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Estrogen Receptor Status, Adrenal Androgens and 7α-Hydroxydehydroepiandrosterone in Breast Cancer Patients*

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Abstract—*Plasma concentrations of dehydroepiandrosterone (DHA), DHA-sulphate (DHAS) and 7αhydroxy-DHA (7αOHDHA) were measured and compared with tumor estrogen receptor (ER) status in 33 postmenopausal patients with breast cancer. Although the plasma concentrations of DHA, DHAS and 7αOHDHA were not different between the ER-positive (ER+) and ER-negative (ER−) patient groups, the ratios of 7αOHDHA/DHAS and of DHA/DHAS were significantly higher ($P < 0.001$ and $P < 0.001$ respectively) in the ER− group. Nine women (normal or with benign breast disease) of similar age and menopausal status had values for plasma 7αOHDHA/DHAS and DHA/DHAS between those of ER+ and ER− patient groups. The measurement of these steroid ratios in the plasma of breast cancer patients thus provides an indirect estimate of ER status. Since DHA and 7αOHDHA are major metabolites of precursor DHAS in mammary tumor tissues, changes in their relative quantities in plasma may reflect the influence of receptor-mediated events on mammary steroid metabolism. Alternatively, the relative increase in tumor metabolism of androgens inferred from high 7αOHDHA/DHAS and DHA/DHAS ratios in the ER− group may disrupt the hormonal microenvironment of the estrogen receptor. These events may, in turn, predispose toward ER status and a poor response to endocrine therapy.*

INTRODUCTION

THE SELECTION of patients with metastatic breast cancer who are likely to respond favourably to endocrine intervention has been the subject of intense research for many years [1]. Two biochemical parameters which have been used for this purpose are the estrogen receptor (ER) content of tumor tissues [2, 3] and the relative concentrations of androgen and corticosteroid metabolites in urine [4]. Surprisingly there appears to be little correlation between these two predictive tests [5, 6], and reports linking androgen metabolism to estrogen production [7–9] and to estrogen receptor activity in mammary tumors [10–12] have not led to a clear understanding of this relationship. There does, however, seem to be little doubt that neoplastic mammary tissues are active in androgen metabolism, synthesising not only estrogens but also a spectrum of C19 steroids [13] which are released into the immediate micro-environment of ER in mammary cells and which may modulate estradiol binding and receptor translocation [10, 11, 14].

Most investigations of androgen metabolism by mammary tissues have, of necessity, used *in vitro* techniques, although *in vivo* perfusion of tumor tissues during surgery has also been reported [15]. The demonstration of DHA and 7αOHDHA as major metabolites of DHAS by human mammary tissues *in vitro* [13] and the measurement of these steroids in patient plasma samples [16] led to the suggestion that the plasma concentrations of these steroids may be related to tumor bulk or activity [17]. This observation raised the possibility that DHAS metabolism by mammary

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tumors *in vivo*, as indicated by the concentrations of DHA and 7αOHDHA in plasma, might be related to tumor ER status. The results presented here suggest that the relative plasma concentrations of 7αOHDHA, DHA and DHAS may be used to distinguish ER status in postmenopausal patients with breast cancer.

MATERIALS AND METHODS

Patients and control subjects

Thirty-three postmenopausal breast cancer patients (age 48–73 yr) were studied by blood sampling 2 weeks before mastectomy. Control subjects (age 48–69 yr) were patients with benign breast disease and hospital staff. Tumor tissue was obtained at surgery for the determination of ER status. There was no significant difference in the clinical estimate of tumor size or histological nodal infiltration with tumor between the ER+ and ER- groups.

Biochemical tests

Estrogen receptor status was determined as described previously [18]. A significant level of ER was defined as ⩾5 fmol/mg cytosol protein [3]. Plasma 7αOHDHA, DHA and DHAS were measured by radioimmunoassays as described previously [17]. Plasma samples were assayed in duplicate and the assay was repeated when duplicates varied by more than 10% from the mean.

