NOTE

Plasma Selectin Levels in Patients with Graves' Disease

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Abstract. Adhesion molecules relate to cell invasion of autoimmune thyroid disease. We studied plasma soluble P-Selectin (platelet activation-dependent granule-external membrane protein), E-Selectin (endothelial leukocyte adhesion molecule) and L-Selectin (leukocyte endothelial cell adhesion molecule-1) levels in patients with Graves' disease before and during methimazole treatment. Plasma P-, E- and L-Selectin levels in patients with untreated Graves' disease were significantly higher than those in normal subjects. Plasma P-Selectin levels decreased when their thyroid functions were normal for more than 6 months after the start of methimazole treatment. No significant change in plasma E- and L-Selectin levels in patients with Graves' disease was found between hyperthyroid state and euthyroid state after the start of methimazole treatment, but plasma L-Selectin levels in patients with untreated Graves' disease were significantly lower than those in the patients in the first euthyroid state. There was no significant correlation between plasma P-Selectin levels and serum FT4 levels, nor between plasma P-Selectin levels and serum FT3 levels. These results suggested that thyroid hormones might reflect expression of P-, L- and E-Selectin from endothelial cells, or lymphocytes, or platelets in patients with Graves' disease.

Key words: P-Selectin, L-Selectin, E-Selectin, Graves' disease

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ADHESION molecules control the extravasation of leukocytes on the circulating cells and on the vascular endothelium, and are involved in autoimmune responses. They consist of three main families, namely, the Immunoglobulin supergene group, Integrin and Selectin families. P-Selectin (granule membrane protein-140), E-Selectin (endothelial leukocyte adhesion molecule-1) and L-Selectin (leukocyte endothelial cell adhesion molecule-1), which belong to the Selectin family, mediate the initial interactions of leukocytes with endothelial cells [1-3]. P-Selectin is a 140 kDa cell surface glycoprotein and is transiently expressed on vascular endothelial cells and on platelets, which mediate rolling of polymorphonuclear cells on endothelial cells. E-Selectin is a 115 kDa glycoprotein which is expressed on vascular endothelial cells in response to IL-1 and tumor necrosis factor [4]. L-Selectin is required for the binding of lymphocytes to high endothelial venules of lymph nodes and of neutrophils to endothelial cells in sites of inflammation as a rolling receptor. Expression of P-Selectin on endothelial cells was reported in Graves' thyroids [5].

In this study, therefore, the changes in plasma soluble P-Selectin (sP-Selectin), E-Selectin (sE-Selectin) and L-Selectin (sL-Selectin) levels, and their relations with thyroid function and TSH receptor antibody were evaluated before and during methimazole treatment in patients with Graves' disease.

Subjects and Methods

The subjects were 5 normal men and 7 normal
women (average age: 49.9 ± 14.2 years old), 37 patients with Graves’ disease (9 men and 28 women, average age: 40.1 ± 14.0 years) and 4 hyperthyroid patients with subacute thyroiditis (SAT) (1 man and 3 women, average age 43.0 ± 4.8 years). Blood samples were obtained in the hyperthyroid state, in the first euthyroid state within 3 months after the start of methimazole treatment and in the euthyroid state over 6 months after start of the treatment in patients with Graves’ disease, while they were in a hyperthyroid state in patients with SAT. The samples were centrifuged, frozen at −20 °C until assayed and measured for thyroid stimulating hormone (TSH), free thyroxine (FT₄), free tri-iodothyronine (FT₃), TSH receptor antibody (TRAb), anti-thyroid microsomal antibody (MCPA), anti-thyroglobulin antibody (TGPA), sP-, sE- and sL-Selectin. The methods used were chemiluminescent immunoassay for TSH (Amerlite TSH 60 kit), RIA for FT₄ (Amerlex M FT₄ kit) and FT₃ (Amerlex M FT₃ kit), passive particle agglutination methods for MCPA and TGPA (Fujirebio), enzyme-linked immunosorbent assay for sP-Selectin (Takara biomedicals, normal reference 12–224 ng/ml), sE-Selectin (R&D Systems, normal reference 9.5–51.6 ng/ml) and sL-Selectin (Bender MedSystems, normal reference 487.3–1096.3 ng/ml), and radioreceptor assay for TRAb (Cosmic Co). The inter- and intraassay coefficients of variations for sP-Selectin, sE-Selectin and sL-Selectin were 1.2% and 4.2%, 1.9% and 4.8%, and 1.6% and 5.2%, respectively.

