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Evaluation of Tissue Plasminogen Activator and Plasminogen Activator Inhibitor-I Levels in Acute Myocardial Infarction

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Abstract

Fluctuations in tissue plasminogen activator (t-PA) activity, t-PA antigen, and plasminogen activator inhibitor-I (PAI-I) antigen levels were evaluated in blood samples obtained from 84 patients with initial uneventful acute myocardial infarction (AMI) and 35 patients with reinfarction and fatal infarction (patients with bad prognoses). Patients with initial AMI had significantly higher levels of t-PA activity than those of 14 patients with angina pectoris. Tissue plasminogen activator activity peaked between day 7 and 19 after the initial attack of AMI. Plasminogen activator inhibitor-I antigen level decreased significantly between day 2 and 19, then returned to the baseline levels of patients with angina pectoris nearly 4 weeks later. The t-PA activity levels of patients with reinfarction were significantly lower than those in patients without events between day 0 and 3 and between day 7 and 19. The percentage stenosis in the coronary arteries measured by coronary angiography was negatively correlated with t-PA activity. This information may help in selecting aggressive treatments such as thrombolysis by recombinant t-PA and predicting the prognosis for patients with AMI.

Key Words
myocardial infarction (acute), immunologic technique, angiography (coronary), plasminogen activators (tissue), plasminogen activator inhibitors

INTRODUCTION

Tissue plasminogen activator (t-PA) is a fibrinolytic enzyme released from vascular endothelial cells, in response to exertion or psychological stress, as well as in coagulation and fibrinolysis abnormalities associated with thrombosis and hemorrhage. Fluctuations in t-PA activity occur in patients with diseases such as liver cirrhosis[1], deep venous thrombosis[2], myocardial infarction[3] and cerebral infarction[4]. Colucci *et al.*[5] showed a relationship between plasminogen activator inhibitor-I (PAI-I) activity and bilirubin. Activated t-PA is inactivated in blood by the specific fast-acting inhibitor (PAI-I), and by nonspecific inhibitors such as α_2-plasmin inhibitor, triglycerides[6] and desmopressin acetate[7], and promoted by high-density lipoprotein cholesterol[6]. We previously described a sensitive and spe-

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cific method for measuring t-PA activity in a diluted sample of whole blood, using a dipstick coated with monoclonal antibody (SP-322)[8–10], combined with an epitope without cross-reaction on a catalytically active residue of t-PA.

Myocardial infarction is now known to be caused mainly by coronary thrombi[11]. Increased thrombogenicity is enhanced by impairment of fibrinolysis. Fast-acting PAI-I may be important in the pathogenesis of myocardial infarction in patients with stable coronary arterial disease[12]. We evaluated the fluctuations of t-PA and PAI-I in blood from patients with initial acute myocardial infarction (AMI) and patients with reinfarction to investigate the equilibrium between coagulative and fibrinolytic enzymes following myocardial infarction, and to clarify whether fibrinolytic disorders are a cause of differences in prognosis between patients with uneventful AMI and those with myocardial infarction resulting in poor outcome (reinfarction or death).

MATERIALS AND METHODS

Patients

We evaluated 84 Japanese patients, 54 males and 30 females (mean age ± standard deviation : 62.0 ±13.4 years old), who were recovering from initial AMI (patients with uneventful course) and 35 patients, 18 males and 17 females (age : 65.3±13.6 years old), with reinfarction and death (patients with bad outcomes). We compared t-PA activity, t-PA antigen, and PAI-I antigen levels in blood samples obtained from these patients with those from 14 patients with angina pectoris (seven males and seven females, age: 60.7±10.0 years old).

The average period from initial AMI to reinfarction in patients with reinfarction was 950.3 ±510.8 days, and from the present attack to death was 51.5±25.6 days. Severity of myocardial infarction varied in both groups. No patients underwent percutaneous transluminal coronary angioplasty.

Twenty blood samples obtained from patients with initial AMI were collected on the day of attack and day 1, 20 samples on days 2 to 3, 13 samples on days 4 to 6, 15 samples on days 7 to 19, 12 samples on days 25 to 33, and 10 samples on days 33 and beyond. Different patients were sampled on each day, excluding six who were sampled twice.