Statistical analysis

The relationship between plasma DHAS and age was determined using linear regression analysis. The difference between mean steroid values in the ER+ and ER- patient groups was assessed by Welch's t statistic, which allows for different variances between groups [19]. Where appropriate the steroid values in the ER+ and ER- patient groups were assessed by analysis of covariance, with age as a covariate.

The plasma steroid levels are expressed in the text as mean ± S.E.M. Since the quantities of DHAS are much greater (100–10,000 times) than those of DHA or of 7αOHDHA, the ratios DHA/DHAS and 7αOHDHA/DHAS are expressed as (0.001 × adjusted mean ± S.E.M.).

RESULTS

As reported in other studies [20], the concentrations of the adrenal androgens DHAS and DHA in human female plasma decreased significantly after age 50 yr (Table 1). This tendency was particularly prominent when correlating the declining concentrations of DHAS in plasma with increasing age ($r = -0.760$, $P < 0.001$; Fig. 1). There was, however, no differentiation between the ER+ and ER- patient groups on the basis of plasma DHAS concentrations. Thus, although the mean ± S.E.M. value for plasma DHAS in the ER+ group (756 ± 93 ng/ml) was higher than in the ER- group (591 ± 103 ng/ml), the difference was not significant. The mean value for plasma DHA in the ER+ group (2.28 ± 0.36 ng/ml) was lower than in the ER- group (3.33 ± 0.83 ng/ml) but again the difference was not significant (Table 1). The mean value for plasma 7αOHDHA was also lower in the ER+ group (154.5 ± 22.6 pg/ml) than in the ER- group (205.4 ± 31.9 pg/ml) but the difference was not significant (Table 1).

Since 7αOHDHA and DHA are metabolites of DHAS the date were analyzed in terms of the ratios 7αOHDHA/DHAS and DHA/DHAS, which, in the simplest interpretation, represent ratios of product to precursor. When these ratios were compared between groups of patients with ER+ and ER- tumors there appeared to be a clear distinction between the two groups, with a consistent increase in the ratios in the ER- group.

In a group of nine control subjects (age 48–69 yr), the mean ± S.E.M. value of the ratio 7αOHDHA/DHAS (0.001 × 0.294 ± 0.040) was intermediate between the mean values of the ER+ (0.001 × 0.224 ± 0.026) and the ER- breast cancer groups (0.001 × 0.437 ± 0.062). The difference between the ER+ and ER- groups in the ratio 7αOHDHA/DHAS was significant not only on the basis of a difference in the mean values ($P < 0.002$, Table 1) but also by analysis of covariance ($P < 0.0002$), with age as a covariate (Fig. 2).

The mean ± S.E.M. value for the ratio DHA/DHAS in the control group (0.001 × 4.41 ± 1.00) was intermediate between the values in the ER+ group (0.001 × 3.12 ± 0.32) and the ER- group (0.001 × 6.63 ± 1.30). There was a significant difference in the mean values of the ratio DHA/DHAS between the ER+ and ER- patient groups ($P < 0.01$, Table 1) but there was no influence of age.

DISCUSSION

The ability of human mammary tissues to metabolize adrenal androgens to a number of products, including estrogens [7–9], testosterone, androstenediol, dihydrotestosterone and 7αOHDHA [13], is well known. There is also evidence of an interaction between some of these metabolites and the cytoplasmic ER either directly, as in the competition between androstenediol for ER [10, 11], or indirectly, as in the correlation between tumor steroid sulfurylation activity and ER content [12, 21]. In addition, there is evidence that most of the circuating estrogen in postmenopausal women is derived from adrenal androgens [22, 23].

