Decreased Serum E-Selectin Concentration After ^{89}Sr-Chloride Therapy for Metastatic Prostate Cancer Bone Pain

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Palliative systemic radionuclide therapy with ^{89}Sr-chloride is a useful intervention for patients with bone pain from metastatic prostatic cancer. Although this radionuclide is highly effective, its mechanism of action remains unresolved. This investigation sought to determine whether systemic radionuclide therapy decreases the production of cell adhesion molecules (E-selectins) that participate in the metastatic process. **Methods:** Sera were collected from 25 men with metastatic (stage IV) prostate carcinoma who received ^{89}Sr-chloride palliative therapy and from 10 age-matched healthy volunteers. The serum concentration of E-selectin was quantified by an enzyme-linked immunosorbent assay. Sera from 5 patients who received 2 courses of radionuclide therapy were also included in the analysis. **Results:** A 2.8-fold decrease in serum E-selectin concentration occurred within 2 mo of radionuclide therapy ($P < 0.0001$). At 10 mo, however, the concentration increased to a mean (±SD) of 151.2 ± 51.3 ng/mL, surpassing the baseline concentration. This pattern coincided with symptomatic improvement and subsequent health status deterioration. For patients who received 2 courses of radionuclide therapy, a second fall in serum E-selectin concentration followed the second radionuclide treatment. **Conclusion:** A significant decrease in serum E-selectin concentration was observed after systemic radionuclide therapy. This finding suggests that expression of cell adhesion molecules, an important determinant of metastatic progression, may be inhibited by ^{89}Sr-chloride.

Key Words: ^{89}Sr-chloride; prostate cancer; metastasis; palliation

J Nucl Med 2000; 41:1021–1024

The management of bone pain from prostate cancer offers many challenges to patients and clinicians. Although opioid analgesia remains the mainstay for palliating the pain associated with advanced prostate carcinoma (stage IV), systemic radionuclide therapy (^{89}Sr, ^{153}Sm, and ^{186}Re) has emerged as a useful therapeutic option (*1–5*). In particular, radionuclide therapy offers significant palliation without the side effects of opioids (e.g., mental status deterioration and constipation). Additionally, for multifocal metastases, systemic radionuclide therapy offers an alternative to wide-field (hemibody) external beam irradiation, which is accompanied by significant bone marrow toxicity (*6*). Evidence suggests that combined chemotherapy and radionuclide therapy may offer, in addition to palliation, some survival benefit to patients with advanced prostate cancer (*7*). Despite these developments, little is known about the mechanisms underpinning the palliative or survival benefits of ^{89}Sr-chloride.

A long list of diagnostic and prognostic markers for human prostate cancer has been identified (*8*). Of these, the role of cell adhesion molecules (CAMs), a complex network of protein and carbohydrate molecules that mediate cell–cell and cell–matrix interactions in tumor invasion and metastasis, may have some bearing on ^{89}Sr-chloride mechanisms of action, because CAMs mediate the adhesion of prostate cancer cells to endothelium and other tissues (*9–11*). This investigation was undertaken to determine whether ^{89}Sr-chloride therapy reduces the production of the adhesion determinant E-selectin, a carbohydrate expressed on human prostate cancer cells and vascular endothelial cells.

MATERIALS AND METHODS

Patients

According to institutional guidelines, 25 men (mean age ± SD, 76 ± 8.3 y) with androgen-independent, histologically confirmed prostatic adenocarcinoma were investigated. All patients had multiple skeletal metastases (Soloway grade 2 or higher) on ^{99m}Tc-methylene diphosphonate bone scintigraphy (*12*). Twenty patients were treated once with ^{89}Sr-chloride (166.5 ± 11.1 MBq); 5 patients were treated twice with this dose at approximately a 10-mo interval. Ten age-matched volunteers with no evidence of prostate cancer served as a control group. All patients reported significant improvement in their quality of life within 2 mo of radionuclide therapy, according to their responses to the McGill Pain Questionnaire (Fig. 1), a validated instrument of monitoring pain response (*13*). No complications or morbidity occurred as a result of radionuclide therapy in this cohort.

Serum Analysis

Sera were obtained at the time of treatment and every 2 mo up to 10 mo after ^{89}Sr-chloride therapy. In 5 patients, who were treated at

Received Jul. 20, 1998; revision accepted Oct. 27, 1999.

