

# Changes in the concentration and distribution of tissue factor pathway inhibitor in Behçet's disease and systemic lupus erythematosus: effect on the prothrombotic state

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## Abstract

**Background**—Tissue factor pathway inhibitor (TFPI) is an anticoagulant which modulates the tissue factor (TF) dependent pathway, acting on the factor VIIa/TF complex, factor Xa, and thrombin. Although most TFPI is found in association with plasma lipoproteins and platelets, the functional pool is bound to vascular endothelium and is released into the circulation on stimulation with heparin or low molecular weight heparin (LMWH).

**Objective**—To assess the vascular endothelial TFPI pool in patients with Behçet's disease (BD) or systemic lupus erythematosus (SLE).

**Methods**—Plasma TFPI concentrations were determined before, and 20 and 60 minutes after subcutaneous LMWH injection in 15 newly diagnosed patients with BD and 12 with SLE, and in 12 healthy controls.

**Results**—Baseline median TFPI was 149.5 ng/ml in healthy subjects, and the percentage change in TFPI at 20 minutes ( $((\text{value at 20th min} - \text{baseline value})/\text{baseline value}) \times 100$ ) was 575.2. TFPI concentrations in patients with BD were initially normal at baseline (136.0 ng/ml), but the percentage change (44.7) was significantly lower than in the patients with SLE and the controls. Baseline TFPI concentrations in patients with SLE (83.0 ng/ml) were lower than in the control group, but the TFPI response to stimulation with LMWH reached a level (626.4%) comparable to that of the controls.

**Conclusion**—Depletion of the functional endothelial pool in BD and low circulating concentrations of TFPI despite an intact pool in SLE may be important in the pathogenesis of thrombosis in these vasculitic syndromes.

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control of haemostasis and thrombosis.<sup>3</sup> Tissue factor pathway inhibitor (TFPI) is a key anticoagulant protein synthesised mainly by the vascular endothelium and modulates the tissue factor (TF) dependent pathway of coagulation, acting on the formation of factor (F)VIIa/TF complex and activation of FX to FXa by the FVIIa/TF complex. TFPI is also a rapid inhibitor of thrombin generation at low concentrations of FVIIa/TF. It has therefore been suggested that TFPI deficiency could lead to a severe prothrombotic state, although a definite clinical deficiency has not been reported in any disease condition.<sup>4,6</sup>

TFPI deficiency is difficult to demonstrate because of its complex distribution in the body. It exists in several pools: more than 80% is associated with plasma lipoproteins and platelets, a smaller amount circulates freely in the plasma, and finally, the functional reservoir is bound to the vessel wall and is released into the circulation on injection of heparin or low molecular weight heparin (LMWH).<sup>7</sup> Heparin and LMWH are both capable of releasing large amounts of endothelium-bound TFPI into the circulation as well as enhancing the anti-Xa activity of TFPI.<sup>7,8</sup>

Assessment of the vascular endothelial TFPI pool by evaluating the basal status and kinetics of TFPI in response to heparin may be helpful in understanding the pathophysiology of the hypercoagulable state in diseases that cause vasculitic endothelial injury and/or dysfunction. Accordingly, we investigated basal plasma TFPI concentrations and the response of the TFPI pool to LMWH stimulation in patients with BD or SLE.

## Patients and methods

### STUDY GROUPS

The study groups consisted of 15 patients with BD (nine men, six women; mean (SD) age 32 (6) years; diagnosed according to the Criteria of the International Study Group), 12 patients with SLE (one man, 11 women; mean (SD) age 29 (5) years; diagnosed according to revised ARA criteria), and 12 healthy volunteers (six men, six women; mean (SD) age 34 (8) years) as controls. Tables 1 and 2 give the disease characteristics of the patient groups.

All patients were newly diagnosed and had not previously received any specific treatment or any kind of medication for their illnesses. To avoid possible confounding factors, subjects were only eligible for the study if they were non-smokers and had no history of thrombosis.

