INCREASED P-SELECTIN PLASMA LEVELS IN PATIENTS WITH THROMBOTIC THROMBOCYTOPENIC PURPURA

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ABSTRACT

Background. Thrombotic thrombocytopenic purpura (TTP) is a rare vascular disorder of unknown etiology. There is evidence to support the hypothesis that platelets and endothelium play a pivotal pathogenetic role. Immunological assays for plasma thrombomodulin and P-selectin levels have recently been made available and they allow simple evaluation of endothelial damage and endothelial/platelet activation, respectively. In this study, we measured the plasma levels of thrombomodulin, P-selectin and von Willebrand factor in 9 TTP patients during active disease and at the time of complete remission (CR).

Methods. Thrombomodulin, P-selectin and von Willebrand factor were measured by enzyme immunoassay.

Results. Mean thrombomodulin and von Willebrand factor plasma values were always within the normal range. P-selectin plasma levels, both in the active phase of the disease and in CR (median 312 and 185 ng/mL, respectively), were significantly higher than in normal controls (mean 96±35 ng/mL, median 88 ng/mL; p<0.05). However, the mean value of P-selectin in CR (median 185 ng/mL) was significantly lower than that observed at diagnosis (p < 0.05). In addition, an inverse relationship between P-selectin plasma levels and platelet count (r= -0.526; p= 0.03) was observed.

Conclusions. These findings suggest that activation of platelets and/or endothelium may play a relevant role in the pathogenesis of TTP.

Key words: thrombotic thrombocytopenic purpura, thrombomodulin, P-selectin
P-selectin is a glycoprotein contained in platelet α-granules and endothelial Weibel-Palade bodies. It belongs to the adhesive molecule family and is involved in leucocyte adhesion. P-selectin is expressed on the cell surface and is secreted as a consequence of cell activation. Because high plasma levels of P-selectin suggest endothelial/platelet activation, this glycoprotein has been proposed as a marker of thrombotic disease. Assays for the evaluation of TM and P-S plasma levels have recently been developed. Von Willebrand factor (vWF) has also been long proposed as a marker of endothelial cell damage. This protein has been detected in platelets and endothelial cells; however, experimental data support the conviction that most, if not all, circulating vWF is likely to originate from endothelium.

In this study we evaluated platelet and endothelial involvement in TTP patients with active disease and at the time of CR by testing TM, P-S and vWF plasma levels.

**Patients and Methods**

**Patients**

This study included nine patients with TTP (2 males and 7 females; median age 39 years, range 20-64). Eight were newly diagnosed and one patient with chronic relapsing TTP was in the acute phase of the disease. All patients were submitted to plasma exchange procedures and prednisone and/or ticlopidine and/or vincristine were administered in a few cases. One patient died early during induction therapy (day +4), whereas the 8 remaining ones reached complete remission (CR). None of these 8 patients in CR has relapsed at a median follow-up of 40 months (range 12-60 months).

**Evaluation of TM, P-S and vWF plasma levels**

Evaluation of TM, P-S and vWF plasma levels was performed before treatment and repeated two or more months after the achievement of CR. Briefly, venous blood samples were collected in 3.8% sodium citrate and the platelet poor plasma was stored at −20℃. Control samples were collected from 26 normal sex- and age-matched subjects.

Plasma thrombomodulin was evaluated by using a commercially available enzyme immunoassay (Diagnostica Stago, Asserachrom Thrombomodulin, France). P-selectin was measured using a sandwich enzyme-linked immunosorbent assay (P-Selectin EIA kit, Takara Biomedicals, Japan), and von Willebrand factor was tested with an enzyme immunoassay (Asserachrom vWF, Boehringer Mannheim, Germany), according to the manufacturer’s instructions.

**Statistical analysis**

Median and interquartile ranges (25th to 75th percentile) were reported in order to describe data.

The Mann-Whitney and Wilcoxon tests for matched pairs were utilized in order to evaluate differences among groups.

BMDP Statistical Software, 1991 ed. (BMDP Statistical Software, Cork, Ireland) was used. The level of significance was set at 0.05. All P-values in this report correspond to two-sided significance tests.

Comparisons among the groups were evaluated by the Student’s t-test.

**Results**

Clinical and laboratory data are reported in Table 1 and Figure 1. Plasma TM levels in TTP patients with active disease and in CR (median = 33 and 24 ng/mL, respectively) were not significantly different from those obtained in normal controls (mean = 30±8 ng/mL, median = 30 ng/mL), and there was no significant difference between baseline and CR values (Table 1). Plasma P-S levels during active disease (median = 312 ng/mL) were significantly higher than normal controls (mean = 96±35 ng/mL; median = 88 ng/mL; p< 0.01), whereas CR values (median =185 ng/mL) were significantly lower than in the active phase of the disease (p< 0.05), although they still remained significantly (p< 0.05) higher than in normal controls. It should be pointed out that during the remission phase 4 out of the 8 patients reached normal values. In addition, we observed an inverse
correlation between P-S plasma levels and platelet counts ($r = -0.526; p = 0.03$).