The results were expressed as the mean ± SEM and statistical significance between the patients and normal subjects was calculated by nonparametric Mann-Whitney test, that between the patients with Graves’ disease was by nonparametric Wilcoxon test or Mann-Whitney test, and the correlation between Selectins levels and thyroid function was by t-test.

### Results

Plasma sP-, sE-, sL-Selectin, serum TSH, FT₃, FT₄ and TRAb levels in patients with Graves’ disease and SAT

Plasma sP-, sE-, sL-Selectin, serum TSH, FT₃, FT₄ and TRAb levels are shown in Table 1. Plasma sP-Selectin levels in hyperthyroid patients with Graves’ disease were significantly higher than in normal subjects (mean ± SEM: 118.0 ± 16.0 ng/ml, \( P<0.001 \)), but not significantly different from those

### Table 1. Plasma P-, E-, L-Selectin, serum TSH, FT₄, FT₃, MCPA, TGPA, and TRAb levels in patients with Graves’ disease before and during treatment with methimazole and in patients with subacute thyroiditis

<table>
<thead>
<tr>
<th></th>
<th>No.</th>
<th>TSH (µU/ml)</th>
<th>FT₄ (ng/dl)</th>
<th>FT₃ (pg/ml)</th>
<th>TRAb (%)</th>
<th>P-Selectin (ng/ml)</th>
<th>L-Selectin (ng/ml)</th>
<th>E-Selectin (ng/ml)</th>
<th>MCPA</th>
<th>TGPA</th>
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<tr>
<td>Normal subjects</td>
<td>11</td>
<td>1.5</td>
<td>0.6</td>
<td>3.4</td>
<td>0</td>
<td>118.1</td>
<td>264.5</td>
<td>22.0</td>
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<td></td>
<td>±0.26</td>
<td>±0.06</td>
<td>±0.14</td>
<td>±16.0</td>
<td>±12.0</td>
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<tr>
<td>Hyperthyroid</td>
<td>21</td>
<td>&lt;0.04</td>
<td>5.6**</td>
<td>15.2**</td>
<td>42.6</td>
<td>289.8***</td>
<td>584.6*</td>
<td>43.9*</td>
<td>9553.0</td>
<td>2809.0</td>
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<tr>
<td>state</td>
<td></td>
<td>±0.54</td>
<td>±1.30</td>
<td>±4.3</td>
<td>±32.0</td>
<td>±134.3</td>
<td>±7.7</td>
<td>±584.0</td>
<td>±2289.1</td>
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<tr>
<td>Treated</td>
<td>22</td>
<td>0.41</td>
<td>1.2</td>
<td>4.2</td>
<td>41.0</td>
<td>219.8***</td>
<td>681.3**</td>
<td>47.1**</td>
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<td>±0.27</td>
<td>±6.8</td>
<td>±20.5</td>
<td>±174.2</td>
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<td>Euthyroid state</td>
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<td>4.04</td>
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<td>4.0</td>
<td>30.1</td>
<td>136.0</td>
<td>464.1*</td>
<td>88.0</td>
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<td>±0.15</td>
<td>±0.36</td>
<td>±10.6</td>
<td>±23.3</td>
<td>±145.4</td>
<td>±62.5</td>
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<td>treatment</td>
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<td>4.2***</td>
<td>10.9***</td>
<td>0</td>
<td>280.8</td>
<td>1191.1*</td>
<td>29.8</td>
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<td>±4.96</td>
<td>±15.3</td>
<td>±428.8</td>
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Data are expressed as the means ± SEM. Significant difference from normal subjects by Mann-Whitney test. *, \( P<0.05 \); **, \( P<0.01 \); ***, \( P<0.001 \).
of patients with SAT. Plasma sP-Selectin levels as well as serum FT₃ and FT₄ levels were reduced significantly in euthyroid patients with Graves' disease over 6 months after start of methimazole treatment, compared with the levels in hyperthyroid patients, and in first euthyroid patients within 3 months after start of the treatment (P<0.05). As shown in Fig. 1, in 10 of 12 patients plasma sP-Selectin levels were diminished in the euthyroid state during methimazole treatment for over 6 months. Plasma sE-Selectin levels in patients with untreated Graves' disease and in the first euthyroid patients were significantly increased compared with normal subjects (P<0.05), and plasma sL-Selectin levels in patients with Graves' disease were significantly higher than those in normal subjects. But plasma sL- and sE-Selectin levels in patients with untreated Graves' disease were not significantly changed in the treated euthyroid state for over 6 months and not different from those in patients with SAT. In patients with SAT, plasma sL-Selectin levels were significantly higher than those in normal subjects.