Behçet's disease (BD) and systemic lupus erythematosus (SLE) are multisystemic disorders which share thrombotic events of arterial and venous origin as a common feature. The mechanisms of thrombotic tendency are not precisely known, but vasculitic endothelial cell injury and/or dysfunction are thought to be important.<sup>1,2</sup>

Vascular endothelial cells are responsible for the secretion of many mediators involved in the

Table 1 Characteristics of disease features of Behçet's disease (n=15)

Oral aphthous lesion	15 (100)
Genital ulceration	9 (60)
Erythema nodosum	6 (40)
Other skin lesions	8 (53)
Pathergy test positivity	8 (53)
Anterior uveitis	5 (33)
Synovitis	3 (20)

Values are expressed as n (%).

Table 2 Characteristics of disease features of systemic lupus erythematosus (n=12)

Malar rash	10 (83)
Discoid lupus	3 (25)
Photosensitivity	9 (75)
Oral ulceration	3 (25)
Arthritis	9 (75)
Serositis	4 (33)
Haematological involvement	6 (50)
Renal involvement	2 (17)
ANA positivity	12 (100)
Anti-dsDNA	8 (67)

Values are expressed as n (%).

ANA = Antinuclear antibodies; dsDNA = double stranded DNA.

Subjects with any coexisting systemic disease and/or receiving drugs affecting coagulation and fibrinolysis were also excluded.

#### STUDY PROCEDURES AND BLOOD SAMPLING

Plasma TFPI concentrations were measured in plasma samples of patients with BD or SLE and healthy volunteers as controls. All tests and sampling procedures were performed in the morning with the subject in the fasting state, to eliminate problems of diurnal variation of the haemostatic system. After a resting period of 30 minutes in the sitting position, blood samples for measuring baseline TFPI levels were obtained. Nadroparine (Fraxiparine), an LMWH, at a dose of 15 000 ICU (5 700 Ju) anti-Xa was injected subcutaneously, and further blood samples were drawn after 20 and 60 minutes. All venepunctures were performed on large antecubital veins, without interruption of venous flow, with a 19G butterfly needle connected to a plastic syringe. The first few millilitres were discarded, and then 9 ml of each sample was transferred to polypropylene tubes containing 1 ml 0.109 M trisodium citrate. The tubes were then centrifuged at 3000 rpm for 15 minutes at 10–18°C, and the supernatant plasma samples stored in plastic tubes at –30°C. TFPI concentrations were measured within 30 days of sampling.

Table 3 Tissue factor pathway inhibitor (TFPI) concentrations in patients with Behçet's disease (BD) or systemic lupus erythematosus (SLE), and controls at baseline, and at 20 and 60 minutes after injection of low molecular weight heparin

	TFPI (ng/ml)			
	Baseline	20th min	60th min	% Change at 20th min*
Control group	149.5 (65.3)	876.5 (389.5)	293.5 (299.3)	575.2 (325.6)
BD group	136.0 (100.0)	205.0 (93.0)†	135.0 (90.0)	44.7 (105.5)†
SLE group	83.0 (75.5)‡	658.0 (273.3)	132.5 (38.8)	626.4 (1067.5)

Values are expressed as median (interquartile difference).

\*Percentage change in TFPI from baseline at 20th minute  $((20\text{th min value} - \text{baseline value})/\text{baseline value}) \times 100$ .

†Significantly lower than control group and patients with SLE.

‡Significantly lower than control group and patients with BD (Kruskal-Wallis analysis followed by post hoc Mann-Whitney U test with level of significance adjusted downward to 0.017).

#### ASSAY OF TFPI

Plasma TFPI concentrations were determined by enzyme linked immunosorbent assay (ELISA) by using the commercially available "IMUBIND TFPI ELISA KIT" (product number 849; American Diagnostica Inc, Greenwich, Connecticut, USA). Assays were performed in duplicate according to the manufacturer's recommendations. Calculations were performed with a curve fitting statistical software package and a computer. The ranges of intra-assay and interassay coefficients of variation in our laboratory were 5.2–8.7% and 6.5–9.4% respectively.

#### STATISTICAL ANALYSIS

With regard to the distribution of TFPI concentrations, the non-parametric Kruskal-Wallis test was used to assess the intergroup differences between patients with BD and SLE and healthy controls at different time points. Statistically significant differences obtained from Kruskal-Wallis analyses were further tested by the Mann-Whitney U test for post hoc pairwise comparisons between groups, with p values adjusted downward to 0.017 (0.05/3—that is, the number of pairwise comparisons among three groups) in order to decrease the possibility of a type I error. Results are expressed as median (interquartile range). The Statistical Package for Social Sciences (SPSS) version 10.0 for Windows was used to analyse the data.

