The Effects of Standard Anthracycline-Based Chemotherapy on Soluble ICAM-1 and Vascular Endothelial Growth Factor Levels in Breast Cancer

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Abstract

Purpose: The circulating soluble form of intercellular adhesion molecule-1 (sICAM-1) and vascular endothelial growth factor (VEGF) are elevated in women with breast cancer and associated with tumor progression and poor prognosis. This study examined the effects of anthracycline-based chemotherapy on plasma sICAM-1 and VEGF, as well as soluble P-selectin, von Willebrand factor, and interleukin-6 levels.

Experimental Design: Twenty-six women diagnosed with stage I-III breast cancer (mean age, 48.4 ± 10.4 years; range, 34–79 years) were studied before (week 1) and at weeks 2 and 3 of cycles 1 and 4 of chemotherapy.

Results: The initial effect of chemotherapy was to reduce sICAM-1 levels; with pretreatment, sICAM-1 levels were decreased at week 2 of both cycles (P values < 0.01). sICAM-1 levels were elevated, however, at the start of cycle 4 as compared with pretreatment (P < 0.01). Chemotherapy led to an increase in sICAM-1 levels in node-positive but not node-negative patients (P < 0.01). VEGF levels decreased at week 2 of cycle 4 (P = 0.001) and remained so at week 3. Similar to sICAM-1, VEGF levels were elevated at the start of cycle 4 as compared with pretreatment (P < 0.006). Soluble P-selectin levels decreased during week 2 of cycle 4 (P = 0.026). Neither interleukin-6 nor von Willebrand factor were significantly changed in response to chemotherapy.

Conclusions: The findings support prior studies suggesting that sICAM-1 levels derive from sources other than endothelial cells. In addition, whereas the more immediate effect of chemotherapy is to reduce sICAM-1 and VEGF, continued treatment may lead to significant elevations.

Introduction

Intercellular adhesion molecule-1 (ICAM-1; Refs. 1–7) and vascular endothelial growth factor (VEGF; Refs. 8–10) expression in breast cancer are predictive of response to chemotherapy and associated with survival (2, 9–11). ICAM-1, a member of the superfamily of immunoglobulin-like adhesion molecules, is expressed on the surface of a wide variety of cells, including leukocytes and endothelial cells. ICAM-1 is a ligand for leukocyte function antigen-1; ICAM-1 leukocyte function antigen-1-mediated cell-cell adhesion is important for numerous immunological functions (12). ICAM-expression has been implicated in tumor progression and metastases, with greater expression being associated with low growth potential, negative lymph node involvement, and good prognosis (1). Levels of the circulating soluble form of ICAM-1 (sICAM-1) are elevated in women with breast cancer (2–7), additionally elevated in patients with versus without metastases (6, 12, 13), and may be predictive of poorer response to chemotherapy and poorer prognosis (2, 11), although findings have not been consistent (7).

The precise source and clinical significance of the elevated sICAM-1 has remained relatively unclear. Some studies suggest that elevated sICAM-1 reflects endothelial and/or other tissue activation (13) More recent studies suggest that sICAM-1 reflects increased inflammation (14). Studies showing that elevated sICAM-1 levels may be related to elevations of proinflammatory cytokines such as interleukin (IL)-6 (3, 11, 15, 16) support this latter contention. von Willebrand factor (vWF) on the other hand, which is found in the Weibel-Palade bodies of secretory organelles of endothelial cells and in platelets and plays a key role in hemostasis, may also be elevated in breast cancer; however, it does not appear to be reflective of inflammation or platelet activation but rather of endothelial activation and/or damage (14, 17). Soluble P-selectin (sP-selectin), on the other hand, is elevated in breast cancer (17, 18) and reflects platelet activation (17). Platelet P-selectin expression supports the formation of blood platelet-tumor cell complexes in the circulation and facilitates metastases (17).

VEGF is an angiogenic cytokine with high relevance to cancer, stimulating the formation of new blood vessels necessary for tumor growth and progression (19). VEGF is an angiogenic cytokine with high relevance to cancer, stimulating the formation of new blood vessels necessary for tumor growth and progression (19).
VEGF expression is elevated in breast cancer and associated with a higher likelihood of recurrence or death (Ref. 8 for review). Plasma levels of VEGF are also elevated in breast cancer, higher in malignant than in benign breast disease, and an independent predictor of poorer survival (9, 10). Elevated plasma VEGF levels have been linked to elevated IL-6 levels in breast cancer (20).

