Changes in the concentration and distribution of tissue factor pathway inhibitor in Behçet’s disease and systemic lupus erythematosus: effect on the prethrombotic 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 (((value at 20th min − baseline value)/baseline value) × 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 ($3.0 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.

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. Vascular endothelial cells are responsible for the secretion of many mediators involved in the control of haemostasis and thrombosis. 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.

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). 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.

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.
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 IU (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 in patients with BD or SLE 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.

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
((value at 20th min − baseline value)/baseline
value) × 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
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which was significantly lower (p<0.001) than
those of healthy controls, but failed to
increase at the 20th minute (205.0 ng/ml),
which was significantly lower (p<0.001) than
those of healthy controls. The percentage
change in TFPI at the 20th minute in patients with SLE
(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 feed-
back inhibition of the FVIIa/TF catalytic
complex. It is the strongest inhibitor of the extrinsic

<table>
<thead>
<tr>
<th>TFPI (ng/ml)</th>
<th>Baseline</th>
<th>20th min</th>
<th>60th min</th>
<th>% Change at 20th min*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>149.5 (65.3)</td>
<td>876.5 (389.5)</td>
<td>293.5 (299.3)</td>
<td>575.2 (325.6)</td>
</tr>
<tr>
<td>BD group</td>
<td>136.0 (100.0)</td>
<td>205.0 (93.0)</td>
<td>135.0 (90.0)</td>
<td>44.7 (105.5)</td>
</tr>
<tr>
<td>SLE group</td>
<td>83.0 (73.5)</td>
<td>658.0 (273.3)</td>
<td>132.3 (38.8)</td>
<td>626.4 (1067.5)</td>
</tr>
</tbody>
</table>

Values are expressed as median (interquartile range).

*Percentage change in TFPI from baseline at 20th minute (((20th min value−baseline value)/baseline value)×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).
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. It has procoagulant, 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. 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. 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. The most common risk factors for thrombosis in patients with SLE are the presence of lupus anticoagulant, positive antikeratin antibodies, decreased protein S concentrations, and thrombocytopenia. Immune complex vasculitis seen in patients with SLE is characterised by decreased protein S concentrations, and positive anticardiolipin antibody titres, with SLE are the presence of lupus anticoagulant: a risk factor for thrombosis in patients with SLE.

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.