The Relationship between Concentrations of Circulating Soluble E-Selectin and Clinical, Pathological, and Biological Features in Patients with Breast Cancer

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ABSTRACT

Increasing evidence suggests that E-selectin contributes to tumor growth and metastasis. E-selectin may increase tumoral angiogenesis and the adhesion of tumoral cells to endothelial cells at distant sites. The aim of this study was to assess the relationship between concentrations of circulating soluble E-selectin (sE-selectin) and clinical, pathological, and biological features in patients with breast cancer (BC). Concentrations of sE-selectin were analyzed by an ELISA method in sera from 113 patients with metastatic BC, 30 patients with primary inflammatory BC, 105 patients with primary noninflammatory BC, and 42 healthy controls. These concentrations were analyzed in terms of the clinical and pathological features of the tumors as well as in terms of the concentrations of serum inflammatory parameters (erythrocyte sedimentation rate, C reactive protein, interleukin 1β, and tumor necrosis factor α), the response to chemotherapy or hormone therapy, and survival. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked received 2/7/97; revised 11/21/97; accepted 11/24/97.

INTRODUCTION

Cellular adhesion molecules are implicated in homotypic or heterotypic cellular interactions. The interaction between endothelial cells and other cells plays an important role in inflammatory responses (1). The endothelial adhesion molecules involved are mainly represented by the intercellular adhesion molecules, vascular cell adhesion molecule type 1, and E-selectin (2). E-selectin is a cell surface adhesion molecule specifically expressed by activated endothelial cells (3). Moreover, E-selectin can be secreted by endothelial cells and can be detected in a soluble form (sE-selectin) in sera (4). E-selectin interacts with the S-LeX and S-Lea factors, carbohydrate ligands expressed by leukocytes. By facilitating leukocyte infiltration, E-selectin is involved in the tissue inflammatory responses (5).

Recent studies have suggested the participation of endothelial adhesion molecules, especially E-selectin, in tumor progression and metastasis (6, 7). Initially, experimental studies suggested that E-selectin played a direct role in the induction of angiogenesis, a crucial phenomenon in tumoral growth and metastasis (8, 9). In some cases, tumoral cells can abnormally express the carbohydrate ligands, and these cells can be attached at distant sites by activated endothelial cells. In patients with colonic cancer, the expression of S-LeX by tumoral cells is known to be associated with an increased risk of liver metastasis.
Table 1  Disease sites and number of disease sites in the 113 patients with metastatic BC

<table>
<thead>
<tr>
<th>Disease sites</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>32 (28.3)</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>40 (35.4)</td>
</tr>
<tr>
<td>Skin</td>
<td>25 (22.1)</td>
</tr>
<tr>
<td>Bone</td>
<td>60 (53.1)</td>
</tr>
<tr>
<td>Lung</td>
<td>41 (36.2)</td>
</tr>
<tr>
<td>Liver</td>
<td>29 (25.7)</td>
</tr>
<tr>
<td>Brain</td>
<td>6 (5.3)</td>
</tr>
<tr>
<td>Other(^a)</td>
<td>9 (8.0)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. of disease sites</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>36 (31.9)</td>
</tr>
<tr>
<td>2</td>
<td>42 (37.2)</td>
</tr>
<tr>
<td>&gt;2</td>
<td>35 (30.9)</td>
</tr>
</tbody>
</table>

\(^a\) Peritoneum in four cases, eye in three cases, and pericardium in two cases.

(10, 11). This could be explained by the abundant expression of E-selectin by the liver sinusoidal endothelium.

An increased expression of E-selectin by endothelial cells in the primary tumor or at distant sites could therefore be associated with more serious tumoral progression and a worse prognosis. This increased expression of E-selectin could be indirectly evaluated by the measurement of sE-selectin concentrations in sera.

The significance of E-selectin has been studied to a lesser extent in the field of BC. However, it has been demonstrated in vitro that BC cell lines can express the S-Le\(^X\) factor and can be attached by endothelial cells (12, 13).

The aim of the present study was to assess the relationship between concentrations of circulating sE-selectin and tumoral extent, metastatic sites, inflammatory parameters, tumoral angiogenesis, response to anticancer treatment, and survival in patients with BC.

