Endothelial-Dependent Fibrinolysis in Subjects with the Lupus Anticoagulant and Thrombosis

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Key words

Fibrinolysis – Thrombosis – Lupus anticoagulant – Endothelium

Summary

To investigate the hypothesis that diminished endothelial fibrinolytic activity contributes to the pathogenesis of thrombosis in patients with the lupus anticoagulant (LA), we assessed the ability of endothelium to release tissue-type plasminogen activator (t-PA) in response to standardized venous occlusion (VO) of the arm, and the extent of inhibition of t-PA, in 11 subjects with LA and a history of thrombosis and in 36 healthy normal subjects. The mean rise in plasma t-PA antigen after VO, the mean plasma free t-PA activity after VO, and the mean plasma t-PA inhibitor level prior to VO were not significantly different in subjects with LA and thrombosis and in normal subjects. Four subjects with LA and thrombosis (36%), and five of 36 healthy control subjects (14%) generated no detectable free t-PA activity after VO ("non-responders"); this difference was not statistically significant. All four "non-responders" with LA and thrombosis had normal t-PA antigen release after VO, indicating that the lack of detectable free t-PA activity after VO was due to increased inhibition of released t-PA. We conclude that abnormally reduced endothelial fibrinolytic activity is not present in the majority of subjects with LA and thrombosis. In the subset of subjects with LA and thrombosis who generate no detectable t-PA activity after VO, a stimulatory effect of LA on endothelial production of t-PA inhibitor cannot be excluded.

Introduction

Anti-phospholipid antibodies, including the lupus anticoagulant (LA) and anti-cardiolipin antibodies (ACA), have emerged as important markers for increased risk of venous and arterial thrombosis (1–5). It is thought that LA and ACA bind to membranes of platelets and/or endothelial cells, thereby disturbing normal platelet and endothelial function in such a way as to promote thrombosis. The demonstration of IgG inhibitors of endothelial prostacyclin production (3, 6), and protein C activation (7–9) in some patients with LA and thrombosis supports this hypothesis, as does the association of LA and ACA with anti-platelet antibodies and immune thrombocytopenia (5, 10).

In addition to production of prostacyclin and activation of protein C, other endothelial functions which if altered by LA or ACA could theoretically influence the risk of thrombosis include inhibition of activated clotting factors by surface-bound anti-

thrombin III (11), activation of coagulation (12), and initiation of fibrinolysis (13). To date, no direct evidence has been presented that any of these endothelial functions are altered in patients with LA and thrombosis. A possible contribution of diminished fibrinolysis to the pathogenesis of thrombosis in patients with systemic lupus erythematosus (SLE) was suggested by Angles-Cano et al., however (14). They reported diminished or absent plasminogen activator activity after venous occlusion (VO) of the upper arm in 24 of 28 patients with SLE, but did not study their patients for the presence of LA or ACA, and did not analyze separately the results of VO in the 5 patients in their series with a history of thrombotic complications. They speculated that diminished fibrinolytic response to venous occlusion was caused by endothelial damage by circulating immune complexes.

Studies of the fibrinolytic system in subjects with arterial thrombosis (15, 16) and venous thrombosis (17–19) have demonstrated two mechanisms of decreased fibrinolysis: diminished endothelial synthesis and/or release of tissue plasminogen activator (t-PA), and increased levels of t-PA inhibitor(s) in plasma, of which the most important, type 1 plasminogen activator inhibitor (PAI-1), is synthesized by endothelial cells (20). Therefore, we felt it was important to study in greater detail the status of endothelial fibrinolysis in subjects with LA and thrombosis, using methods which permit assessment of both the capacity of endothelium to release t-PA in response to a defined stimulus, and the extent of inhibition of t-PA after release. We report herein studies of fibrinolysis in 11 subjects with LA and thrombosis, and 36 normal, healthy subjects. Endothelial t-PA release was assessed by measuring t-PA antigen before and after VO; the extent of inhibition of released t-PA was assessed both indirectly by measuring the level of free (uninhibited) t-PA activity after VO, and directly by measuring overall t-PA inhibitor activity of plasma.

Patients, Materials and Methods

Patients

11 adult subjects with LA and a history of thrombosis (including 10 with spontaneous and/or recurrent venous thromboembolism, and 1 with a spontaneous thrombotic stroke without other known risk factors), and 36 healthy adult subjects without history of thrombosis were studied. Thrombosis was documented in all subjects by one or more of the following: Doppler or impedance plethysmographic studies of the legs, venography, ventilation/perfusion lung scanning, pulmonary angiography, or CT scanning of the head. All patients with thrombosis were studied at least two weeks after their last acute thrombotic event, and all were receiving warfarin at the time blood samples for fibrinolytic assays were obtained, with the exception of the subject with a thrombotic stroke, who was receiving aspirin. Only one subject was receiving steroid therapy (20 mg of prednisone every other day). The presence of LA was documented by the kaolin clotting time of Exner (21). ACA was measured by an enzyme-linked immunosorbent assay (22) by Dr. Francisco Quismorio of the Division of Rheumatology at USC School of Medicine.