Table 1. The concentration of DHAS, DHA and 7αOHDHA and their ratios in plasma from patients with ER+ and ER- mammary tumors

Age (yr)	ER+	DHAS (ng/ml)	DHA (ng/ml)	7αOHDHA (pg/ml)	7αOHDHA/DHAS (×0.001)	DHA/DHAS (×0.001)
48	16	1013	-	152.5	0.1505	-
52	10	1341	5.46	137.0	0.1021	4.0716
52	41	1506	4.33	344.3	0.2286	2.875
53	5	1017	1.82	128.0	0.1258	1.7896
55	52	768	1.56	41.0	0.0533	2.0313
56	36	1327	4.90	322.0	0.2426	3.6925
58	8	832	2.71	170.4	0.2048	3.2572
58	146	1348	4.16	330.3	0.2450	3.0861
59	17	1224	3.41	308.1	0.2517	2.7859
59	62	583	0.22	70.0	0.1200	0.3774
61	33	679	1.67	135.0	0.1988	2.4595
62	16	224	1.07	52.0	0.2321	4.7768
63	275	513	-	248.3	0.4840	-
64	70	497	0.83	82.0	0.1649	1.6700
65	6	286	1.02	127.0	0.4440	3.5664
66	66	295	1.20	104.0	0.3525	4.0678
66	356	286	0.50	27.0	0.0994	1.7483
66	36	352	2.07	114.0	0.4090	5.8807
67	102	516	2.72	109.4	0.2120	5.2173
68	36	514	1.48	59.0	0.1147	2.8794
Mean ± S.E.M.		756 ± 93	2.28 ± 0.36	154.5 ± 22.6	0.2242 ± 0.026	3.126 ± 0.32
Age (yr)	ER-	DHAS (ng/ml)	DHA (ng/ml)	7αOHDHA (pg/ml)	7αOHDHA/DHAS (×0.001)	DHA/DHAS (×0.001)
54	0	885	3.37	235.0	0.2655	3.8079
55	0	1017	11.08	332.0	0.3264	10.8948
56	0	949	4.31	241.9	0.2549	4.5416
58	0	853	3.39	433.7	0.5084	3.9742
59	0	237	0.49	118.0	0.4978	2.0675
59	0	588	1.32	173.0	0.2942	2.2449
60	1	1245	5.80	332.1	0.2667	4.6586
63	0	109	1.87	92.0	0.8440	7.1560
63	0	676	-	40.0	0.0591	-
64	0	323	1.47	126.0	0.3900	4.5511
65	0	322	1.83	220.5	0.6847	5.6832
66	4	347	3.96	248.0	0.7147	11.4121
73	0	136	1.17	78.8	0.5794	8.6029
Mean ± S.E.M.		591 ± 103	3.33 ± 0.83	205.4 ± 31.9	0.4374 ± 0.062	6.632 ± 1.30

Blood samples were obtained 2 weeks before mastectomy. The plasma levels of DHAS, DHA and 7αOHDHA were determined as described in Materials and Methods.

There is also a considerable body of literature, some of it controversial, indicating a link between decreased urinary androgen metabolite excretion and failure of breast cancer patients to respond to endocrine therapy [4, 24]. There has been, however, no success in attempts to demonstrate a difference between ER- and ER+ breast cancer patients by measurement of urinary androgen metabolite concentrations [5, 6] or any plasma steroid concentrations.

In this investigation we have shown that most postmenopausal patients with ER- breast cancer have higher plasma 7αOHDHA and DHA concentrations, in relation to plasma DHAS, than found in patients with ER+ tumors. In isolation the concentrations of DHAS, DHA and 7αOHDHA do not distinguish between ER- and ER+ patients. This is not surprising since the output of DHAS and, to a lesser extent, DHA from the human adrenal cortex would be far greater than any direct effect on plasma levels of synthesis or metabolism of androgens by the mammary tumor. Mammary tumor tissue does, however, metabolize DHAS very actively to DHA and 7αOHDHA [13]. Thus any assessment of the effect of tumor activity on androgen metabolism is best related to the concentration of the major precursor (DHAS) available to the tumor tissue from the circulating blood. A previous report from this laboratory has demonstrated that the plasma concentration of 7αOHDHA is closely correlated with the concentrations of DHA and DHAS in plasma [17]. In that study it was found that an increasing concentration of plasma DHAS