**PATIENTS AND METHODS**

**Patients.** This retrospective study involved 113 patients with histologically documented metastatic BC (age, 26–84 years; mean age, 55.3 ± 12.1 years), 30 patients with histologically documented inflammatory BC (age, 26–65 years; mean age, 49.4 ± 10.8 years), 105 patients with histologically documented primary noninflammatory BC (age, 31–82 years; mean age, 47.4 ± 8.1 years), and 42 healthy laboratory and clinical personnel and blood donors (age, 20–75 years; mean age, 46.2 ± 16.2 years). All patients and healthy controls gave their informed consent.

The disease sites and the number of disease sites in patients with metastatic BC are presented in Table 1. All patients with inflammatory BC had clinical evidence of breast erythema and/or edema. Tumoral size was <2 cm in 5 patients (16.6%), between 2 and 5 cm in 12 patients (40.0%), and >5 cm in 13 patients (43.3%). Enlarged axillary lymph nodes were detected in 21 patients (70.0%). In patients with primary noninflammatory BC, tumoral size was <2 cm in 24 patients (23.0%), between 2 and 5 cm in 59 patients (56.7%), and >5 cm in 21 patients (20.1%). Enlarged axillary lymph nodes were detected in 59 patients (56.7%).

To avoid a possible rise in sE-selectin levels associated with impaired liver or kidney function, patients with impaired liver (bilirubin > 35 \(\mu\)mol/l) or kidney function (creatinine > 140 \(\mu\)mol/l) were excluded from the study.

**Treatment.** In the metastatic BC group, 77 of 113 patients (68.1%) received a palliative chemotherapy (anthracyclin-based chemotherapy in 63 cases, methotrexate-based chemotherapy in 10 cases, vinorelbine alone in 3 cases, and mitomycin alone in 1 case), 34 of 113 patients (30.0%) received hormone therapy, 1 patient just had radiation therapy, and 1 patient died before any anticancer therapy.

In the inflammatory BC group, 21 of 30 patients (70.0%) received anthracyclin-based chemotherapy, and 9 of 30 patients (30.0%) received methotrexate-based chemotherapy.

In the group of 105 patients with primary noninflammatory BC, 37 received chemotherapy for tumoral reduction before surgery; 31 of 37 patients (83.8%) received anthracyclin-based chemotherapy; and 6 of 37 (16.2%) patients received methotrexate-based chemotherapy.

Response to treatment was assessed using the WHO criteria (14). In all groups of patients, treatment was stopped in cases of documented tumoral progression. In patients with metastatic BC, chemotherapy was stopped after six courses in cases of stable disease or in cases of serious toxicity according to WHO criteria (14). The maximum cumulative dose was 500 mg/m\(^2\) for doxorubicin and 900 mg/m\(^2\) for epirubicin. Patients with inflammatory or primary noninflammatory breast tumors received a maximum of six courses of chemotherapy.

**Serum Inflammatory Parameters.** The erythrocyte sedimentation rate and the C reactive protein concentration were measured in all of the patients. A measurement of serum IL-1\(\beta\) and TNF-\(\alpha\) was also performed using an ELISA method (Immunotech, Marseille, France).

**Tumoral Angiogenesis.** Tumoral angiogenesis was assessed in the 68 patients presenting primary noninflammatory BC who were treated by primary surgery. Four-\(\mu\)m-thick sections were cut from the formalin-fixed, paraffin-embedded tumor blocks. These sections were stained for anti-factor VIII-related antigen with polyclonal antisera (DAKO, Trappes, France), using a standard immunohistochemical method (15). Factor VIII antigen-stained slides were scored by using the microvessel-counting protocol and criteria developed by Weidner et al. (16). The region of the tumor that seemed to have the greatest density of microvessels was selected using a low-power microscopic examination. Three microscopic fields were analyzed at \(\times 200\) microscope magnification, and the most densely vascular field was recorded for statistical analysis.