Twelve blood samples obtained from patients with reinfarction and death were collected on the day of attack and day 1, 9 samples on days 2 to 3, 7 samples on days 4 to 6, 6 samples on days 7 to 19, and 4 samples on days 33 and beyond. Different patients were sampled on each day, excluding three sampled twice.

Assay for t-PA activity

Blood samples were collected between 9:00 and 11:00 by venipuncture using a vacutainer containing 1.5 m*l* and a dipstick coated with the monoclonal antibody, SP-322. After incubation for 1 hour at room temperature, the dipstick was washed with Tris-hydrochloride buffer (pH 7.4) containing 0.05% of polyoxyethylene sorbitan laurate. A fragment of the dipstick was placed into a microtiter plate well and incubated with 20 mM of Tris-hydrochloride buffer containing 0.45 mM S-2251 (Kabi Vitrum, Stockholm, Sweden), 120 μg/m*l* of Bromcyanoid fibrinogen and 0.042 μM Lys-plasminogen as a substrate solution. After incubation for 4 hours, the development of color in the wells was measured using the optical density at 405 nm. Tissue plasminogen activator activity was determined from a standard curve.

Assay for tissue plasminogen activator antigen

Blood samples used for the assay of t-PA activity were centrifuged at 3,000 rpm at 4°C for 30 minutes. Supernatants were stored at −80°C until the t-PA antigen and PAI-I antigen were measured. The t-PA antigen level was determined by an enzyme-linked immunosorbent assay (ELISA) using SP-322 as the primary antibody, and goat-antihuman melanoma t-PA polyclonal antibody conjugated with biotin as the second antibody. After incubation, a streptavidin-biotin peroxidase complex was added to each well with 3,3',5,5'-tetramethylbenzidine.

Assay for PAI-I antigen

Plasminogen activator inhibitor-I antigen level was determined by ELISA using the reagents (IMMULYSE PAI-I) obtained from Biopool AB (Umea, Sweden). The t-PA index[6,13,14] and T/P 100 were calculated as follows :

t-PA index = (t-PA activity level, ng/m*l*)/(t-PA antigen, ng/m*l*) × 100

T/P 100 = (t-PA antigen level, ng/m*l*)/(PAI-I antigen, ng/m*l*) × 100

Percentage stenoses of the coronary arteries

Coronary angiography was performed in 33 patients with initial AMI. Percentage stenosis of the coronary arteries was measured using callipers, and the value of the most stenosed artery was used for the analysis.

Statistical analysis

Conventional methods were used for calculating the trimmed mean (5%) and the 5% trimmed range. Mean values for measured variables were compared using one-way analysis of variance and Scheffe's test. Coefficients of skew and kurtosis were calculated to test the deviation from a normal distribution. Correlations between percentage stenoses of the coronary arteries and t-PA, PAI-I, t-PA index and T/P 100 were estimated.

Transformation calculations (logarithmic, reciprocal, square, square root or exponential) were carried out on the individual values of skewed variables before statistical computations and significance testing. The maximum correlation funcitons were determined from 11 bivariate regression models. The analysis was based on 33 patients who underwent coronary angiography, for whom there was data. $p<0.05$ was accepted as statistically significant level.

RESULTS

Mean values of t-PA activity, t-PA antigen, PAI-I antigen, t-PA index and T/P 100

Table 1 shows the mean values of t-PA activity, t-PA antigen, t-PA index, PAI-I antigen and T/P 100 in blood obtained from 84 patients who had initial AMI with uneventful courses, 35 patients with reinfarction and infarction followed by death (a group with bad prognoses), and 14 patients with angina pectoris. The 84 patients with initial AMI had significantly higher values of t-PA activity ($p<0.034$) and T/P 100 ($p<0.025$) than the 14 patients with angina pectoris. The t-PA activity ($p<0.001$), t-PA antigen ($p<0.001$) and t-PA index ($p<0.004$) levels of the 35 patients with bad outcomes were significantly lower than those of patients with uneventful courses. The t-PA antigen ($p<0.019$) and PAI-I antigen ($p<0.016$) levels of patients with bad outcomes significantly exceeded those of patients with angina pectoris (**Table 1, Fig. 1**).