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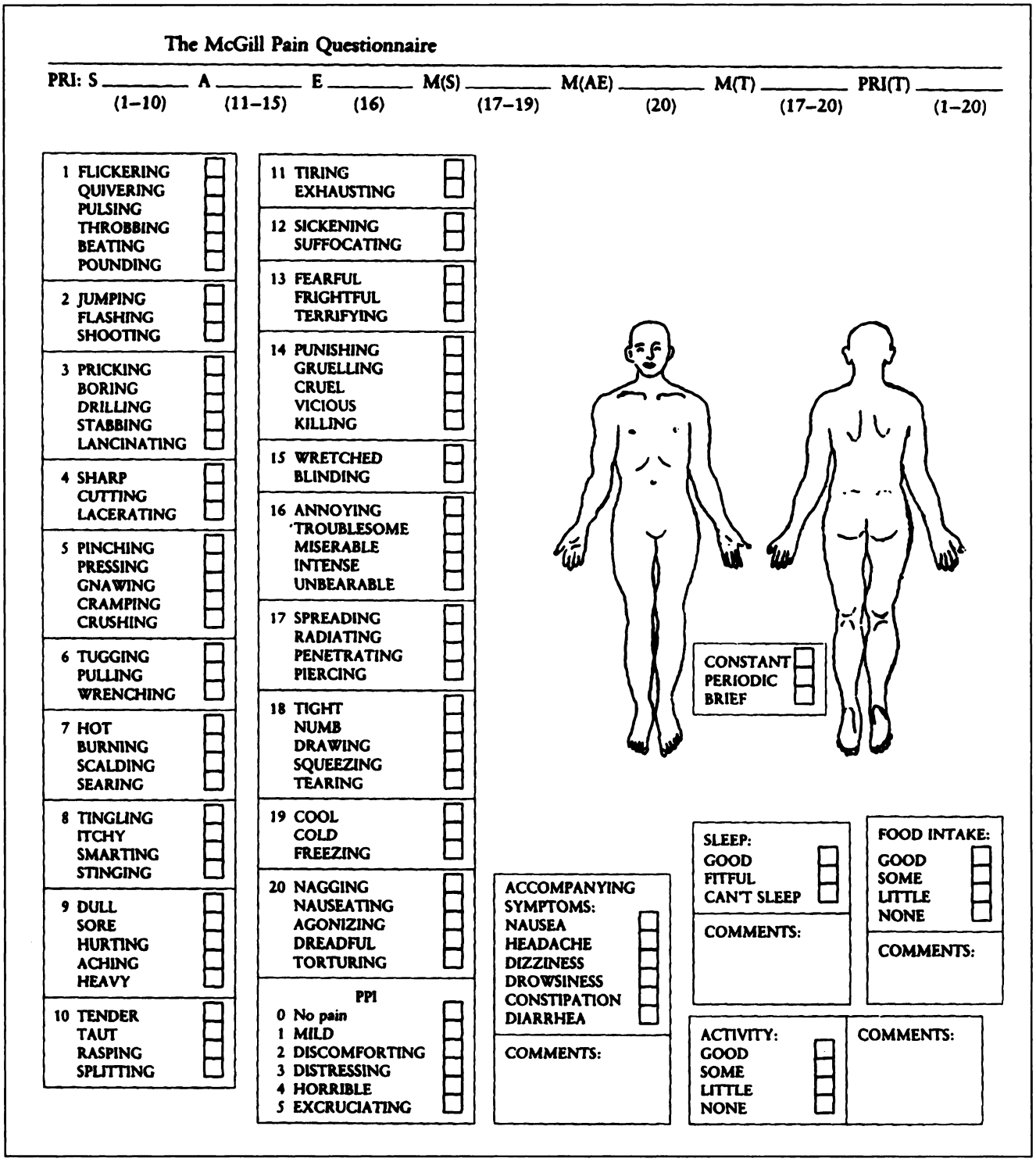

FIGURE 1. McGill Pain Questionnaire. PRI = pain rating index; S = sensory; A = affective; E = evaluative; M = musculoskeletal; T = total; PPI = present pain intensity.

the 10-mo stage, additional serum samples were obtained until 14 mo. Fresh or frozen (−70°C) sera were analyzed for E-selectin concentration using a commercially available enzyme-linked immunosorbent assay kit according to the manufacturer's specifications (British Biotechnology Products, Ltd., Abingdon, UK). Patient sera (0–200 ng/mL) were serially diluted with the zero standard available from the diagnostic assay. Parallel inhibition curves were obtained, signifying that patient serum E-selectin bound with the same affinity as standard human E-selectin. Inter- and intra-assay variability was 7.5% and 8.3%, respectively. Serum prostate-specific antigen (PSA) was also measured at the same time as E-selectin, according to common laboratory methods (*14*).