Despite studies demonstrating their diagnostic and potentially useful prognostic value, few studies have examined the effects of anthracycline-based chemotherapy on sICAM-1 or VEGF. As part of a larger study on sleep and fatigue in breast cancer, this study was designed to determine the effects of a standardized course of adjuvant or neoadjuvant anthracycline-based chemotherapy on these important factors in women with breast cancer. vWF, IL-6, and sP-selectin were also determined with the intention of differentiating possible sources of as well as mechanisms of chemotherapy-induced changes in sICAM-1 and VEGF.

PATIENTS AND METHODS

Women diagnosed with stage I through III-A breast cancer and referred for adjuvant or neoadjuvant anthracycline-based chemotherapy were invited to participate. Subjects were recruited from the University of California, San Diego Cancer Center and from oncologists in the San Diego area. Exclusion criteria included pregnancy, women undergoing bone marrow transplants, metastatic breast cancer, confounding underlying medical illnesses such as renal failure, significant preexisting anemia, or other physical or psychological impairments that would limit participation. The study was approved by the University of California, San Diego Human Research Protection Program and the University of California, San Diego Cancer Center Human Research Protection Program of Participating Oncologists.

Breast cancer disease staging was performed by the referring medical oncologist typically using the American Joint Committee on Cancer Staging Manual 5th edition, although not specified in the protocol. Neoadjuvant patients received anthracycline-based chemotherapy after a biopsy to confirm invasive disease and after clinical staging. Adjuvant patients received anthracycline-based chemotherapy after clinical staging and definitive surgical treatment with either lumpectomy or mastectomy and axillary staging with sentinel node biopsy or axillary lymph node dissection according to institutional standards.

Women were studied during cycles 1 and 4 of adjuvant or neoadjuvant anthracycline-based chemotherapy. The individual anthracycline regimens were not limited in the protocol, but most patients received doxorubicin/cyclophosphamide, cyclophosphamide/doxorubicin/5-fluorouracil, or 5-fluorouracil/epirubicin/cyclophosphamide combination chemotherapy regimens (chemotherapy regimens for each subject are presented in Table 1). Standard clinical doses of doxorubicin/cyclophosphamide

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Abbreviations: AC, doxorubicin/cyclophosphamide; CAF, cyclophosphamide/doxorubicin/5-fluorouracil; FEC, 5-fluorouracil/epirubicin/cyclophosphamide combination chemotherapy.

* Neoadjuvant chemotherapy following biopsy.
† Patients who subsequently received two additional cycles of CAF or FEC.
‡ Patients who went on to be treated with either Taxol or taxotere;
Chemotherapy and sICAM-1

Typically include fluorouracil (500 mg/m\(^2\)) and epirubicin (50 mg/m\(^2\)), the most commonly used regimen, would typically include doxorubicin (60 mg/m\(^2\)) and cyclophosphamide (500 mg/m\(^2\)) day 1 of each cycle (21). Standard doses of cyclophosphamide/doxorubicin/5-fluorouracil would include cyclophosphamide (500–600 mg/m\(^2\)), doxorubicin (50–60 mg/m\(^2\)), and 5-fluorouracil (500–600 mg/m\(^2\)) i.v. day 1 of each cycle (22). Standard doses of 5-fluorouracil/epirubicin/cyclophosphamide would include fluorouracil (500 mg/m\(^2\)), epirubicin (50–100 mg/m\(^2\)), and cyclophosphamide (500 mg/m\(^2\)) day 1 of each cycle (23). Adriamycin and taxotere cycles were administered according to National Surgical Adjuvant Breast and Bowel Project Protocol B-30 consisting of Adriamycin (50 mg/m\(^2\)) and taxotere (75 mg/m\(^2\)) i.v. day 1 of each cycle. Within each cycle, blood samples were collected during week 1 (immediately before the administration of chemotherapy), during week 2 to examine effects close to the nadir blood count, and during week 3 to gauge changes during the recovery phase of chemotherapy. Patients data were gathered on 26 women with breast cancer (23 adjuvant patients and 3 neoadjuvant patients). Blood from 15 women without breast cancer or other illness (mean age, 42 years) was used to determine normal control values for each analyte before the start of chemotherapy (Table 2).