Fluctuations of t-PA activity, t-PA antigen, PAI-I antigen, t-PA index and T/P 100 levels in blood from patients with initial AMI and uneventful courses

Concentrations of t-PA activity peaked between days 7 and 19, then decreased after day 33 to the baseline levels occurring in patients with angina pectoris. Between days 7 and 33, such levels significantly exceeded those of patients with angina pectoris. Concentrations of t-PA antigen were significantly higher on the day of the attack and day 1 than those of patients with angina pectoris. Levels of the t-PA index peaked between days 7 and 19, then decreased gradually. Concentrations of PAI-I antigen were significantly lower on days 2 to 19 than those of patients with angina pectoris. The values of T/P 100 of patients with AMI were higher on the day of attack to day 19 than those of patients with angina pectoris (**Table 2, Fig. 1**).

Fluctuations of t-PA activity, t-PA antigen, PAI-I antigen, t-PA index and T/P 100 in blood from patients with reinfarction and death

The mean t-PA activity in blood obtained from patients with reinfarction was significantly lower ($p<0.048$), and t-PA antigen ($p<0.032$) or PAI-I antigen ($p<0.004$) was significantly higher on the day of attack and day 1 than in patients with uneventful initial AMI. Mean values of the t-PA antigen ($p<0.004$) and PAI-I antigen ($p<0.062$) in patients with bad prognoses were significantly higher on days 2 to 3 than those of patients with uneventful initial AMI. The mean values of the t-PA activity ($p<0.009$) and the t-PA index ($p<0.007$) were significantly lower, and those of the t-PA antigen ($p<0.038$) and PAI-I antigen ($p<0.001$) of patients with reinfarction were significantly higher on days 7 to 19 than those of patients with uneventful initial AMI (**Table 2, Fig. 2**).

Coronary stenoses and fibrinolytic system

Results of coronary angiography using the percutaneous transfemoral technique revealed significant negative correlations between percentage stenoses of the coronary arteries and t-PA activity ($p<0.005$) or t-PA index ($p<0.001$; **Fig. 3**)

DISCUSSION

Juhan-Vague *et al.*[2,15] found that an impaired re-

Table 1 Levels of tissue plasminogen activator (t-PA) activity, t-PA antigen, t-PA index, plasminogen activator inhibitor-I (PAI-I) antigen and T/P 100 in blood from patients with uneventful initial myocardial infarction and patients with reinfarction and death

	t-PA activity (ng/m*l*)	t-PA antigen (ng/m*l*)	t-PA index	PAI-I antigen (ng/m*l*)	T/P 100
Uneventful course (n=84)	0.78 (0.10)	8.4 (0.5)	11.4 (1.3)	14.3 (1.0)	76.4 (5.0)
Reinfarction (n=35)	0.15 (0.09)	14.2 (1.9)	2.69 (0.64)	47.1 (7.4)	54.1 (9.5)
Angina pectoris (n=14)	0.28 (0.07)	6.2 (0.6)	6.69 (2.54)	23.2 (3.0)	35.2 (5.8)

Values are standard error of the mean (SEM).
T/P 100=(t-PA antigen level, ng/m*l*)/(PAI-I antigen, ng/m*l*)×100.

