antigen levels may indicate an increased risk of stroke.

The results from the previous prospective studies in which a high plasma t-PA mass concentration was found to be predictive of stroke (9,18,25,26) and myocardial infarction (6-8,33,34) are in accordance with our finding that stroke patients have a higher acquired t-PA mass concentration level (19).

Our findings are also in accordance with those of earlier studies and show that high t-PA antigen levels can be found in the same group of individuals examined in either the acute or subacute phase after stroke (19,21,26,35). Our results show that infarct size is not related to t-PA concentration in the acute and subacute stage of stroke. There were no correlations between MMS and plasma t-PA levels. These findings show that t-PA levels are not an indicator for prognosis in ischemic cerebrovascular disease.

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