**sE-Selectin Assay.** Serum samples were obtained from the patients with BC before anticancer treatment. Serum samples were also obtained from the healthy controls. Blood (5 ml) was collected in EDTA and centrifuged at 3000 \(\times\) g for 10 min. Sera were stored at \(-20^\circ\)C until analysis. Concentrations of sE-selectin were measured in duplicate using a commercial ELISA kit (R&D Systems, Abington, United Kingdom). The typical sensitivity of the ELISA kit used in this study is <2 ng/ml, which is sufficient to detect minimal physiological concentrations. Briefly, microtiter ELISA plates coated with a specific capture monoclonal antibody were used. Standards and samples were added to the plate and then incubated for 1.5 h at
room temperature. After washing, bound sE-selectin was detected by incubation with a specific antibody conjugated to the enzyme horseradish peroxidase. After removal of unbound material by aspiration and washing, the amount of conjugates bound to the well was detected by reaction with a substrate (tetramethylbenzidine). The reaction was stopped by the addition of 1 M HCl, and \(A_{450}\) was measured. Concentrations higher than the mean value obtained in the healthy control group + 3 SDs were considered elevated.

**Statistical Analysis.** The mean concentrations of sE-selectin in each group were compared using the Mann-Whitney test. Age, serum inflammatory parameter concentrations, and sE-selectin concentrations were compared using the nonparametric Spearman test. Tumor microvessel count and sE-selectin concentrations were compared using the linear regression test after logarithmic transformation of the values. The survival curves were estimated using the Kaplan-Meier method. Unadjusted differences in these estimates were assessed with the log-rank test. Evaluation of the prognostic factors was assessed using the multivariate Cox method.

**RESULTS**

**Concentrations of Circulating sE-Selectin**

The mean concentrations of circulating sE-selectin were 21.8 ng/ml (± 15.1, SD) in the healthy control group, 26.4 ng/ml (± 14.7) in the primary noninflammatory BC group, 27.5 ng/ml (± 19.0) in the inflammatory BC group, and 33.5 ng/ml (± 25.9) in the metastatic BC group (Fig. 1). The mean sE-selectin concentration found in the metastatic BC group was significantly higher than the mean found in the healthy control group (\(P < 0.01\)). Concentrations higher than 67.1 ng/ml (mean in the healthy control group + 3 SDs) were considered elevated. Elevated concentrations of sE-selectin were found in 2 of 104 patients (2%) with primary noninflammatory BC, 2 of 30 patients (6%) with primary inflammatory BC, and 7 of 113 patients (6%) with metastatic BC.

**Tumoral Extent, Response to Chemotherapy, and Prognosis**

**Patients with Metastatic BC.** The mean level of sE-selectin was significantly higher in patients with liver metastasis than in patients without liver metastasis (55.3 ± 34.8 versus 26.0 ± 16.6 ng/ml; \(P < 10^{-7}\); Fig. 2). No significant difference was found for other disease sites. Mean sE-selectin concentrations in patients with one, two, and more than two disease sites were 26.4 ± 17.7, 34.1 ± 25.9, and 40.1 ± 31.3 ng/ml (\(P = 0.08\)), respectively.

The tumoral response to therapy was evaluable in 106 of 113 patients. An objective response (complete or partial response) was observed in 29 of 106 patients (27.3%), stabilization or minor response was observed in 30 of 106 patients (28.3%), and progression was observed in 47 of 106 patients (44.3%). The mean level of circulating sE-selectin in these three groups was 32.4 ± 29.6, 32.4 ± 22.7, and 34.8 ± 26.2 ng/ml, respectively (non-significant differences).

The univariate analysis of overall survival showed the prognostic value of sE-selectin concentration (\(P < 0.05\); Fig. 3), the number of disease sites (\(P < 0.05\)), and an objective response to treatment (\(P < 10^{-3}\)). In the multivariate analysis, a prognostic value was found only for the number of disease sites (\(P = 0.04\); risk ratio, 1.36), the presence of liver metastasis (\(P < 10^{-3}\); risk ratio, 3.38), and an objective response to therapy (\(P < 10^{-3}\); risk ratio, 0.52).

**Patients with Inflammatory BC and Patients with Primary Noninflammatory BC.** No correlation was found between the concentrations of circulating sE-selectin and tumoral size, inflammation grade, lymph node extension, response to anticancer therapy, and overall survival.