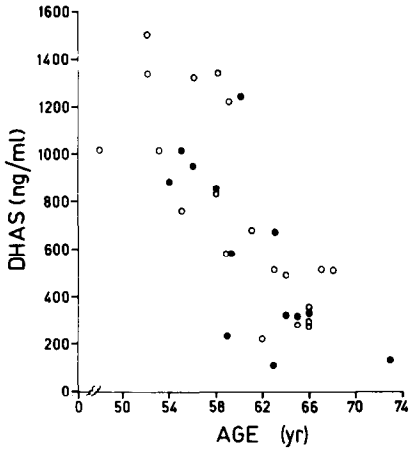

Fig. 1. The plasma levels of DHAS compared with patient age and ER status. DHAS was measured in plasma samples obtained 2 weeks before tumor excision and determination of ER status. There was a decrease in plasma DHAS with increasing age (r = -0.760, P < 0.001) but there was no difference in DHAS levels between the ER- (●) and ER+ (○) patient groups.

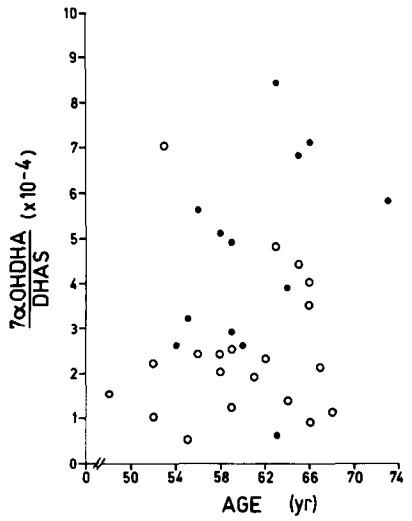

Fig. 2. The ratio of plasma 7αOHDHA/DHAS compared with patient age and ER status. The 7αOHDHA and DHAS levels were measured in plasma samples obtained 2 weeks before tumor excision and determination of ER status. The ratio 7αOHDHA/DHAS was greater in plasma from patients with ER- (●) tumors than in those with ER+ (○) tumors (P < 0.0002 by analysis of covariance, with age as a covariate).

would lead to an elevation in plasma 7αOHDHA and that the size of the primary tumor and the metastatic load also influenced plasma 7αOHDHA levels. In the present study we have compared two patient groups with similar tumor load and nodal infiltration but which differed in the tumor ER status. The results suggest that patients with ER- status achieve higher plasma concentrations of 7αOHDHA and DHA in relation to the concentration of DHAS than patients with ER+ tumors.

These data may be explained in several ways. There could be an inductive effect of estrogens in ER+ tumors to partially suppress sulfatase and 7α-hydroxylase activity, which would decrease the metabolism of DHAS to DHA and 7αOHDHA. The same effect would be achieved by increasing the rate of sulfurylation of DHA to DHAS in mammary tumor tissues [21]. In most *in vitro* studies, however, sulfatase activity greatly exceeds sulfurylating activity [13].

Alternatively, the raised plasma levels of 7αOHDHA and DHA in relation to DHAS in ER- patients may simply reflect an increase in metabolism of all androgens, not only DHAS, to a variety of metabolites, including estrogens (estradiol, estrone) and other steroids capable of modulating tumor ER levels (androstenediol, androstanediol and dihydrotestosterone). The production of these agents in the immediate microenvironment of the receptor and the sites of synthesis, translocation and recycling of ER [11] may cause sufficient disruption of the normal regulatory processes to lower the cytoplasmic ER content. Thus, in patients with ER- tumors the increased conversion of DHAS, the androgen at the highest concentration in plasma, to its major mammary metabolites 7αOHDHA and DHA results in a small rise in the plasma concentrations, detectable only if compared to the precursor (DHAS) concentration. The much smaller conversion to estrogens and to androgens capable of binding to ER are almost certainly not detectable in plasma, but the local concentrations in the tumor tissue [25] and in the micro-environment of the receptor may have significant effects.

The possible diagnostic potential of these observations is difficult to assess. There does appear to be good discrimination between patients with ER+ and ER- tumors by the ratios 7αOHDHA/DHAS and DHA/DHAS. There is, however, no evidence to suggest that these measurements would add to the accuracy of predicted response to endocrine therapy already available from the ER status alone.

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