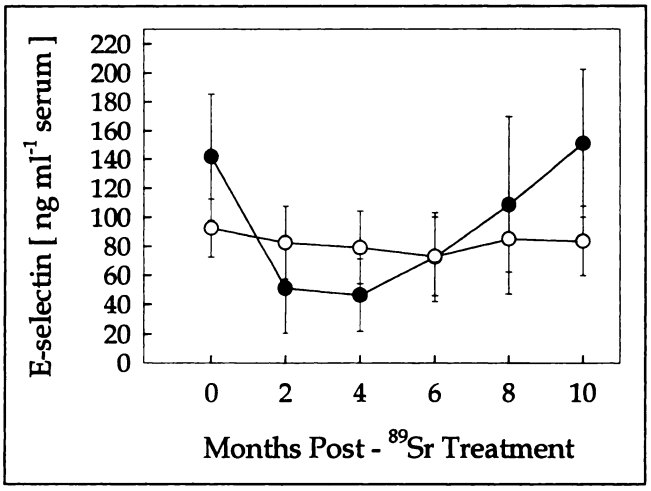

FIGURE 2. Time-interval changes in serum E-selectin concentration after systemic radionuclide therapy. ○ = healthy volunteers (n = 10). ● = patients with androgen-independent metastatic prostate cancer (n = 25).

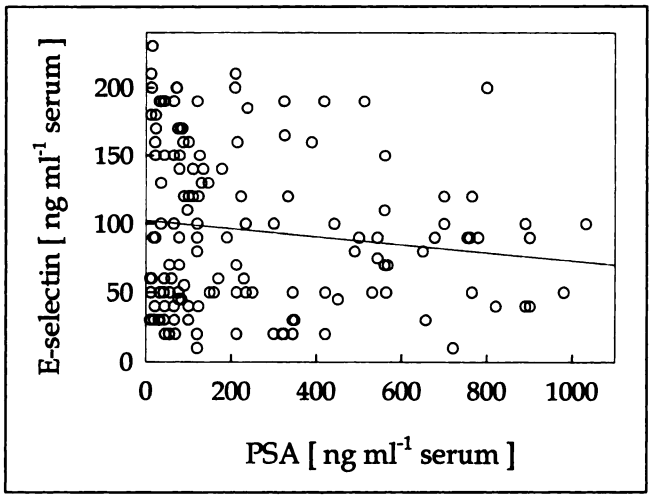

FIGURE 4. Linear regression analysis of serum PSA versus E-selectin concentration.

Statistical Methods

Descriptive methods were used to obtain the mean and SD for the indicated data. Significance between means was established by 1-way ANOVA and the Tukey test, including normality and equal variance testing. Significance was established for an α of 0.05, and the power for the statistical comparisons was >0.85 at an α of 0.05.

RESULTS

Changes in serum E-selectin levels during the 10-mo interval after radionuclide therapy are indicated in Figure 2. No statistically significant change occurred in serum E-selectin concentration over that interval for the control group. The baseline, pretreatment E-selectin concentration, which was 1.5 times greater for the prostate cancer group (141.6 ± 43.7 mg/mL) than for the control group (92.5 ± 20.0 ng/mL; $P < 0.002$), was consistent with the literature (*7*). A 2.8-fold decrease in serum E-selectin concentration occurred within 2 mo of radionuclide therapy ($P < 0.0001$). At 10 mo, however, the concentration increased to 151.2 ± 51.3 ng/mL, surpassing the baseline concentration. This pattern coincided with symptomatic improvement and subsequent deterioration of health status. Figure 3 shows the results from 5 patients who received 2 doses of radionuclide therapy. The pattern indicates a second fall in serum E-selectin concentration after the second radionuclide treatment.

No correlation between serum E-selectin concentration and PSA was observed (Fig. 4). The Pearson product moment correlation of these data did not show a significant relationship between any pair of variables ($P > 0.05$; $r = 0.131$; $r^2 = 0.0173$; Spearman rank coefficient of correlation = −0.0998).

DISCUSSION

The results suggest that systemic radionuclide therapy disrupts the production of E-selectin adhesion molecules. This disruption is reproducible after the initial and second treatment with ^{89}Sr-chloride (Fig. 3), and the duration of decreased serum E-selectin concentration corresponds to the 100-d deposition interval of ^{89}Sr in metastatic lesions (*15*). These data do not reveal whether this disruption is a primary effect, indicating action of the radionuclide on the vascular endothelium or metastasized tumor cells, or a secondary effect, indicating mediation by other molecules (e.g., prostacyclins). Prostacyclins have known antimetastatic activity, and they may exert this effect, in part, by inhibiting CAM-mediated adherence of prostate cancer cells to endo-

