Defective fibrinolysis in Behçet's syndrome: significance and possible mechanisms

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SUMMARY Reduced fibrinolytic activity has frequently been reported in Behçet's syndrome, but both the underlying mechanism and its relation to the development of thrombosis are unclear. The fibrinolytic activity was studied in seven patients with Behçet's syndrome and 12 patients with idiopathic oral ulceration. The patients with Behçet's disease had significantly reduced resting tissue plasminogen activator (t-PA) both by euglobulin fibrin plate lysis and enzyme linked immunosorbent assay (ELISA), but the tissue plasminogen activator inhibitor (t-PAI) was not significantly different from the control value. After stimulation by venous occlusion the euglobulin fibrin plate lysis results showed three good responders and four poor responders, but neither t-PA antigen nor t-PAI concentrations showed any clear difference between the two groups. Interestingly, three out of the four patients with a history of thrombosis were good responders. The group with oral ulceration showed no significant difference from the controls for any of these measurements. These findings suggest that the reduced resting fibrinolytic activity is usually due to decreased production of t-PA, but that the defective response to stimulation may be multifactorial in origin. The fibrinolytic abnormalities appear unrelated to the development of thrombosis.

Behçet's syndrome is an uncommon systemic vasculitic disorder complicated in about one third of cases by venous thrombosis.¹ The reduced fibrinolytic activity well reported in this syndrome²⁻⁴ may contribute to these thrombotic events,^{2,4} and both the thrombotic tendency and the abnormal fibrinolysis are reported to be related to disease activity.^{1,2} An associated failure of F VIII R:Ag production has been cited to support the theory that vasculitic damage may lead to decreased production of tissue plasminogen activator (t-PA) by the endothelium, and hence the reduced fibrinolysis.⁴ The possibility of a fibrinolytic inhibitor has also been proposed,² and increased plasma t-PAI, the rapidly acting inhibitor of t-PA, is a likely candidate because it has been shown to rise in a non-specific acute phase manner in a variety of disorders⁵ and also because it is thought to have a role in the pathogenesis of idiopathic deep venous thrombosis.⁶ The distinction between these two mechanisms is important because

it may have implications for the treatment and prevention of thrombosis in patients with Behçet's disease. We therefore assessed the relative contributions of diminished t-PA production and increased concentrations of t-PAI to abnormal fibrinolysis in seven patients with Behçet's disease by both functional and immunological measurement of t-PA, and also by measurement of t-PAI.

Patients and methods

Seven patients with Behçet's disease defined by the criteria of Mason and Barnes⁷ were included in the study. Four of these patients had a well recorded history of thrombosis (Table 1). We also studied 12 patients with idiopathic oral ulceration and 12 normal subjects as controls.

Citrated blood for fibrinolytic studies was taken from an antecubital vein between 0830 and 1030 after an overnight fast and immediately cooled. Platelet poor plasma was obtained by centrifugation at 4°C for 10 minutes at 3000 g twice and then stored at -70°C until tested.

The venous occlusion test was carried out with

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sphygmomanometer set midway between systolic and diastolic pressures for 10 minutes after 20 minutes' rest in a semirecumbent posture. Blood was taken before and immediately after venous occlusion. Consent was obtained from all patients.

TISSUE PLASMINOGEN ACTIVATOR

Functional assay: euglobulin fibrin plate lysis.

Three 25 µl aliquots of freshly prepared euglobulin fraction of fresh plasma were pipetted onto the surface of a fibrin plate containing freshly prepared plasminogen and incubated at 37°C for 24 hours. Two perpendicular diameters of each of the subsequent lytic areas were then measured and the lytic activity expressed as the mean of the six diameters.

Immunochemical assay: enzyme linked immunosorbent assay (ELISA)

Microtitre plates were coated with goat anti-t-PA immunoglobulin and incubated for three hours at 25°C. Unbound immunoglobulin was then removed by three saline/Tween washes (0.15 M NaCl containing 0.5 g/l Tween 20). The coated plates were incubated for 18 hours at 25°C with diluted test samples/standards. After further washing the resultant reactions were developed for three hours by the addition of peroxidase-linked anti-tPA conjugate, excess of which was then removed by washing. The reaction product was visualised by the substrate *o*-phenylenediamine, and sulphuric acid was used to halt the reaction after 30 minutes in the dark. The final coloured product was measured by absorbance readings at 492 nm.

TISSUE PLASMINOGEN ACTIVATOR INHIBITOR ACTIVITY ASSAY

Test plasma (25 µl) was added to 25 µl of t-PA (100 units/ml) and incubated for 10 minutes at room temperature. Acetate buffer (50 µl) was added and the resulting mixture incubated for 10 minutes at room temperature before rapid freezing and storage at -70°C. For the t-PAI assay the samples were thawed and diluted with trometamol (TRIS) buffer. Aliquots (200 µl) of this diluted plasma were added to a reaction mixture consisting of plasminogen, substrate S2251, and t-PA stimulator. After a one hour incubation at 37°C the reaction was stopped by the addition of 100 µl of 20% acetic acid, and absorbance readings were taken at 405 nm. A standard curve was constructed by assaying pooled normal plasma, pretreated and diluted to give a range of t-PAI concentrations from 0 to 80 IU/ml, from which values for the test samples could be calculated.

Activated partial thromboplastin time, prothrombin time, and thrombin time were measured by standard methods⁸ and plasminogen, α₂-antiplasmin, and α₂-macroglobulin by chromogenic substrates using commercially available kits (Behring). For statistical analysis the differences between sets of data were measured with the Mann-Whitney U test.