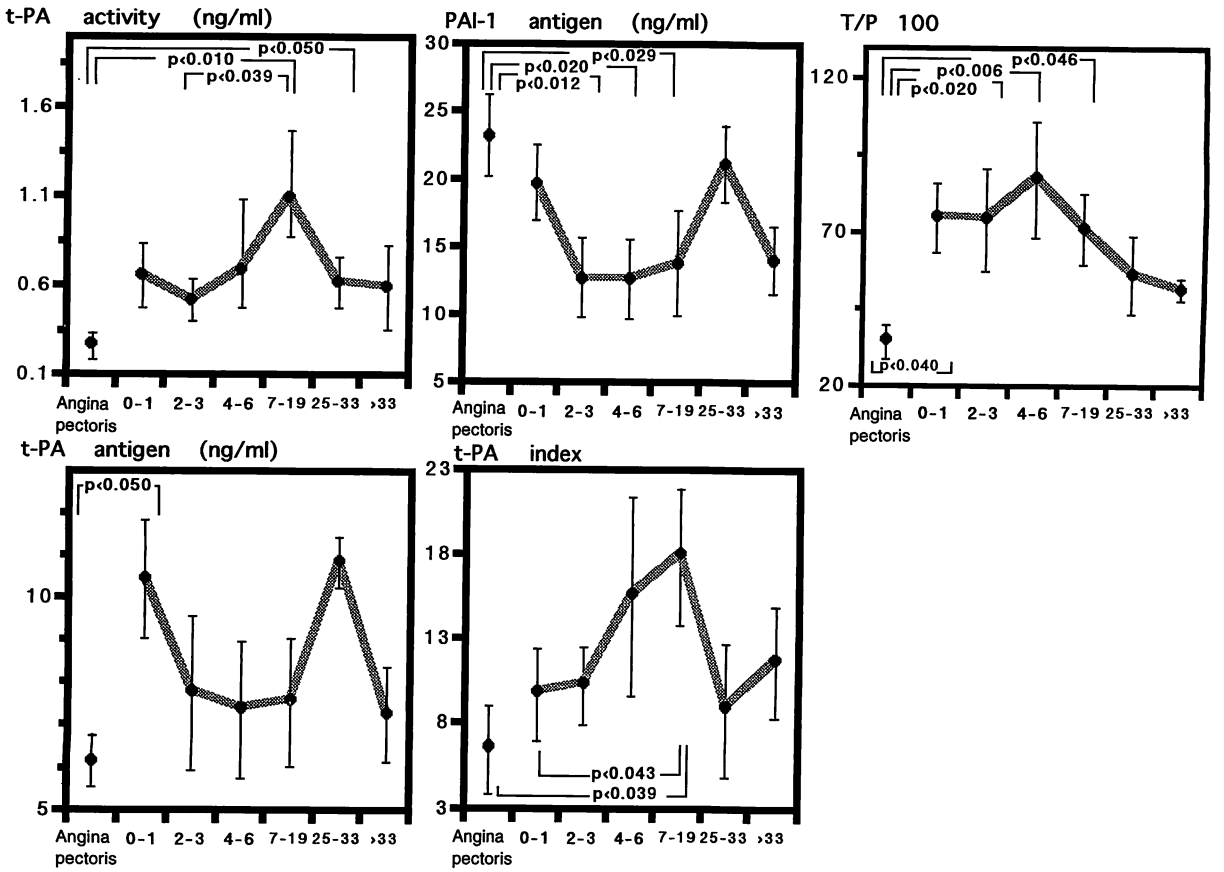

Fig. 1 Fluctuations of fibrinolytic enzyme levels in blood obtained from patients with acute myocardial infarction and uneventful courses

Differences in fibrinolytic parameters were compared between patients with initial AMI and patients with angina pectoris.
Values are mean ±SEM. Abbreviations as in Table 1.

lease of t-PA was responsible for the defect in fibrinolysis observed in about 10% of patients with recurrent deep vein thrombosis. Paramo *et al.*[16] showed that PAI-I levels are increased in patients with angiographically documented coronary atherosclerosis and suggested that elevated levels of this inhibitor might be a risk factor for the development of coronary artery disease. Hamsten[17] reported that patients who had survived a myocardial infarction showed low t-PA activity after venous occlusion