**Serum Inflammatory Parameters**

The mean (± SD) erythrocyte sedimentation rate was 18 ± 19 mm/h in the patients with primary noninflammatory BC, 23 ± 20 mm/h in the patients with primary inflammatory BC, and 34 ± 41 mm/h in the patients with metastatic BC. The mean serum C reactive protein concentration was 4 ± 5.9 ± 12, and 16 ± 24 mg/l, respectively. The mean serum IL-1β level was 0.8 ± 3.7, 1.1 ± 5.1, and 4.7 ± 17.4 pg/ml, respectively. The mean serum TNF-α concentration was 15.0 ± 30.8, 32.4 ± 55.4, and 40.5 ± 142.0 pg/ml, respectively.

No correlation was found between the concentrations of these inflammatory parameters and the concentration of sE-selectin in any of the groups of patients.

**Tumoral Angiogenesis**

In the group of 68 patients assessed for angiogenesis, the mean microvessel count was 73.1 ± 43.9 per ×200 field (range, 23–230). The mean sE-selectin concentration was 26.7 ± 16.3 ng/ml (range, 7–100). There was a significant negative correlation between the microvessel counts and the sE-selectin concentrations (r = −0.47; \(P = 10^{-4}\); Fig. 4).

**DISCUSSION**

Increasing evidence suggests that endothelial cell adhesion molecules play a role in tumor metastasis. In an in vitro model, Steinbach et al. (17) found that the adhesion of renal cancer cell lines to cytokine-activated HUVECs was mediated in part by the interaction of the tumoral S-Le^X^ ligand and endothelial E-selectin. In a study performed on melanoma cell lines, Miller et al. (18) found a lack of tumoral expression of S-Le^X^, and this was associated with a lack of adhesion to cytokine-activated HUVECs. In the same study, tumoral cells transfected with S-Le^X^ exhibited E-selectin-dependent adhesion to HUVECs. In the study of Biancone et al. (19), normal mice and transgenic mice (which constitutively expressed cell surface E-selectin in all tissues) were injected with melanoma cell lines transfected with the S-Le^X^ gene. Normal mice developed lung metastasis exclusively, whereas transgenic mice developed massive liver metastasis. Ye et al. (20) assessed the expression of E-selectin in patients with colorectal cancer. A high expression of E-selectin in primary tumors and liver metastasis was observed. Moreover, patients with liver metastasis had significantly higher levels of circulating sE-selectin. The pathological study of Fox et al. (21) was performed on human breast tumors. A vascular expression of E-selectin was detected in 52% of the tumors compared with 21% in the normal breast tissues. In the study of Banks et al.
In the present study, we assessed the clinical and biological significance of sE-selectin in three groups of patients with BC: (a) metastatic BC; (b) primary inflammatory BC; and (c) primary noninflammatory BC. The concentrations of circulating sE-selectin were higher in patients with metastatic BC than in the healthy controls ($P < 0.01$). We compared the concentrations of sE-selectin with the metastatic localizations and found that high concentrations of circulating sE-selectin were mainly found in patients with liver metastasis ($P < 10^{-5}$). High con-
Concentrations of sE-selectin in patients with metastatic BC with (n = 29) or without (n = 84) liver metastasis. Horizontal bars, the mean titers in each group.

Fig. 2. Concentrations of sE-selectin in patients with metastatic BC with (n = 29) or without (n = 84) liver metastasis. Horizontal bars, the mean titers in each group.

Concentrations of sE-selectin seemed to be associated with a high number of disease sites (P = 0.08). However, this tendency was only related to the fact that patients with numerous metastatic sites had a higher probability of having liver metastasis. After exclusion of the patients with liver metastasis, we found that patients with other metastatic sites had a mean concentration of sE-selectin similar to the mean concentration found in patients without metastasis.