Results

Table 1 shows the fibrinolysis results, which indicate that patients with Behçet's disease had a significantly

Table 1 Fibrinolysis results

Patient No	Age	Previous venous thrombosis	EFP* (mm)		t-PA* (ng/ml)		t-PAI* (IU/ml)	
			Basal	Stimulated	Basal	Stimulated	Basal	Stimulated
Behçet's disease								
1	38	RVT*	8.9	16.1	2.5	6.4	4.7	0
2	37	DVT*	8.6	13.3	5.3	8.6	17	2
3	36	DVT	5.8	12.3	7.8	8.8	23	4
4	36	DVT and PE*	<2.0	<2.0	9.0	14.0	110	104
5	40	No	11.2	12.2	2.3	2.3	0	3
6	28	No	6.5	8.7	3.2	6.3	26	5
7	33	No	7.8	8.9	2.3	3.8	0	2
Median			7.8	12.2	3.2	6.4	23	3
OU* group (n=12)								
Median			9.3	13.0	5.2	9.9	15.5	8.5
(Range)			<2-12.1	7.1-23.1	2.4-26.1	5.5-30.6	0-98	0-105
Control (n=10)								
Median			10.2	13.6	7.3	10.4	9.5	5.2
(Range)			5.9-13.9	10.8-21.1	6.3-10.7	7.6-20.8	1.5-26	0-21

*EFP=euglobulin fibrin plate lysis; t-PA=tissue plasminogen activator; t-PAI=tissue plasminogen activator inhibitor; RVT=retinal vein thrombosis; DVT=calf deep venous thrombosis; PE=pulmonary embolus; OU=oral ulceration.

reduced resting t-PA activity as measured by euglobulin fibrin plate lysis with a median of 7.8 mm compared with a control value of 10.2 mm ($p < 0.05$). Immunological assay by ELISA showed a similar reduction (median 3.2 ng/ml, control 7.3 ng/ml; $p < 0.05$). The median t-PAI concentration in these patients was normal. Results for the group with oral ulceration showed no significant difference from the control group for any of these variables.

After stimulation by venous occlusion the euglobulin fibrin plate lysis results for the whole group of patients with Behçet's disease were not significantly different from the control value (median 12.2 mm and 13.6 mm respectively), but the t-PA antigen was reduced (median 6.4 ng/ml v control 10.4 ng/ml; $p < 0.05$). The results of euglobulin fibrin plate lysis in these patients fell into two distinct groups; three patients (Nos 1–3) were good responders with a mean increase in euglobulin fibrin plate lysis response of 6.1 mm, but the remaining four (Nos 4–7) were poor responders with a mean increase of only 1.0 mm (control group 3.6 mm). In contrast, the t-PA antigen concentrations showed no clear difference between the two groups. Similarly, t-PAI after occlusion showed no consistent difference between good and bad responders or, indeed, the control group. Interestingly, three of the four patients with a history of thrombosis were good responders, the remaining patient (No 4) had a good t-PA antigen response but grossly raised t-PAI both before and after stimulation. The group with oral ulceration again showed no significant difference from the control group for these measurements. Other fibrinolytic variables, such as plasminogen, α_2 -antiplasmin, and α_2 -macroglobulin, were all normal.

Discussion

The defective fibrinolysis seen in patients with Behçet's disease contrasts with the normal results for patients with isolated oral ulceration and supports the concept of Behçet's syndrome as a distinct entity. The unstimulated fibrinolytic activity in patients with Behçet's disease shows significantly low euglobulin fibrin plate lysis response and t-PA antigen concentrations, and in six of the seven patients no significant increase in t-PAI. This suggests that the reduced resting fibrinolytic activity could indeed be related to decreased endothelial cell production of t-PA, presumably secondary to vasculitic damage to the endothelium. Defective endothelial production of t-PA is also implied by the finding that the t-PA antigen concentration was low even after stimulation by venous occlusion (6.4 ng/ml, control 10.4 ng/ml; $p < 0.01$). Similar findings of reduced t-PA production have been reported in

other conditions associated with vasculitis, such as systemic lupus erythematosus.⁹ The euglobulin fibrin plate lysis response after stimulation was more variable; four patients showed a defective increase in euglobulin fibrin plate lysis response (poor responders), including one patient (No 4) with markedly raised t-PAI concentration (110 IU/ml) who showed no detectable euglobulin fibrin plate lysis response at all. This was the only patient in the group of poor responders with a history of thrombosis. The remaining three patients (Nos 1–3) were still able to mount a normal euglobulin fibrin plate lysis response despite a low t-PA antigen response, but the underlying reason for this is unclear. It is possible that other plasma fibrinolytic mechanisms, such as the urokinase pathway, may be involved to account for the normal euglobulin fibrin plate lysis response.

It has previously been suggested that defective fibrinolysis may be a contributory factor to the development of thrombosis in patients with Behçet's disease.⁴ Our results, however, support the view of Conard *et al.*, who suggested that defective fibrinolysis was not directly related to the development of thrombosis,¹⁰ implying that other as yet unidentified factors are involved. The number of patients studied in most of these reports is small and larger studies would clearly be necessary to clarify the significance of the abnormal fibrinolysis. This is potentially of major therapeutic importance as drugs such as tizanazolol, a fibrinolytic enhancing agent which decreases t-PAI concentrations, have already been used in an attempt to prevent thrombosis in patients with a thrombotic tendency.¹¹

In conclusion, our results suggest that in patients with Behçet's disease the reduced resting fibrinolytic activity is related to decreased endothelial production of t-PA, presumably as a result of vasculitic damage, but that the defective response to stimulation may be multifactorial in origin. The reduced fibrinolytic activity appears to be unrelated to the thrombotic tendency.

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