Table 2 Day-to-day fluctuations in fibrinolytic enzyme level

A. Uneventful course

Days after the attack	Sample number	t-PA activity (ng/m*l*)	t-PA antigen (ng/m*l*)	t-PA index	PAI-I antigen (ng/m*l*)	T/P 100
0–1	20	0.67 (0.18)	10.5 (1.4)	9.9 (2.7)	19.8 (2.8)	75.1 (11.2)
2–3	20	0.53 (0.12)	7.8 (1.8)	10.4 (2.3)	12.8 (2.9)	74.6 (16.7)
4–6	13	0.79 (0.30)	7.4 (1.6)	15.7 (5.9)	12.7 (2.9)	87.6 (18.9)
7–19	15	1.18 (0.30)	7.6 (1.5)	18.1 (4.1)	13.9 (3.9)	71.2 (11.5)
25–33	12	0.63 (0.14)	10.9 (0.6)	9.0 (3.9)	21.2 (2.8)	56.7 (12.4)
>33	10	0.60 (0.24)	7.3 (1.1)	11.8 (3.3)	14.1 (2.5)	51.9 (3.5)

B. Reinfarction

Days after the attack	Sample number	t-PA activity (ng/m*l*)	t-PA antigen (ng/m*l*)	t-PA index	PAI-I antigen (ng/m*l*)	T/P 100
0–1	12	0.07 (0.01)	17.0 (3.6)	0.78 (0.29)	55.1 (9.3)	39.7 (11.2)
2–3	9	0.18 (0.08)	17.9 (5.3)	3.91 (1.41)	37.4 (16.5)	86.0 (28.9)
4–6	7	0.34 (0.06)	8.2 (4.1)	8.06 (1.72)	11.3 (5.4)	68.6 (15.3)
7–19	6	0.17 (0.10)	16.1 (3.4)	1.89 (1.43)	55.1 (8.9)	35.7 (9.9)
>33	4	0.32 (0.24)	8.9 (1.5)	3.23 (2.30)	94.6 (50.7)	26.8 (11.3)

Values are mean ± SEM. Abbreviations as in Table 1.

and high levels of release of t-PA antigen 3 years after the infarction. Gram *et al.*[3] reported that a subgroup of patients who survived AMI were characterized by abnormally low fibrinolytic activity and normal euglobulin activity in the presence of excess C1-inhibitor.

We investigated t-PA and PAI-I levels in blood obtained from patients with or without previous myocardial infarctions, and analyzed the correlations between levels of t-PA activity or the t-PA index in blood obtained from patients with AMI and the severity of coronary arterial stenoses. Patients with uneventful initial AMI had significantly higher values of t-PA activity and T/P 100, and lower values of PAI-I antigen, than patients with angina pectoris. The values of t-PA activity, t-PA index and T/P 100 of patients with reinfarction were all significantly lower than those of patients with uneventful AMI. The levels of t-PA antigen and PAI-I antigen of patients with bad prognoses significantly exceeded those of patients with uneventful initial AMI. These results suggest that fibrinolytic agents as a supplement to other therapies may be effective, especially for patients with reinfarction. We tried to clarify the fluctuations of t-PA and PAI-I in blood after AMI with an uneventful course. We found that t-PA activity levels peaked between days 7 and 19, then decreased to the baseline level seen in patients with angina pectoris. Concentrations of PAI-I antigen decreased significantly between days 2 and 19, compared with those of patients with angina pectoris, suggesting that days 7 to 19 may be a period requiring supplementary t-PA for patients with bad prognoses. T/P 100 levels of patients with uneventful courses were significantly higher from the day of attack to day 19, in parallel with the t-PA index, and returned to the baseline after more than a month, which may show that t-PA and its specific inhibitor are more activated and effective for about a month than under usual conditions. The mean value of t-PA activity in blood obtained from patients with bad outcomes showed significantly lower levels from the day of attack and day 1, as well as days 7 to 19 than in patients with uneventful courses. Mean values of t-PA index in blood obtained from patients with bad outcomes were lower on days 7 to 19 than those with initial AMI. Concentrations of t-PA antigen and PAI-I antigen in blood obtained from patients with bad outcomes were higher on the day of attack to day 3, and days 7 to 19