#### Results

Baseline median TFPI concentration was 149.5 ng/ml in healthy subjects. The TFPI response to LMWH showed a peak at the 20th minute (876.5 ng/ml) and a relative fall at the 60th minute (293.5 ng/ml). The median percentage change in TFPI at the 20th minute  $((\text{value at } 20\text{th min} - \text{baseline value})/\text{baseline value}) \times 100$  was 575.2 (table 3).

Baseline TFPI concentrations in patients with BD (136.0 ng/ml) were initially comparable to those of healthy controls, but failed to increase at the 20th minute (205.0 ng/ml), which was significantly lower ( $p < 0.001$ ) than in patients with SLE and control groups. The percentage change in TFPI at the 20th minute in patients with BD (44.7) was also significantly lower ( $p < 0.001$ ) than those of patients with SLE and controls (table 3).

In patients with SLE, baseline TFPI concentrations (83.0 ng/ml) were significantly ( $p < 0.005$ ) lower than those of healthy controls. However, at the 20th min after stimulation, they increased (658.0 ng/ml) comparably to those of healthy controls. The percentage change in TFPI in patients with SLE (626.4) was also comparable to that of the controls (table 3).

#### Discussion

TFPI is a multivalent Kunitz-type proteinase inhibitor, which regulates the initiation of coagulation by producing FXa mediated feedback inhibition of the FVIIa/TF catalytic complex. It is the strongest inhibitor of the extrinsic

pathway of coagulation. The active role of vascular endothelium together with circulating platelets and proteins of the coagulation and fibrinolytic system in the maintenance of haemostatic balance is well known.<sup>3</sup> It has pro-coagulant, anticoagulant, and fibrinolytic properties through the production, secretion, and receptor mediated binding of proteins involved in haemostasis.

The role of TFPI has been described in diabetes mellitus and its vascular complications, in acute coronary events, and in cases of disseminated intravascular coagulation caused by various underlying diseases.<sup>9-11</sup> Vasculitic disorders may affect the endothelium, resulting in derangement of the haemostatic process. It is therefore rational to investigate TFPI kinetics in multisystemic vasculitic diseases associated with a prethrombotic/hypercoagulable state.

In BD, fibrinogen levels as well as von Willebrand factor are often raised, and, as acute phase reactants, both may exhibit good correlation with disease activity. Likewise, hypofibrinolysis and defective fibrinolytic response to venous occlusion as well as DDAVP infusion have also been shown to be part of generalised endothelial cell dysfunction.<sup>1 12 13</sup> However, there are no conclusive data on the status of TFPI in BD. In our study, although baseline values were normal, the expected response to LMWH was blunted in these patients. This finding may be explained by the hypothesis that vasculitic injury depletes the vascular TFPI pool and alters endothelial response to exogenous stimuli. As all the patients in our study had not had a previous thrombotic event, this may be regarded as a clue to the early changes in endothelial dysfunction in the procoagulant phase of BD.

Arterial and venous thrombotic events in SLE are well known, but the pathogenesis of this entity is yet to be determined.<sup>2</sup> The most common risk factors for thrombosis in patients with SLE are the presence of lupus anticoagulant, positive anticardiolipin antibody titres, decreased protein S concentrations, and thrombocytopenia.<sup>14</sup> Immune complex vasculitis seen in patients with SLE is characterised by complement activation and leucocyte infiltration into vessel walls with subsequent vessel damage.<sup>15</sup>

In our study, we found that baseline TFPI concentrations in patients with SLE were lower than in the control group. On the other hand,

TFPI increased dramatically after LMWH injection and reached levels comparable to those of the healthy controls. This finding suggests that the vascular endothelial pool of TFPI remained unchanged in SLE. However, the underlying mechanisms and significance of low circulating TFPI levels in the pathogenesis of thrombosis in SLE is yet to be determined.

In conclusion, the findings of a depleted endothelial TFPI pool in BD and low circulating levels of TFPI despite an intact endothelial pool in SLE require further examination to clarify the pathogenesis of the thrombotic tendency in these two distinct vasculitic syndromes.

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