Correlation between sP-, sE- or sL-Selectin and thyroid hormones, MCPA, TGPA, or TRAb

There was no significant correlation between plasma sP-Selectin and serum FT₄ levels, between plasma sP-Selectin and serum FT₃ levels, and between plasma sP-Selectin and serum TRAb levels in patients with untreated Graves' disease. No correlation between plasma sL-Selectin and serum FT₃ levels, or serum FT₄ levels was found in patients with untreated Graves' disease, or between plasma sE-Selectin and serum FT₄ levels, although there was a significant positive correlation between plasma sE-Selectin and serum FT₃ levels as shown in Fig. 2 (r=0.558, P<0.001). There was no correlation between sP-Selectin and sE-Selectin levels, and between sP-Selectin and sL-Selectin levels in patients with Graves' disease. There was no significant correlation between those Selectins and MCPA, or TGPA levels.

Discussion

Selectins guide non-activated polymorphonuclear (PMN) cells to the areas of inflammation in creating loose contacts with the endothelial layer. L-Selectin mediates rolling of PMNs on endothelial cells together with P- and E-Selectin [6]. It has been observed that L-, E- and P-Selectin may exist in a soluble, nonmembrane-associated form in normal healthy subjects [7-10]. In this study, plasma sL-, sE- and sP-Selectin levels were measured in patients with Graves' disease before and during methimazole treatment, in order to make clear the relations between Selectins and thyroid function, TGPA, MCPA or TRAb.
Plasma sP-Selectin levels in patients with untreated Graves’ disease were significantly higher than those in normal subjects and those in euthyroid patients with Graves’ disease treated with methimazole, although they were not higher than those in patients with subacute thyroiditis. In 10 of 12 patients with untreated Graves’ disease, plasma sP-Selectin levels were noticeably diminished when their thyroid functions became normal during methimazole treatment for over 6 months. In patients with Graves’ disease we observed no significant correlation between plasma sP-Selectin and serum FT3 levels or serum FT4 levels. These results indicated that in hyperthyroidism plasma sP-Selectin levels were increased under the influence of thyroid hormone levels but did not suggest a relationship between P-Selectin and the autoimmune process because of no significant correlation between P-Selectin and TRAb, TGPA or MCA. The reason why plasma sP-Selectin levels increased in patients with untreated Graves’ disease was not so clear. We thought that thyroid hormones as well as some cytokines such as interleukin-1 alpha, which was demonstrated in thyroid follicular epithelial cells [11], affected the expression, secretion and degradation of P-Selectin, which was similar to polymorphonuclear elastase [12], and that thyroid hormones might cause the secretion of a soluble form of P-Selectin as a result of alternative splicing [13]. Although sE- and sL-Selectin levels, not sP-Selectin, were noticeably increased in patients with Graves’ disease before treatment with methimazole [14], there were some differences in the duration of follow up of the patients and of the quantity of methimazole administered, and their antibody by Bender MedSystems used for ELISA may be different from ours by Takara Co.

Plasma sL- and sE-Selectin levels in patients with untreated Graves’ disease did not differ from those in euthyroid patients with Graves’ disease who received methimazole treatment for over 6 months, although the plasma levels in patients with untreated Graves’ disease were significantly higher than those in normal subjects. A significant correlation between plasma sE-Selectin levels and serum FT3 levels was found in patients with untreated Graves’ disease. There was no correlation between plasma sP-Selectin levels and sL-Selectin levels, or sE-Selectin levels. We thought that these Selectins have independent shedding pathways and that their shedding was too rapid in patients with Graves’ disease, although L-Selectin was associated with PMN recognition of both P- and E-Selectin [15]. Plasma sE-Selectin levels were affected by thyroid hormone levels in patients with untreated Graves’ disease, since there was a significant correlation between plasma sE-Selectin and serum FT3 levels, as levels of E-Selectin paralleled the clinical cure [14]. In patients with Graves’ disease thyroid hormones could lead to the secretion of E-Selectin as an endothelial protein [16] and these Selectins may be elevated under the influence of thyroid hormones and an autoimmune inflammatory process.

L-Selectin is useful in monitoring certain types of inflammatory disorders [17]. In patients with SAT, plasma sL-Selectin levels were significantly higher than in normal subjects.

In conclusion, we have shown that in patients with untreated Graves’ disease, plasma soluble P-Selectin levels as well as E- and L-Selectin levels were increased significantly but were decreased by treatment with methimazole, although not related to TRAb. It is emphasized that it is necessary to study the relationship between Selectins and some cytokines, because adhesion molecules may be important in the pathogenesis of immune-mediated thyroid disease.

Acknowledgment

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References

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