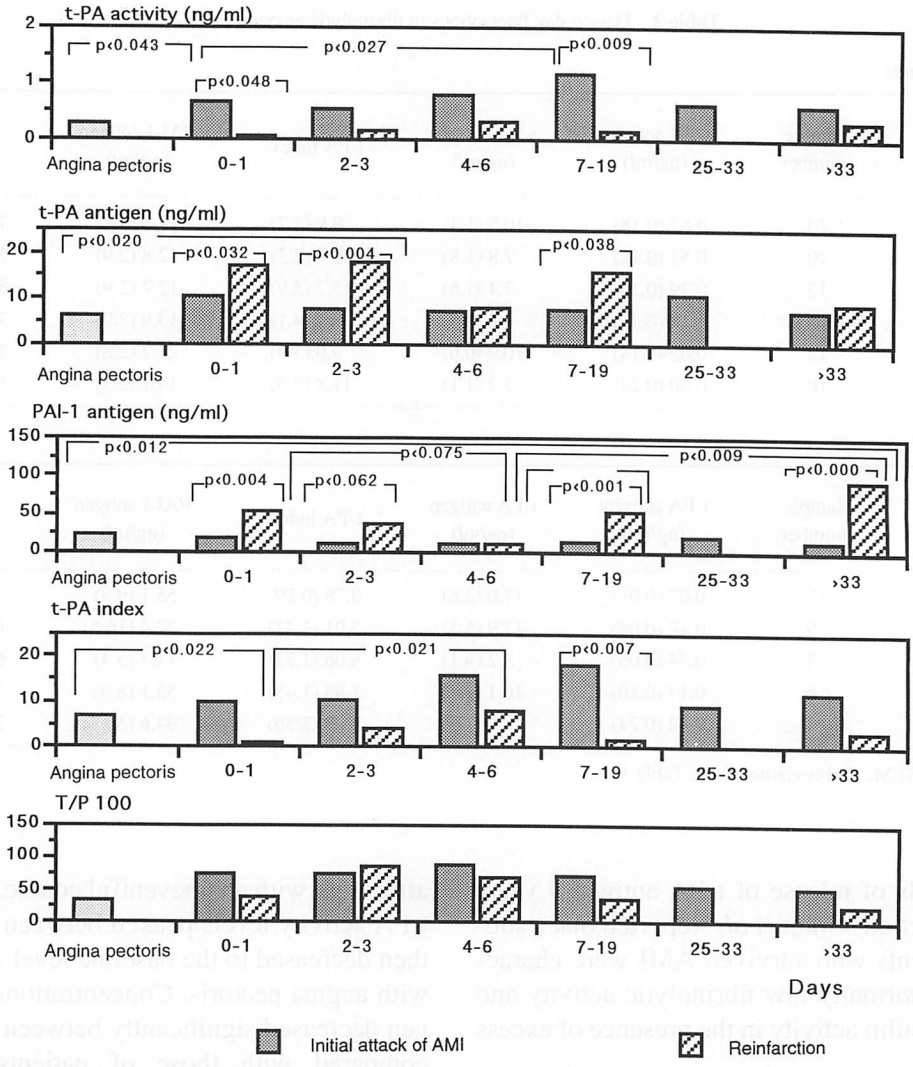

Fig. 2 Fluctuations in t-PA and PAI-I levels in blood obtained from patients with reinfarction and bad outcomes

Mean differences in fibrinolytic parameters were compared between patients with reinfarction and those with uneventful initial myocardial infarction, or those with angina pectoris on each day.
AMI = acute myocardial infarction. Other abbreviations as in Table 1.

than those from patients with uneventful courses. These results suggest that fibrinolytic enzymes may be activated immediately after the attack to help recovery from the initial event as an early phase effect, then may be reinforced for some reason such as secondary wound healing after 1 or 3 weeks as a late phase effect in the clinical course.

Percentage stenosis of the coronary arteries showed significant and negative correlations with t-PA activity and t-PA index levels. This may explain why the incidence of increased thrombogenicity in patients with AMI is increased by the presence of sclerotic changes in the coronary arteries.

