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Clinical Study

Dynamic changes in plasma tissue plasminogen activator, plasminogen activator inhibitor-1 and beta-thromboglobulin content in ischemic stroke

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ABSTRACT

The aim of this paper is to investigate the corresponding variations of plasma tissue plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1) activities, and beta–thromboglobulin (β-TG) content in patients during different stages of ischemic stroke. Ischemic stroke is a common disease among aging people and its occurrence is associated with abnormalities in the fibrinolytic system and platelet function. However, few reports focus on the dynamic changes in the plasma fibrinolytic system and β-TG content in patients with ischemic stroke. Patients were divided into three groups: acute, convalescent and chronic. Plasma t-PA and PAI-1 activities were determined by chromogenic substrate analysis and plasma β-TG content was detected by radioimmunoassay. Patients in the acute stage of ischemic stroke had significantly increased levels of t-PA activity and β-TG content, but PAI-1 activity was significantly decreased. Negative correlations were found between plasma t-PA and PAI-1 activities and between plasma t-PA activity and β-TG content in patients with acute ischemic stroke. There were significant differences in plasma t-PA and PAI-1 activities in the aged control group, as well as in the acute, convalescent and chronic groups. It can be speculated that the increased activity of t-PA in patients during the acute stage was the result of compensatory function, and that the increase in plasma β-TG level not only implies the presence of ischemic stroke but is likely a cause of ischemic stroke. During the later stages of ischemic stroke, greater attention is required in monitoring levels of PAI-1.

1. Introduction

Stroke is one of the leading causes of death around the world as well as being the primary cause of adult disability [1]. China has a high incidence of stroke, greater than 336 per 100,000 population in 2010 [2]. The occurrence of ischemic stroke is associated with abnormalities in fibrinolysis and platelet function [1,2]. Coagulation and fibrinolysis are precisely regulated by a combination of substrates, activators, inhibitors, cofactors and receptors under physiological conditions. Activation of coagulation ultimately produces thrombin resulting in the formation of thrombus by the conversion of fibrinogen to fibrin and by platelet activation [3]. Although plasminogen can also be converted to plasmin by urokinase in plasma [3], tissue plasminogen activator (t-PA), a serine protease, is the most important activator of fibrinolysis *in vivo* and converts plasminogen into fibrinolytically active plasmin. It is produced mainly in vascular endothelial cells but also in smooth muscle cells, monocytes and megakaryocytes. Plasminogen activator inhibitor-1 (PAI-1) is a single chain glycoprotein which is synthesized mainly in endothelial and hepatic cells but also in smooth muscle cells and adipose tissue. It has an inhibitive effect on serine proteases and is considered to be the major inhibitor of fibrinolysis and a marker of vascular endothelial dysfunction. Both t-PA and PAI-1 are regulative substances of the fibrinolytic system *in vivo* [4,5]. Both clinical and animal studies have demonstrated that during cerebral ischemia, plasma t-PA and/or PAI-1 levels change significantly [6–8]. Beta–thromboglobulin (β-TG) is the most abundant specific protein within the platelet α-granule and its presence in serum reflects platelet activation [9]. An earlier study found that in ischemic cerebrovascular diseases, plasma β-TG levels are significantly elevated [10]. These blood biomarkers play an important role in thrombogenesis and their measurement has been widely adopted in the field of cardiovascular and cerebrovascular diseases [11–14]. However, there are very few studies evaluating the simultaneous determination and correlation of these

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three blood biomarkers in patients with ischemic stroke. In addition, few studies have examined the relationship during different stages of ischemic stroke and the fibrinolytic system.

In order to investigate dynamic changes in the fibrinolytic system and in platelet function in patients with ischemic stroke, we measured the plasma activities of t-PA and PAI-1, and β-TG content in patients with acute ischemic stroke and analyzed the correlation between the three biomarkers. We then evaluated the plasma activities of t-PA and PAI-1 in patients during the convalescent and chronic stages of ischemic stroke.

2. Patients and methods

2.1. Patients

Blood samples were collected from 24 patients with ischemic stroke (middle cerebral artery occlusion) during different stages of the disease. The acute stage is within 2 days of ischemic stroke onset, the convalescent stage two weeks after the onset of ischemic stroke and the chronic stage 6 months following onset. Patients were taken to the Emergency Department at Shanghai East Hospital, Tongji University School of Medicine eleven patients were men and 13 were women. Mean age was 63 years (range: 55–86). The inclusion criterion was patients with ischemic stroke confirmed by head CT scan. Exclusion criteria were cerebral hemorrhage, hemorrhagic transformation, progressive ischemic stroke or repeated stroke and cardio embolism. Among the patients with ischemic stroke, six had hypertension, 11 had hyperlipidemia and five had diabetes (Table 1). All patients were discharged from hospital, had recovery of limb function, limb function and could walk independently.