### Assays

Whole blood was preserved with EDTA. After centrifugation, the plasma was stored at \(-80^\circ\)C until assay. sICAM-1, VEGF, sP-selectin, IL-6 (R&D Systems, Minneapolis, MN), and vWf (Diagnostica Stago, Inc., Parsippany, NJ) were determined by commercial ELISA (24, 25). All samples from a given patient were assayed together. Intra-assay coefficients of variation for sICAM-1, VEGF, sP-selectin, IL-6, and vWf are 3.9, 5.0, 7.8, 2.3, and 2.6%, respectively. The respective interassay coefficients of variation are 6.0, 6.6, 4.7, 4.3, and 4.6%.

### Data Analysis

Separate one-way ANOVA tests were used to compare breast cancer patients to healthy controls on blood factors before chemotherapy. Treatment data were analyzed by two-way (cycle by week) and three-way (cycle by week by group or treatment) repeated measures ANOVA (SPSS Statistical Software, Chicago, IL). Posthoc \(t\) tests were used to follow-up significant ANOVA findings. Pearson correlations were used to examine relationships between tumor size and blood factors. Statistical significance for all analyses was considered to be at \(P < 0.05\).

### RESULTS

Table 1 presents the individual patient characteristics of age, disease stage, tumor size, number of positive nodes, estrogen and progesterone receptor status, type of surgery, and chemotherapy regimen. The mean age of the sample was 48.4 years (SD \(\pm 10.4\); range, 34–79 years).

Before chemotherapy, compared with the healthy control women, women with breast cancer had significantly elevated circulating sICAM-1 levels [285 ng/ml (SD = 75) versus 231 ng/ml (SD = 45) (\(F = 15.2, P < 0.001\))]. VEGF levels [96 pg/ml (SD = 115) versus 20.4 pg/ml (SD = 9.55) (\(F = 6.2, P < 0.01\))], and sP-selectin levels [92 ng/ml (SD = 48) versus 53 ng/ml (SD = 29) (\(F = 29.1, P < 0.001\))]. There were no significant differences between the two groups in IL-6 or vWf.

Compared with pretreatment (week 1), sICAM-1 levels were significantly decreased at week 2 of cycle 1 of chemotherapy (\(F = 20.4, P < 0.001\)) (Fig. 1) but returned to pretreatment levels by the end of week 3. A similar effect was seen during cycle 4, with an initial significant drop at week 2 (\(F = 12.8, P = 0.001\)), followed by a recovery at week 3. In contrast, sICAM-1 levels during the first week of cycle 4 were significantly higher than the pretreatment level at the first week of cycle 1 (\(F = 8.3, P < 0.01\)).

During cycle 4, VEGF levels were significantly decreased at week 2 compared with week 1 (\(F = 15.3, P = 0.001\)) and remained so at week 3 (Fig. 2). Compared with pretreatment (week 1), VEGF levels were decreased but not significantly so at week 2 of cycle 1. Similar to sICAM-1, VEGF levels during the first week of cycle 4 were significantly elevated when compared with pretreatment (\(F = 9.2, P < 0.006\)).

sP-selectin levels were unchanged in response to cycle 1 of chemotherapy. Levels were elevated but not significantly at the start of cycle 4 (\(P = 0.11\)). There was a significant drop in sP-selectin at week 2 of cycle 4 as compared with week 1 (\(F = 0.05\)).

