Plasma Levels of Estrone, Estrone Sulfate, and Estradiol and the Percentage of Unbound Estradiol in Postmenopausal Women with and without Breast Disease


Department of Chemical Pathology [M. J. R., R. W. C., C. T. N., V. H. T. J.] and Academic Surgical Unit [H. A. F. D.], St. Mary's Hospital Medical School, London W2 1PG, England

ABSTRACT

To investigate the possibility of increased tissue exposure to estrogen in breast cancer patients, plasma levels of estrogens and the percentage of unbound estradiol were measured in postmenopausal women with benign or malignant breast disease and compared with levels in normal postmenopausal women. The percentage of unbound estradiol in breast cancer patients [1.85 ± 0.35% (S.D.)] was significantly higher ($p < 0.001$) than in normal postmenopausal women [1.52 ± 0.33%] and was still significantly higher when patients were matched with control subjects for weight ($p < 0.001$) or ideal body weight ($p < 0.001$). The binding capacity of sex hormone binding globulin was similar in both groups of women. No significant differences in the plasma levels of estrone, estradiol, or estrone sulfate were detected between breast cancer and normal subjects.

It is concluded that, given similar concentrations of estradiol in plasma of normal and breast cancer subjects, the significant increase found in the unbound estradiol fraction may result in a very small increment in tissue exposure to estrogens in breast cancer subjects. However, even such a small increase in tissue exposure to estradiol may be significant, given the length of time required for breast tumor development.

INTRODUCTION

The circumstantial evidence suggesting that estrogens may be involved in the development of tumors in hormone-dependent tissues such as the breast has resulted in a search for evidence of estrogen excess in women with breast cancer (10). Several studies have been made of urinary estrogen excretion by women with and without breast cancer (24), but few investigations have been carried out to measure plasma levels of estrogens. In the present study, plasma levels of estrone and estradiol have been measured in postmenopausal women with benign or malignant breast disease and have been compared with levels in normal postmenopausal women. Plasma levels of estrone sulfate have also been measured in subjects with breast cancer, insomuch as this hormone is a potential source of unconjugated estrone and estradiol. Because measurement of plasma estrogen concentrations may not indicate the level of biologically available estrogen (21), the percentage of unbound estradiol was measured. In addition, for some patients, the fraction of estradiol bound to albumin was also determined.

A preliminary account of some of the results obtained in this study has been presented (11).

SUBJECTS

Patients for the present study were recruited from postmenopausal women [56 ± 10 (S.D.) years old] attending a breast clinic. Blood (20 ml) was taken between 2 p.m. and 4:30 p.m. Steroid analyses were carried out before the subject's clinical status was known. It was subsequently established that some of the women had breast cancer, some had benign breast disease, and others were women who had previously had breast cancer (4 months to 18 years previously) who were undergoing follow-up examination. No distinction has been made in the results between women with breast cancer and those who had previously had breast cancer.

Blood samples were obtained from normal postmenopausal women (57 ± 11 years old) between 9 a.m. and 6 p.m. These women were in good health and without any endocrinological disorder. None of the subjects had received any hormone replacement therapy in the 3 months preceding the study.

Blood obtained from patients and control subjects was centrifuged, and the plasma was removed and stored at −20°C until assayed.

MATERIALS AND METHODS

Plasma levels of estrone, estradiol, and estrone sulfate were measured by methods described previously (4, 15). The percentage of unbound estradiol was measured in undiluted plasma using a Dianorm dialysis machine (23). Analysis of a plasma pool gave values of 8.3% ($n = 10$) and 8.7% ($n = 15$) for the intra- and interassay coefficients of variation for the measurement of the unbound fraction by this method. The fraction of estradiol not bound to SHBG3 was measured using a precipitation technique (16), and intra- and interassay coefficients of variation were 4.2% ($n = 21$) and 5.5% ($n = 24$), respectively. The binding capacity of SHBG was measured by the method of Rosner (20) as modified by Anderson et al. (2). A detailed description of the techniques used to measure the fractions of unbound and non-SHBG-bound estradiol has been published previously (11). A subject's ideal body weight was calculated by comparison of a subject's weight with tables for the average weight of women of the same age (6).

Statistics. Data were analyzed using Student’s t test and linear regression, using the method of least squares.

RESULTS

Plasma Estrogen Concentration. Plasma levels of estrone, estradiol, and estrone sulfate are shown in Table 1. Plasma levels of estrone and estradiol in postmenopausal women with benign or malignant breast disease and plasma levels of estrone

3 The abbreviation used is: SHBG, sex hormone binding globulin.
Table 1

<table>
<thead>
<tr>
<th>Stratum</th>
<th>Estrone (pg/ml)</th>
<th>Estrone sulfate (pg/ml)</th>
<th>Estradiol (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>28.2 ± 11.2</td>
<td>302 ± 197 (46)</td>
<td>14.0 ± 6.0 (32)</td>
</tr>
<tr>
<td>Benign</td>
<td>21.8 ± 5.4</td>
<td>307 ± 177 (17)</td>
<td>11.4 ± 5.2 (10)</td>
</tr>
<tr>
<td>Cancer</td>
<td>31.4 ± 13.4</td>
<td>307 ± 177 (17)</td>
<td>13.5 ± 6.1 (43)</td>
</tr>
</tbody>
</table>

a Mean ± S.D.

Numbers in parentheses, number of subjects.