Von Willebrand factor plasma levels always remained in the normal range and there was no

difference between active disease phase (D) and complete remission (CR) and were not different with respect to the normal controls. P-selectin in active phase of TTP was significantly higher ($p=0.001$) than in normal controls. P-selectin in CR was significantly higher ($p=0.05$) than in normal controls but significantly lower ($p=0.05$) than in active phase.

Table 1. Clinical and laboratory data for the 9 TTP patients studied.

<table>
<thead>
<tr>
<th>SEX-AGE</th>
<th>SEVERITY SCORE</th>
<th>Hb (g/dL)</th>
<th>PLATELETS (10^9/L)</th>
<th>LDH (U/L)</th>
<th>THROMBOMODULIN (ng/mL)</th>
<th>P-SELECTIN (ng/mL)</th>
<th>vWF (%)</th>
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<tr>
<td>F-42</td>
<td>5</td>
<td>6.4</td>
<td>12.9</td>
<td>20</td>
<td>205</td>
<td>2000</td>
<td>123</td>
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<td>F-14</td>
<td>6</td>
<td>6.1</td>
<td>13.5</td>
<td>16</td>
<td>229</td>
<td>2800</td>
<td>148</td>
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<tr>
<td>M-32</td>
<td>7</td>
<td>8.0</td>
<td>15.0</td>
<td>12</td>
<td>191</td>
<td>884</td>
<td>160</td>
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<tr>
<td>M-40</td>
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<td>7.6</td>
<td>12.0</td>
<td>4</td>
<td>289</td>
<td>1446</td>
<td>173</td>
</tr>
<tr>
<td>F-64</td>
<td>6</td>
<td>6.4</td>
<td>11.8</td>
<td>10</td>
<td>160</td>
<td>1700</td>
<td>213</td>
</tr>
<tr>
<td>F-52</td>
<td>6</td>
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<td>14.8</td>
<td>14</td>
<td>178</td>
<td>2000</td>
<td>178</td>
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<tr>
<td>F-20</td>
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<td>8.4</td>
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<td>6.1</td>
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<td>12</td>
<td>1900</td>
<td>1446</td>
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<tr>
<td>MEDIAH</td>
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<td>1700</td>
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<tr>
<td>25%</td>
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<td>12.2</td>
<td>12</td>
<td>198</td>
<td>1446</td>
<td>142</td>
<td>29</td>
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</table>

According to Rose et al. (Am J Med 1987); Normal value 30±8 ng/mL; Normal value 96±35 ng/mL; Normal value 108±10%; Thrombomodulin and von Willebrand factor plasma levels did not show significant differences between active disease phase (D) and complete remission (CR) and were not different with respect to the normal controls. P-selectin in active phase of TTP was significantly higher ($p=0.001$) than in normal controls. P-selectin in CR was significantly higher ($p=0.05$) than in normal controls but significantly lower ($p=0.05$) than in active phase.

Von Willebrand factor plasma levels always remained in the normal range and there was no

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Figure 1. Plasma P-selectin levels in TTP patients during active disease phase and in complete remission.
significant difference between baseline and CR values. We did not find any significant correlation between either TM and P-S levels at diagnosis or P-S levels at diagnosis and the severity score.

Discussion

TTP is a disease in which platelets and endothelium play a relevant pathogenetic role; however, the exact mechanism of their involvement is poorly understood. The availability of laboratory tests for TM (marker of endothelial activation/damage) and P-S (marker of endothelial and/or platelet activation) have brought a better understanding of the pathogenesis of this disease. It has been reported that plasma TM in TTP is increased in patients with active disease and that its level significantly decreases when CR is achieved.\(^1\) In our experience, mean plasma TM values were found to be within the normal range both at diagnosis and in CR. However, it should be underlined that conflicting results may be due to different methods employed for detection of TM plasma levels. The assay reported here had already been successfully used to monitor TM levels in patients with veno-occlusive disease following bone marrow transplantation.\(^1\) Interestingly, plasma vWF levels in our patients were also in the normal range both during the active phase of the disease and in CR.

In our study, plasma P-S was always increased in patients with active TTP and significantly decreased when CR was obtained, reaching the normal range in 4 out of the 8 patients in CR. An inverse relationship (p=0.03) between plasma P-S level and platelet count was observed in our patients and, interestingly, the only patient with a rapidly fatal disease had the highest basal P-S level (534 ng/mL). In agreement with our data, Katayama et al. previously showed high P-S levels during the active phase of TTP and a normalization of the protein in the three patients studied in remission phase.

These results seem to support the hypothesis that hematological remission in TTP may be associated in some patients with the persistence of abnormal endothelial and/or platelet activation. This is also corroborated by the persistence of high TGF-β1 plasma levels in some patients in CR.\(^1\) Our findings suggest that platelet and endothelial cell activation appears to be the main mechanism of platelet aggregation rather than damage to endothelial cells. These results are further confirmed by the normal vWF levels observed both in active disease and in CR.

The only patient in our study with a fulminating disease course did not have a particularly high severity score as compared to the other patients, but she did have the highest basal P-S plasma level. In a larger series of TTP patients it would be useful to evaluate the prognostic value of P-S level at diagnosis as well as the predictive value of the P-S level in CR on the probability of relapse.

In conclusion, our study suggests that activation of platelets and/or endothelium may play a relevant role in the pathogenesis of TTP.

References