### Table 2

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<tr>
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<td>Week 2</td>
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<tr>
<td>sICAM-1 (ng/ml)</td>
<td>285 (75)</td>
<td>256 (69)†</td>
</tr>
<tr>
<td>sP-selectin (ng/ml)</td>
<td>96 (115)</td>
<td>65 (56)</td>
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<tr>
<td>IL-6 (pg/ml)</td>
<td>92 (48)</td>
<td>79 (37)</td>
</tr>
</tbody>
</table>

* Week 1 sample collected before chemotherapy.
† Decreased as compared with week 1 (\(P < 0.01\)).
§ Decreased as compared with week 1 of cycle 4 (\(P < 0.02\)).
§§ Increased as compared with week 1 of cycle 1 (\(P < 0.01\)).
Neither IL-6 or vWf were significantly changed in response to chemotherapy. Additionally, more exploratory analyses were run to examine possible effects of stage of disease, node status, presence or absence of estrogen and/or progesterone receptors, type of chemotherapy, and type of surgery. As is evident from the data presented in Table 1, the sample sizes for some of these analyses were somewhat limited (stages I/II (n = 19) versus stages III/III-A (n = 7); node positive (n = 15) versus node negative (n = 10); estrogen receptor positive (n = 13) versus negative (n = 13); progesterone receptor positive (n = 10) versus negative (n = 16); and surgery (only 3 patients received neoadjuvant therapy). These analyses revealed that sICAM-1 levels were higher before and during cycle 1 in node-positive versus node-negative patients (F = 7.81, P = 0.01; Fig. 3). Node-positive patients showed a significant drop in sICAM-1 at week 2 of cycle 1, whereas node-negative patients did not (F = 5.1, P < 0.05). This group difference was no longer evident by the start of cycle 4 because chemotherapy induced an increase in sICAM-1 levels in node-negative patients (F = 8.3, P < 0.01).

5.99, P = 0.026). Neither IL-6 or vWf were significantly changed in response to chemotherapy.

Fig. 1 Compared with pretreatment, sICAM-1 levels were elevated at the start of cycle 4 (P < 0.01). Compared with pretreatment (cycle 1, week 1), sICAM-1 levels were lower following week 2 of cycle 1 and week 2 of cycle 4 (P values < 0.01; mean ± SE).

Fig. 2 Compared with pretreatment (cycle 1, week 1), VEGF levels were elevated at the start of cycle 4 (P < 0.01). Compared with week 1 of cycle 4, VEGF levels were lower at week 2 of cycle 4 (P < 0.01) and remained low at week 3 (mean ± SE).

DISCUSSION

Our findings are consistent with those of prior studies documenting that circulating sICAM-1 and VEGF levels are elevated in women with breast cancer as compared with those without breast cancer (2–9). We observed that the cumulative effect of three cycles of standard chemotherapy was to further elevate both sICAM-1 and VEGF in these breast cancer patients. In contrast, the acute effect of chemotherapy was to reduce these factors; we observed an initial decline of both sICAM-1 and VEGF within 1 week after cycle 1 and/or 4 of chemotherapy treatment. Also consistent with prior studies (6, 11, 13), we found that sICAM-1 levels were elevated in node-positive versus node-negative patients. In contrast to node-positive women, women with negative nodes showed a significant increase in sICAM-1 after the first three cycles of chemotherapy. sICAM-1 levels are reported to be higher in metastatic breast cancer patients who do not respond to subsequent chemotherapy treatment (6).

Prior breast cancer studies suggest that elevated levels of sICAM-1 and VEGF are associated with elevated levels of cytokines such as IL-6 (3, 11, 16). We did not observe a chemotherapy-induced increase in IL-6. Such an increase could have been interpreted as a potential mechanism for the treatment-associated elevation of sICAM-1, as well as VEGF. Other studies with high-dose chemotherapy and with taxanes indicate that the proinflammatory cytokines IL-6 and tumor necrosis factor α may be increased in response to certain treatment regimes (26, 27). Our data do not support a conclusion that proinflammatory cytokines are contributing to the change in sICAM-1 or VEGF levels during chemotherapy.

We found no significant effect of chemotherapy on vWf. As with ICAM-1, vWf is a product of activated endothelium.
mediating thrombus formation at sites of vascular injury (28). The fact that it was unchanged with treatment lends additional support to the conclusion that the increased sICAM-1 levels are not indicative of chemotherapy effects on the endothelium per se (16). P-Selectin, too, is expressed on activated endothelial cells as well as activated platelets (29). Prior studies have shown that it is elevated in response to anthracycline chemotherapy (30, 31). We found that sP-selectin was elevated at the start of cycle 4, although not significantly. This could have reflected limited power of our sample. Together with the sICAM-1 and vWf findings, our data support prior observations that the elevation of sP-selectin in breast cancer likely reflects platelet and not endothelial activation (17).