We had 35 healthy volunteers consisting of 21 men and 14 women from Shanghai Geriatric University with a mean age of 62 years (range: 55–80). Subjects from the control group did not suffer from liver, kidney, blood, cardiac, cerebrovascular or thrombotic diseases.

The patient control group (non-ischemic stroke) consisted of 20 patients (12 men and eight women) with a mean age of 61 years (range: 54–83). Among the patients, eight had hypertension, six had hyperlipidemia and six had diabetes.

Table 1
Details of patients with acute ischemic stroke

Characteristic	Patients
Mean age (years)	63.4 ± 8.3
Sex (%)	45.8 M, 54.2F
Weight (kg)	64.1 ± 13.2
NIHSS score (mean)*	12.0 ± 6.3
Systolic blood pressure (mmHg)	153.6 ± 18.4
Diastolic blood pressure (mmHg)	84.8 ± 14.5
Hyperlipidemia (%)	45.8
Diabetes (%)	20.8
Previous use of aspirin or antiplatelet drugs (%)	12.2
History of stroke (%)	8.9
Smoking status (%)	
Never smoked	54.2
Ex-smoker	16.4
Current smoker	29.4
Type of stroke (%)	37.5 (L-MCAO), 62.5 (R-MCAO)

All values are reported as the mean ± standard deviation, unless otherwise specified.
L-MCAO = left-middle cerebral artery occlusion, NIHSS = National Institutes of Health Stroke Scale, R-MCAO = right-middle cerebral artery occlusion.
* Scores on the NIHSS range from 0 to 42, with higher values reflecting more severe neurological impairment (<5 mild impairment, ⩾25 very severe impairment).

All patients and healthy controls received no statins, calcium channel blockers or other drugs that may influence the level of blood biomarkers for the 2 weeks prior to study entry. The protocol was approved by the Local Ethics Committee of Shanghai East Hospital, Tongji University School of Medicine.

2.2. Methods

2.2.1. Sample processing

Plasma was obtained by venous blood collection from all subjects using size 8 needles. The first 2 ml of blood was added to a test tube containing anti-coagulant agent (0.13 M natrium citricum; anti-coagulant agent to blood 1:9 v/v) to be used for the determination of the plasma t-PA and PAI-1 activities. Next, the syringe was detached from the needle and 2 ml venous blood was freely dripped into a separate plastic test tube containing anti-coagulant agent (ethylenediaminetetraacetic acid, theocin and prostaglandin E1; anti-coagulant agent to blood 1:9 v/v) for the determination of plasma β-TG content. Blood and anti-coagulant agent were mixed gently. Plasma was collected by centrifugation (3000 rpm, 20 min) at 4 °C before being subpackaged. Acetic acid buffer (pH 3.9) and a portion of plasma were mixed in equal volumes for the determination of t-PA activity. All samples were stored at −70 °C until analysis.

2.2.2. Determination

The plasma activities of t-PA and PAI-1 were determined by chromogenic substrate analytical method. The kits used in this study were obtained from the Laboratory of Molecule Heredity at Fudan University School of Medicine.

Under the action of an enzyme such as fibrin, t-PA rapidly activates plasminogen to form plasmin which undergoes hydrolysis as But-CHT-Lys-pNA generating free paranitroaniline (pNA). pNA has a strong absorption peak at 405 nm wavelength. Therefore, plasma activity of t-PA can be measured indirectly. Briefly, the plasma sample was diluted 1:60 with buffer, t-PA standard (100 International units [IU]/ml) was diluted to 1.0 IU/ml with acidified and t-PA-free plasma, a series of t-PA standards were prepared by buffer solution (unit of activity is 0–0.04 IU/ml). The standard and sample wells for the ELISA were set up and 100 μl of t-PA standard and sample were added to the wells, respectively. Next, 100 μl of plasminogen chromogenic substrate mixture was added to each well and the ELISA plates were incubated in a 37 °C water bath for 5 h. The sample absorbance was then measured on a microplate reader at 405 nm. The measurement of plasma PAI-1 activity was identical to that of t-PA according to the instructions [15]. The plasma activities of t-PA and PAI-1 were expressed in IU and arbitrary units (Au), respectively. The 1.0 Au of PAI-1 was defined as the amount required for 1.0 IU activity of t-PA to be inhibited by PAI-1.

The plasma content of β-TG was determined by radioimmunoassay according to the instruction manual [16]. The kits used were obtained from the Ruijin Hospital, Shanghai Jiaotong University School of Medicine. The plasma content of β-TG was expressed as ng/ml.

2.2.3. Statistical analysis

All statistical analyses were performed using SPSS Statistics software (version 14.0; IBM Corporation, Armonk, NY, USA). The plasma activities of t-PA, PAI-1, and β-TG content in healthy controls and patients were expressed as the mean ± standard deviation (mean ± SD). Differences among the groups were assessed using a one-way analysis of variance with *post hoc* multiple comparisons, the average fold change at each stage was assessed by non-parametric tests and differences between the two separate groups were assessed by independent sample *t*-test. Correlation