Recent conflicting findings on the association between coronary artery disease and decrease in fibrinolysis suggest that the methods used to assay t-PA activity and methods of blood sampling may be critical. We attempted to take blood samples without venous occlusion and to measure the t-PA activity of whole blood immediately after venipuncture using an antibody (SP-322 method). This antibody combines with an epitope without cross-reaction on a catalytically active residue of t-PA which helps to minimize the inhibitory effect of PAI-I[18,19]. Most myocardial infarctions are caused by coronary thrombi, and only rarely by vasospasm. Increased thrombogenicity can be linked to impaired fibrinolysis[11]. Our present study describes the day-to-day fluctuations of t-PA and PAI-I in blood obtained from patients with AMI and uneventful

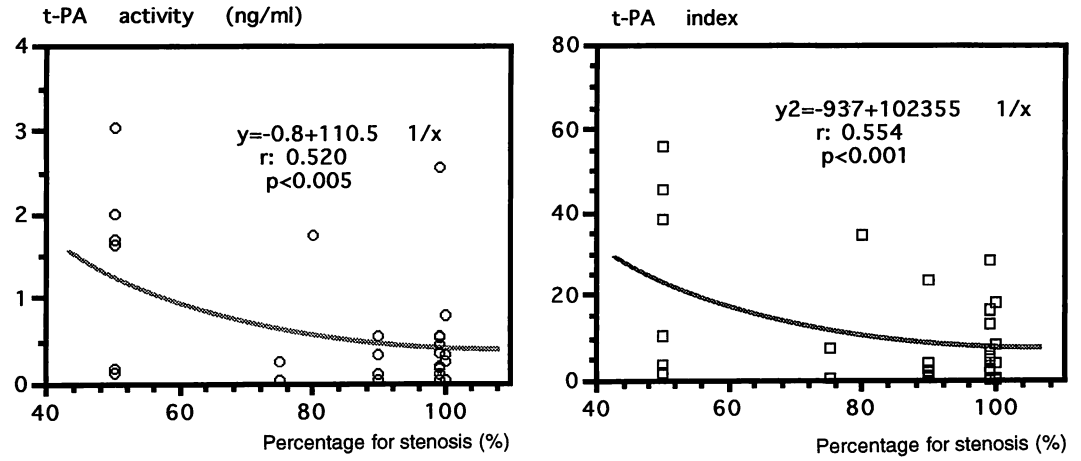

Fig. 3 Correlations between t-PA activity, t-PA index levels and percentage stenosis of coronary arteries
Abbreviations as in Table 1.

courses as compared with those who had reinfarction and bad outcomes. We examined the difference in the levels of t-PA and PAI-I in blood from the two groups. We found that t-PA activity and t-PA index levels were significantly lower, and t-PA antigen and PAI-I antigen levels significantly higher in blood obtained from patients with reinfarction and death than from those with initial AMI. The percentage stenoses of the coronary arteries were negatively correlated with t-PA activity and t-PA index levels. These results suggest that the measurements of fibrinolytic parameters (t-PA activity and antigen, PAI-I) may be invaluable for the diagnosis of atherosclerotic changes of vessels and evaluation of the prognosis perhaps for AMI[20]. Such information may be useful in decisions about aggressive treatment such as thrombolysis using recombinant t-PA, as well as predicting the prognosis.

要　約

急性心筋梗塞時の組織プラスミノーゲン活性化因子ならびにプラスミノーゲン活性化因子インヒビターI値の検討

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初発急性心筋梗塞84例および再梗塞35例を対象として，組織プラスミノーゲン活性化因子(t-PA)活性および抗原ならびにプラスミノーゲン活性化因子インヒビターI (PAI-I)値の変動を検討した．初発心筋梗塞の患者は14例の狭心症患者より有意なt-PA活性の上昇を示した．t-PA活性は発症後7–19日にピークに達し，約4週後に初期値に復した．再梗塞の患者のt-PA活性値は初発梗塞患者に比べ0–3日目と7–19日目に低値を示した．冠動脈造影上の狭窄率はt-PA活性値と有意な負の相関を示した．これらの所見は遺伝子組み替え型t-PAなどの療法の決定や急性心筋梗塞の予後判定に有用な情報であると考えられた．

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