Regarding prior studies examining chemotherapy on sICAM-1 and VEGF, in a study that included five breast cancer patients, Wang et al. (32) showed that sICAM-1 levels decreased during the neutropenia phase after high-dose chemotherapy but then normalized during the postneutropenia phase after autologous hematopoietic stem cell transplantation. We are interpreting our observation of a similar initial drop in sICAM-1 after acute chemotherapy as reflecting the generalized immunosuppressive effects of the anthracyclines. Several studies have prospectively examined the effects of differing chemotherapy regimens on serum VEGF levels. Colleoni et al. (33) examined the effects of low-dose oral cyclophosphamide and methotrexate on VEGF levels in patients with metastatic breast cancer. Serum VEGF levels were decreased to similar levels at 2 months in both responders and nonresponders; patients on therapy showed further reductions at 6 months. Lissoni et al. (34) examined the effects of Taxol monochemotherapy on serum VEGF levels in 14 patients with metastatic breast cancer. VEGF mean values significantly decreased during Taxol therapy in patients with a partial response or stable disease, whereas no decline was observed in patients with progressive disease. Finally, Verheul et al. (35) examined the effects of six cycles of chemotherapy with cyclophosphamide and doxorubicin supported by granulocyte macrophage colony-stimulating factor on serum VEGF levels and platelet counts in 27 breast cancer patients. Within each cycle, serum VEGF showed an initial significant decline (at day 8) followed by subsequent significant increases at days 15–20. The peak levels tended to decrease with progressive cycles. These latter findings of an initial decline of serum VEGF followed by an increase are more consistent with our findings. The Verheul et al. (35) study also reported evidence for VEGF transport by platelets and suggested that future studies should examine plasma rather than serum levels of VEGF to more accurately assess circulating VEGF.

There are several limitations of this study that should be noted. Blood was sampled at only weekly intervals after cycles 1 and 4. The clinical setting of this study precluded more frequent sampling. Although we observed significant initial treatment effects on sICAM-1, sP-selectin, and VEGF, it is possible that we missed more acute treatment effects on the other parameters we assessed, namely IL-6 and vWf. We feel it is unlikely, however, that our sampling intervals would have missed potential cumulative effects on IL-6 or vWf such as was observed for sICAM-1 and VEGF. Another limitation is that we did not have a control group of healthy subjects with repeated blood sampling to coincide with the sampling in the treatment group. In addition to running statistical analyses across the entire cohort of subjects, we also ran analyses on subgroups of patients, including examining stages of disease, node status, and type of chemotherapy. Given the limited sample size across some of these groupings, we view these particular analyses as more exploratory, meritng additional review with a larger group of patients. A larger sample size would also permit the addition of potentially important subgroup analyses such as examining possible effects of different types of chemotherapy in node-negative versus node-positive patients or different types of chemotherapy in estrogen-positive versus nonpositive status patients.

What might be the significance of elevated sICAM-1 and VEGF levels in response to a standard course of anthracycline-based chemotherapy? We observed that chemotherapy led to a significant rise in sICAM-1 levels in node-negative women. These elevated levels were comparable with those of the node-negative women, a group with poorer prognosis (6, 12, 13, 36). Regarding clinical criteria, the most consistent finding in the breast cancer literature is that elevated sICAM-1 levels are associated with positive nodes and metastases. Our findings extend these observations by showing that chemotherapy may, at least temporarily, elevate sICAM-1 in node-negative women to values similar to those seen in node-positive women. As with circulating VEGF, which exerts angiogenic effects (37, 38), sICAM-1 is biologically active (39). sICAM-1 exerts immunomodulatory effects, including suppression of natural killer cell and T-cell function, inhibiting leukocyte binding to target cells, including formation of natural killer cell/tumor cell conjugates (40–42). Prior studies indicate that sICAM-1 and VEGF levels are predictive of prognosis (2, 9–11). Future studies from our group will be directed toward determining whether these chemotherapy-induced changes in sICAM-1 and VEGF levels are reproducible in larger numbers of patients and whether they are associated with clinical outcomes.

REFERENCES

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