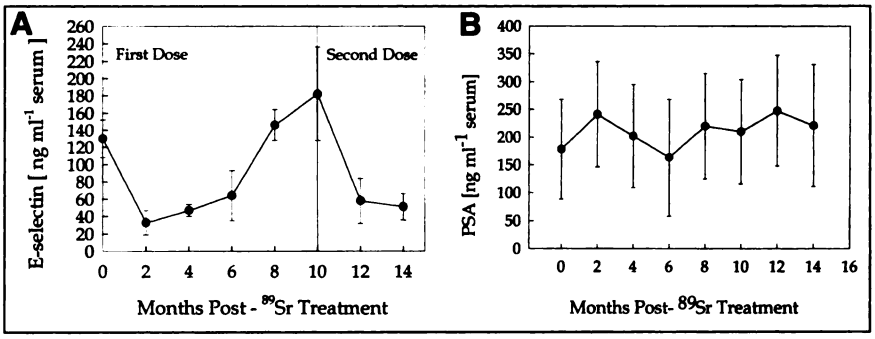

FIGURE 3. Time-interval changes in serum E-selectin (A) and PSA (B) concentrations after 2 courses of systemic radionuclide therapy. Data represent patients with metastatic prostate carcinoma (n = 5).

thelial cells in target organs (*16*). In addition, discordance between serum E-selectin and PSA concentration (Fig. 4) implies distinct mechanisms for each in the setting of skeletal metastatic disease and may partly explain metastatic progression despite PSA normalization.

Accordingly, the mechanisms underlying CAM-mediated cell adhesion are themselves extraordinarily complex, but their significance in cancer metastases requires some understanding to be appreciated. E-selectin is a cell surface glycoprotein found on vascular epithelial cells, as well as on prostate cancer cells (e.g., prostate cancer cell lines, PC-3LNCaP, and DU145). E-selectin is expressed in response to inflammatory cytokines (e.g., interleukin-1 and tumor necrosis factor–α).

Serum E-selectin concentration and serum PSA levels do not correlate, for reasons that are likely multiple. PSA is a serine protease produced at high concentrations by normal and malignant prostatic epithelium. It is secreted mainly into seminal fluid. Only minor amounts of PSA leak into circulation from normal prostate glands, but the release of PSA from diseased prostate glands is increased. Consequently, the histologic origin of the 2 serum markers is quite different. Similarly, ^{89}Sr is localized in regions of osteoblastic activity that generally neighbor vascular endothelium. Although no direct evidence exists that ^{89}Sr localizes in endothelial cells, the current results may be related to cell-mediated mechanisms linked by the proximity of such cells (osteoblasts and endothelial cells).

A wide range of cells interacts with E-selectins, including neutrophils, monocytes, T-cells, natural killer cells, and an assortment of carcinoma cells such as breast and colon as well as melanomas. The carbohydrate determinants, sialyl Lewis A and sialyl Lewis X, frequently expressed on such human cancer cells, serve as the ligands for the E-selectins. The initial adhesion mediated by E-selectins triggers the activation of integrin molecules through the action of several common cytokines and results in the extravasation of cancer cells. Cancer cells also produce humoral factors that facilitate endothelial cell surface expression of E-selectins. The degree of surface carbohydrate ligand expression is highly correlated with the frequency of hematogenous metastases and the prognostic outcome of patients (*11*).

E-selectin also participates in a variety of chronic inflammatory diseases (e.g., rheumatoid arthritis, psoriasis, and asthma) in a manner that suggests it plays a role in endothelial proliferative mechanisms such as those common during angiogenesis. Importantly, angiogenesis-related metastatic proliferation may be blocked through inhibition of E-selectin production (*16*).

The adhesion of cancer cells to vascular endothelium, an important event in hematogenous metastasis, may be disrupted by radionuclide therapy. Increased expression of CAMs leads to augmentation in the adhesiveness of cancer cells to vessel walls, and radionuclide therapy appears to block this expression. Inhibition of such processes may undermine the metastatic course and, in the case of prostate cancer, retard the spread of bony metastatic disease. Such a radionuclide-mediated process may explain halted progression of metastatic skeletal disease in patients with index pain sites who were treated with ^{89}Sr-chloride (*17*).

The current data represent an initial step in linking radionuclide therapy to the events associated with cancer metastasis. Although the exact mechanisms accounting for the palliative and disease-controlling effects of systemic radionuclide therapy are likely to be deciphered at the molecular level, the current data point to some directions for further exploration.

CONCLUSION

Measurement of serum E-selectin concentration showed that production of this cell adhesion determinant decreases immediately after systemic radionuclide therapy with ^{89}Sr-chloride. This finding also occurs in patients who are treated a second time, thereby establishing a direct association between such therapy and E-selectin production.

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