Abnormal 24-Hr Mean Plasma Concentrations of Dehydroisoandrosterone and Dehydroisoandrosterone Sulfate in Women with Primary Operable Breast Cancer

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ABSTRACT

The 24-hr mean plasma concentrations of dehydroisoandrosterone (DHA) and dehydroisoandrosterone sulfate were measured in 11 women with primary operable breast cancer, ages 31 to 78 years, and in 37 normal women, ages 21 to 75 years. In contrast to the marked and progressive decline of DHA and dehydroisoandrosterone sulfate concentration with age in the normal women, the concentrations of both steroids were age invariant in the cancer patients. The premenopausal patients had subnormal plasma DHA and dehydroisoandrosterone sulfate levels, while the post menopausal patients had supranormal levels. Since the plasma DHA/androsterone ratio was normal in the premenopausal patients and significantly elevated in the postmenopausal patients, it is postulated that the subnormal plasma adrenal androgen levels in the premenopausal patients were due principally to diminished production of these steroids, while the elevated plasma levels in the postmenopausal patients were due principally to slowed metabolic removal. Reports in the literature that DHA inhibits the development of breast cancer in mice suggest that the subnormal plasma DHA levels in premenopausal breast cancer may have clinical significance.

INTRODUCTION

One of the first hormonal groups studied in breast cancer was the adrenal androgens. Allen et al. (1) found that the ratio of 11-deoxy-17-ketosteroids (derived from adrenal androgens) to 11-oxo-17-ketosteroids (derived from cortisol) was considerably lower in patients who did not have a therapeutic response to adrenalectomy or hypophysectomy than in those who did. Juret et al. (10) and Kumaoka et al. (13), while confirming the predictive value of the level of urinary androgen metabolite excretion in patients undergoing adrenalectomy or hypophysectomy, reported that the corticoid excretion values added no useful information and could be omitted.

In 1962, Bulbrook et al. (7) reported that women with primary operable breast cancer excreted subnormal amounts of 11-deoxy-17-ketosteroids in the urine prior to mastectomy and suggested that this abnormality might precede the clinical onset of the disease. To explore this possibility, these workers set up a large-scale study on the island of Guernsey, Great Britain, in which urines were collected from about 5000 healthy women who were then followed clinically for up to 9 years. At the end of that time, as subsequently reported (6, 27) of the women had developed breast cancer, most or perhaps all of them at either premenopausal age or just beyond. The excretion of various metabolites in the urines collected initially (i.e., 5 months to 9 years before the clinical diagnosis of the cancer) was compared with that of 187 carefully matched controls from the same population who had not developed cancer, and it was found that the excretion of both etiocholanolone and androsterone (the 2 principal urinary metabolites of the adrenal androgens) was significantly lower in the women who had gone on to manifest breast cancer. These results appear to confirm that subnormal urinary excretion of adrenal androgen metabolites is present before clinical breast cancer develops. A follow-up paper from this group (26) suggests that this abnormality may be genetic in origin. It was found that a group of 52 unaffected sisters of the Guernsey study women who developed breast cancer likewise showed subnormal urinary excretion of androsterone and etiocholanolone.

More recently, with the development of suitable analytical methods, the attention of workers in this field has turned to the measurement of plasma adrenal androgen levels. Wang et al. (25) initially reported normal plasma levels of DHAS and its metabolite androstenedione sulfate in women with breast cancer, but after Brownsey et al. (5) had reported subnormal DHAS levels in these women, Wang et al. (28) confirmed this finding in a later restudy; they also found subnormal levels of androsterone sulfate. Šonká et al. (22) reported subnormal plasma levels of DHA in breast cancer, but their method, using solvolysis, measured both DHA and DHAS, and since the latter is added no useful information and could be omitted.

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We were prompted to restudy plasma DHA and DHAS levels in women with breast cancer for 3 reasons.

1. Reported plasma levels have been measured in ‘‘spot’’ samples, but studies from this and other laboratories (2-4, 9, 10, 12, 14, 17, 23, 29-31) have shown that the levels of many hormones, specifically including DHA (19), fluctuate markedly (up to several hundred %) during a 24-hr cycle, so that spot values may be unrepresentative and therefore misleading. To avoid this problem, we have developed the approach of measuring 24-h mean plasma concentration by sampling blood...
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every 20 min, pooling aliquots of the 72 samples, and determining the concentration of hormone in the pooled sample (32).

2. The groups of patients reported in most studies have been heterogeneous, sometimes complicated by other diseases and/or medications, and sometimes poorly or not at all characterized by stage of disease. We have studied only well-characterized patients with primary operable breast cancer and normal controls, with both groups rigorously selected to exclude factors known or suspected to alter endocrine function (e.g., significant past or present disease; abnormal thyroid, kidney, or liver function; medications; history of endocrine therapy; and history of chemotherapy).