The present study suggests that in patients with metastatic BC, circulating sE-selectin is mainly secreted in the liver. sE-selectin is probably issued from liver endothelial cells, because these cells are known to express high amounts of E-selectin. This is consistent with the results previously found in patients with colorectal cancer. However, in certain cases, an abnormal expression of E-selectin by liver metastatic cells cannot be excluded, because in the study of Fox et al. (21) performed on primary breast tumors, an abnormal expression of E-selectin by tumoral cells was detected in 7% of the tumors.

The high expression of E-selectin by liver endothelial cells
Fig. 3 Overall survival in patients with metastatic BC. We compared the survival of the 76 patients who had a level of sE-selectin of <40 ng/ml with the survival of the 37 patients who had a level of sE-selectin of ≥40 ng/ml.

Fig. 4 Correlation between concentrations of circulating sE-selectin and tumoral microvessel count in 68 patients with primary noninflammatory BC who had a primary surgery. Because the distribution of sE-selectin concentrations and of microvessel counts were log normal, the values were transformed to logarithms and compared using the linear regression test ($r = -0.47; P = 10^{-4}; r^2 = 0.228; y = -0.487x + 2.358$).
could also enhance the adhesion of other circulating tumoral cells expressing the E-selectin ligands.

The shedding of sE-selectin by liver endothelial cells could conversely induce a blockage of the E-selectin ligands on circulating tumoral cells and subsequently prevent their adhesion to endothelial cells at metastatic sites.

In patients with nonmetastatic BC, the presence of a significant tumoral inflammatory process was not associated with increased shedding of sE-selectin, because the concentrations of sE-selectin were similar in patients with primary noninflammatory BC and patients with primary inflammatory BC. This result is perhaps surprising, because in inflammatory breast tumors, the presence of an inflammatory infiltrate is probably associated with secretion of IL-1, TNF-α, and IFN-γ, which are potent inducers of E-selectin expression by endothelial cells (3). However, in the present study, we found no correlation between concentrations of sE-selectin and levels of inflammatory parameters (erythrocyte sedimentation rate, C-reactive protein, IL-1β, and TNF-α). The study suggests that sE-selectin shedding is independent of inflammation in patients with BC. This is consistent with the results of the study of Wittig et al. (24). In their study involving patients with colorectal cancer, no correlation was found between sE-selectin levels and inflammatory parameters. In contrast, in patients with renal carcinoma, Muraki et al. (25) found a correlation between levels of sE-selectin and inflammatory markers. These results suggest that the relationship between endothelial expression and shedding of E-selectin and inflammatory reactions depends on the type of cancer.

Because E-selectin has been suggested to play a role in angiogenesis, we compared the concentrations of sE-selectin and tumoral angiogenic activity. Surprisingly, concentrations of sE-selectin were negatively correlated with this tumoral angiogenesis. The explanation for this negative correlation remains unclear. Excessive shedding of sE-selectin in serum could have an inhibitory effect on angiogenesis. sE-selectin could recognize its S-LeX or S-Lea counterpart on circulating mononuclear cells and/or neighboring tumoral cells. This mechanism could inhibit the endothelial fixation of these cells and reduce their activating properties.

The interaction of endothelial E-selectin and tumoral carbohydrate ligands (S-LeX and S-Lea) could play a role in tumor metastasis. Assessment of the carbohydrate counterpart expression is also important, but there is some genetic heterogeneity among the Lewis genes in the human population. Mitsuoka and Kannagi (26) measured the concentrations of sE-selectin in patients with colorectal cancer and assessed the phenotype and genotype of the E-selectin carbohydrate ligands. The highest frequency of metastasis was found in patients with high expression of S-Lea and high levels of sE-selectin. The expression of the carbohydrate ligands must also be determined in patients with BC.

To conclude, the present study concerning patients with BC suggests that at advanced stages, high concentrations of sE-selectin are associated with liver metastasis, whereas at early stages, high concentrations of circulating sE-selectin are associated with a low tumoral angiogenesis. These results suggest a functional discrepancy between endothelial cell surface E-selectin and circulating sE-selectin. Although several studies have previously demonstrated that the expression of cell surface E-selectin enhances the metastatic process, the shedding of sE-selectin in circulation may be considered a mechanism of inhibition of tumor progression. Further evaluation is needed to assess the potential role of sE-selectin in the inhibition of angiogenesis.

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