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Blood Samples

Blood was collected by standard venipuncture from resting subjects into $\frac{1}{10}$ volume of balanced citrate anticoagulant, immediately centrifuged for 10 minutes at 12,400 g at 4° C, and the resultant platelet-poor plasma stored at −80° C until assayed.

Venous Occlusion Test

A blood pressure cuff was placed on the upper arm and inflated to a pressure of 100 mm Hg for exactly 10 minutes. Blood was taken from the occluded arm just prior to deflating the cuff.

Fibrinolytic Assays

t-PA antigen and activity were measured in the euglobulin fraction of plasma as previously described (23), using modifications of the assays of Bergsdorf et al. (24) and Mahmoud and Gaffney (25), respectively. Overall t-PA inhibitory activity of plasma was assayed as previously described (23) using a modification of the method of Juhan-Vague (26).

Statistical Methods

The significance of differences among mean values for t-PA antigen, activity, and inhibitor were assessed using the two-tailed Student t test, with p <.05 considered significant. The significance of differences in the frequency of detectable t-PA activity post-VO was assessed using the chisquare test, with p <.05 considered significant.

Results

The results of fibrinolytic assays are shown in Table 1. Mean pre-VO and post-VO t-PA antigen levels were significantly higher in the subjects with LA and thrombosis than in normal subjects; however, the mean increment in t-PA antigen post-VO was not significantly different between the two groups.

The mean t-PA activity post-VO was not significantly different between the two groups. Four subjects with LA and thrombosis (36%) and five healthy control subjects (14%) showed no detectable t-PA activity post-VO despite a rise in t-PA antigen. These differences were not statistically significant.

The mean t-PA inhibitor levels were not significantly different between the two groups.

Discussion

Our observations do not support the hypothesis that impaired endothelial fibrinolytic activity contributes to the pathogenesis of thrombosis in the majority of subjects with LA. Endothelial synthesis and release of t-PA was not reduced in these subjects; indeed, the mean pre- and post-VO t-PA antigen levels were actually significantly higher in these subjects than in our healthy control subjects. Although the significance of these elevations is unknown, a similar finding of increased pre-VO t-PA antigen in subjects with myocardial infarction at a young age has been ascribed to increased endothelial stimulation by high levels of circulating catecholamines (15). It is conceivable that increased baseline t-PA antigen levels in subjects with LA and thrombosis could represent a protective or adaptive response to an ongoing stimulus to thrombosis. Inhibition of t-PA was also not increased in the majority of subjects with LA and thrombosis studied, as evidenced by a normal mean level of free t-PA activity post-VO, and a normal mean overall t-PA inhibitor activity level in plasma.

We cannot exclude the possibility that warfarin may have in some way stimulated endothelial production of t-PA in our subjects, and that t-PA release might be abnormally reduced in nonwarfarinized subjects with LA-associated thrombosis. However, we are not aware of any published evidence that warfarin stimulates endothelial t-PA production either in vivo or in vitro.

Table 1 Fibrinolytic parameters in subjects with the lupus anticoagulant and thrombosis, and in normal subjects (ng/ml mean ± SD)

Parameter	LA/Thrombosis (n = 11)	Normals (n = 36)	p
TPA antigen			
Pre-VO	8.1 ± 2.4	5.2 ± 2.6	<.01
Post-VO	21.8 ± 12.0	14.1 ± 9.0	<.05
Increment	14.7 ± 10.9	9.0 ± 8.9	NS*
TPA activity			
Pre-VO	0.1 ± 0.3	0	NS
Post-VO	5.6 ± 10.3	4.1 ± 7.0	NS
Increment	5.5 ± 10.4	4.1 ± 7.0	NS
TPA Inhibitor	16.2 ± 8.4	15.4 ± 11.0	NS

* NS = not significant

We recognize that the relatively small number of subjects with LA and thrombosis studied limits our ability to detect small differences in fibrinolytic parameters between these subjects and normal subjects. In particular, the study of larger numbers of subjects would be useful in determining whether the trend toward a higher percentage of fibrinolytic "non-responders" (subjects with no detectable t-PA activity post-VO) in the group with LA and thrombosis represents a statistically significant difference. Nevertheless, the majority of subjects with LA and thrombosis we studied had normal endothelial release of t-PA antigen and normal generation of free t-PA activity after VO, thus demonstrating no evident impairment of endothelial-dependent fibrinolysis by current assay techniques. Further studies using cultured endothelial cells would be of interest in determining whether serum or IgG from fibrinolytic "non-responder" subjects with LA and thrombosis has a stimulatory effect on endothelial production of PAI-1.

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