3. The distinction between premenopausal and postmenopausal breast cancer has often not been taken into consideration, despite the fact that various lines of evidence point to the possibility that premenopausal and postmenopausal breast cancer may represent 2 biologically and epidemiologically distinct diseases (8, 15). In the present study, we considered the results for premenopausal and postmenopausal breast cancer patients separately and found different DHA and DHAS abnormalities in these 2 groups.

MATERIALS AND METHODS

Patients and Normal Controls. Since the 24-hr mean plasma levels of both DHA and DHAS show an inverse linear correlation with age (32), we studied enough healthy controls (n = 37) over the age range of 21 to 75 to define the regression line adequately. All subjects met the screening criteria outlined in "Introduction." The group of breast cancer patients consisted of 11 women, ages 31 to 78 years, with clinically operable Stage 1 or 2 breast cancer studied preoperatively. Screening criteria were as outlined under "Introduction."

Analytical Methods. The 24-hr multiple-blood-sampling technique was as described previously by this laboratory (32). DHA was determined by radioimmunoassay as described by Rosenfeld et al. (20), and DHAS was determined by radioimmunoassay as described by Nieschlag et al. (16). To help define the mechanism of the abnormalities in DHA and DHAS levels that will be described in "Results," we also determined the 24-hr mean plasma concentration of androsterone in all patients and controls, using the radioimmunoassay method of Kream et al. (11).

Statistical Methods. Regression equations for DHA and DHAS versus age in patients and controls were determined by the method of least squares, using a computer program. Significance of the correlations was determined from the correlation coefficient (r). Significance of the difference between patients and controls with respect to DHA levels and the DHA/androsterone ratio was determined with Student's 2-tailed t test.

RESULTS

Plasma DHA and DHAS Levels. In contrast to the marked and progressive decline of DHA and DHAS concentration with age in normal women (Charts 1 and 2), the concentrations of both steroids were age invariant in the patients over the age range of 32 to 78 (Charts 3 and 4). The levels of both steroids were subnormal in premenopausal patients and supranormal in postmenopausal patients (this is shown for DHA in a conventional scattergram in Chart 5).

Ratio of Plasma DHA to Plasma Androsterone. In premenopausal cancer patients, the DHA/androsterone ratio was essentially identical to that of premenopausal controls (Chart 6). In postmenopausal patients, the ratio was significantly higher than that of postmenopausal controls (Chart 7).

DISCUSSION

The plasma DHA and DHAS levels we observed in breast cancer patients were differently abnormal in premenopausal and postmenopausal patients: subnormal in the former and supranormal in the latter. It is pertinent to note that several workers (8, 15) have suggested a "2-disease" theory of breast...
The principal factors that could raise or lower plasma DHA and DHAS levels are changes in their production rate and/or their metabolic removal rate. Since androsterone is a major plasma metabolite of DHA, we felt that examination of the plasma DHA/androsterone ratio could shed light on the role these factors play in the DHA (and presumably the DHAS) abnormalities. The fact that the DHA/androsterone ratio was normal in the premenopausal patients (the same was true of subsequently to develop breast cancer had subnormal urinary excretion of adrenal androgen metabolites years earlier relates to premenopausal breast cancer and is therefore quite compatible with our finding of subnormal plasma adrenal androgen levels in this condition.

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the DHA/androsterone sulfate ratio) speaks against a significant role for increased metabolic removal rate as a factor and therefore suggests by exclusion that the subnormal plasma DHA levels in these patients were due principally to diminished DHA production. Conversely, the finding that the DNA/androsterone sulfate ratio was markedly elevated in the postmenopausal breast cancer patients (the same was true of the DHA/androsterone sulfate ratio) suggests that the metabolic conversion of DHA to androsterone was slowed in these patients; this effect would probably be sufficient to account for their elevated plasma DHA levels without postulating any increase in DHA production (though the latter possibility cannot be excluded).

If further studies should confirm that there are indeed 2 different mechanisms for the different plasma adrenal androgen abnormalities in premenopausal and postmenopausal breast cancer patients, namely decreased production in the former and slowed metabolic removal in the latter, a biochemical basis would have been provided for the '2-disease' distinction in breast cancer.

A very large amount of data accumulated over a 23-year period, including the findings of the present study, points to the existence of a deficiency of adrenal androgens in women with premenopausal breast cancer. Prospective studies and familial studies suggest that the deficiency antedates the clinical appearance of the disease and may well be a genetic marker. Of course, a genetic marker may be just that—a marker, with no implications for the pathophysiology, prevention, or treatment of the disease. However, the intriguing recent observation by Schwartz (21) that administration of DHA strongly inhibits the development of breast cancer in a strain of mice that is normally prone to the disease suggests that the subnormal levels of DHA in premenopausal breast cancer may indeed have pathophysiological significance and even tempt one to consider the possibility of prophylactic trials of DHA, at least in high-risk young women.

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REFERENCES


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