Percentage of Unbound Estradiol, Percentage of Non-SHBG-bound Estradiol and SHBG Binding Capacity. The fraction of unbound estradiol in plasma from normal women and patients with breast disease is shown in Chart 4 and Table 2. The fraction of unbound estradiol was significantly higher ($p < 0.001$) in the breast cancer group than in normal women and also in women with benign breast disease ($p < 0.02$).

Because the binding capacity of SHBG is reduced in obese women, the unbound estradiol fraction was compared in a group of weight-matched controls and breast cancer patients and also in a group matched for ideal body weight (Chart 5), and a significant difference ($p < 0.001$) was still found.

The fraction of estradiol not bound to SHBG (i.e., unbound plus mainly albumin bound) was also measured in the women with breast disease (Table 2), but there was no significant difference between women with benign or malignant disease. There was no significant difference in the binding capacity of SHBG (expressed as ${\mu g}$ 5 $\alpha$-dihydrotestosterone per 100 ml plasma) in patients with breast cancer ($2.1 ± 1.2$ (S.D.)), in those with benign breast disease ($2.5 ± 1.8$), or in normal postmenopausal women ($2.0 ± 1.4$) (Table 2). For patients with breast disease, significant negative correlations were found between the fraction of unbound estradiol and SHBG binding capacity ($r = -0.36; p < 0.01$) and also between SHBG binding capacity and subjects' percentage of ideal body weight ($r = -0.33; p < 0.05$).
M. J. Reed et al.

DISCUSSION

The concentration of unbound estradiol was calculated from the fraction of unbound estradiol and plasma level of estradiol for patients with breast disease. These values are shown in Table 3 together with the calculated concentrations of non-SHBG-bound, SHBG-bound, and albumin-bound estradiol. Although no significant difference was found between the concentrations of these fractions in women with benign or malignant disease, it is apparent that the concentration of estradiol bound to albumin (mean, 482 pg/100 ml) is 22 times greater than the concentration of unbound estradiol (mean, 21.8 pg/100 ml). No significant correlation was found between the concentration of unbound estradiol and subjects' body weight ($r = 0.23$, not significant) (Chart 6).

The recent studies of Pardridge et al. (17) have suggested that the unbound fraction in cancer patients suggests that breast tissue may receive increased estrogen exposure although the difference between the 2 groups is very small. Others (8, 12) have failed to find a significant difference between plasma levels of estradiol in postmenopausal breast cancer patients and normal controls. In 2 recent reports (7, 14), significantly elevated plasma levels of estradiol were found in postmenopausal women with breast cancer. In these 2 studies, the mean plasma estradiol levels of approximately 25 pg/ml in normal postmenopausal women and 50 pg/ml in postmenopausal breast cancer subjects were much higher than those found in the present study. There is no obvious reason for the difference between these results and our own, other than possible differences in methodology. However, recent studies (3, 13, 18) have reported mean values for estradiol concentrations in plasma from normal postmenopausal women of 13.9, 13.4, and 7.2 pg/ml, respectively, which are similar to those reported in our study.

The reason for the increase in the fraction of unbound estradiol seen in breast cancer patients is not known. Although patients with breast cancer tended to be older than control subjects, it is unlikely that this could account for the difference inasmuch as no correlation was found between the fraction of unbound estradiol and age in breast disease and control subjects. In the present study, however, there was no difference in the binding capacity of SHBG between cancer patients and normal women, in contrast to the results obtained by Moore et al. (14). Although weight affects the binding capacity of SHBG (5), a significant difference was still found in the present study when patients were carefully matched with controls for weight and ideal body weight. It is possible that, as suggested by Sitteri (21), differences in plasma lipids may account for the higher percentage of unbound estradiol seen in breast cancer patients.

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that, in addition to the free fraction, steroids bound to albumin may be biologically available. In the present study, although the concentration of the albumin-bound fraction of estradiol was 22 times greater than the unbound fraction, no difference between patients with benign or malignant breast disease was detected. Because it is possible that women with benign breast disease are at an increased risk for developing breast cancer (9), further studies are required to resolve the importance of the albumin-bound estradiol fraction in the development of breast cancer.

Plasma levels of estrone have been measured previously in postmenopausal women with breast cancer (1, 7). Drafta et al. (7) found significantly higher plasma levels of estrone in postmenopausal women with breast cancer.

As far as we are aware, only one other investigation has been carried out to measure levels of estrone sulfate in postmenopausal women with breast cancer (19). It has been shown that in vitro estrone sulfate is a potential source of estrone and estradiol in MCF7 human breast cancer cell lines (22). In the present study, however, no difference in the plasma levels of estrone sulfate was detected, thus confirming the results obtained by Remy-Martin et al. (19). In the small number of subjects studied thus far, no significant correlation was found between plasma levels of estrone sulfate and patient’s weight, as reported previously for normal postmenopausal women (15).

In conclusion, the results of the present study suggest that breast tissue in patients with breast cancer may be exposed to a small increase in the amount of biologically active estradiol compared with that in women without breast cancer, although the difference is very small. However, it is possible that even such a small increase may be significant during the long period required for the development of breast cancer. Although SHBG-bound or albumin-bound estradiol fractions may also be involved in determining tissue estrogen exposure, it remains important to discover the mechanism responsible for the increase in the unbound fraction of estradiol seen in many breast cancer patients, because this may provide further insight into factors that govern the development of this disease.

REFERENCES

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