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The Relationship of Plasma 7α-Hydroxy Dehydroepiandrosterone to Disease Stage and Adrenal Androgens in Breast Cancer Patients

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Abstract—*The concentrations of 7α-hydroxy dehydroepiandrosterone (7α-hydroxy DHA) were measured in the plasma of patients presenting with different stages of breast cancer. The results were compared with the concentrations in control subjects and patients with benign breast disease. The concentrations of 7α-hydroxy DHA in plasma were also compared with the concentrations of dehydroepiandrosterone (DHA) and dehydroepiandrosterone sulphate (DHAS) in plasma.*

Plasma 7α-hydroxy DHA was lower in patients presenting with early disease without axillary node involvement than in the control or benign disease groups but no significant difference was found between the control or benign disease group and the early disease nodal positive group or advanced disease groups. Plasma 7α-hydroxy DHA was positively correlated with plasma DHA and, to a lesser extent, with plasma DHAS. The greater increases in plasma 7α-hydroxy DHA compared to plasma DHA in patients presenting with distant metastases and with large tumours were significantly different to the control and benign disease group but there was no significant difference between the early disease groups and the control and benign disease group. The greater increase in plasma 7α-hydroxy DHA compared to plasma DHAS in patients presenting with metastases was significantly different to the control group, but there was no significant difference in any of the other patient groups.

INTRODUCTION

THE CONVERSION of dehydroepiandrosterone (DHA) to 7α-hydroxy dehydroepiandrosterone (7α-hydroxy DHA) by animal [1, 2] and human tissues [3–6] suggests widespread 7α-hydroxylase activity but no *in vivo* studies have been carried out to determine the importance of 7α-hydroxylation in DHA metabolism. In some human tissues *in vitro*, particularly amniotic epithelium [7] and mammary tissues [8], 7α-hydroxylation appears to be a major pathway of DHA metabolism. It has also been shown that there is greater production of 7α-hydroxy DHA by malignant human mammary tumour tissues than by benign human mammary tumour tissues when dehydroepiandrosterone sulphate (DHAS) is used as the incubation substrate [8]. These observations led to the suggestion that malignant human mammary tumour tissues may produce enough 7α-hydroxy DHA from circulating DHAS to cause an elevation in plasma concentrations of 7α-hydroxy DHA.

The measurement of plasma 7α-hydroxy DHA in an unselected group of breast cancer patients suggested that there is less 7α-hydroxy DHA in the plasma of breast cancer patients than in control subjects and patients with benign breast disease [9]. There was, however, a much greater range of plasma 7α-hydroxy DHA values in the breast cancer patients than in both the benign disease and control groups and the possibility that some mammary tumours may produce enough of this steroid to cause high concentrations in plasma was unresolved.

In the study reported here, plasma 7α-hydroxy DHA has been determined in patients presenting with different stages of breast

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cancer and has also been compared to plasma DHA and DHAS to obtain information on which of these precursors are of most importance in influencing plasma 7α-hydroxy DHA.

MATERIALS AND METHODS

Blood samples were obtained from patients with breast cancer and with benign breast disease, before surgical or radiation therapy, and from a control group comprising hospital staff and hospitalised patients without endocrine or malignant disease. Samples were also obtained from breast cancer patients 2–20 weeks after therapy. Blood was collected between 9 a.m. and 5 p.m. into heparinised tubes and centrifuged to separate plasma which was stored at −20°C until analysed. The patients were classified according to the TNM system [10] into different disease stage groups. The presence of distant metastases was established by radiographic or bone scanning techniques.

Plasma 7α-hydroxy DHA [9], DHA and DHAS [11] were determined by radioimmunoassay. An antiserum to DHA, allowing the determination of DHA in dried [hexane/ethylacetate (9:1)] extracts without chromatography, was obtained from I.S.I., Calif., U.S.A. In each assay a standard plasma sample was measured in triplicate and inter- and intra-assay coefficients of variation were <15% and <8% respectively in all three assays. Patient plasma samples were assayed in duplicate and were repeated when duplicates varied by more than 10% from the mean. Statistical comparison between patient groups and controls was by Student's *t*-test. The correlation between plasma steroid concentrations in the different groups was by linear regression analysis and differences in the regression lines were tested by analysis of covariance.

RESULTS

Comparison of plasma 7α-hydroxy DHA in breast cancer patients, benign breast disease patients and in control subjects

Plasma 7α-hydroxy DHA was compared between groups of patients presenting with different stages of breast cancer. The patient groups were also compared with the group of control subjects and the group of patients with benign breast disease. The mean plasma 7α-hydroxy DHA concentration in the early disease nodal negative (T_{1-2}, N_0, M_0) group was significantly lower than in the control group ($P<0.05$) and in the benign disease group ($P<0.02$, Table 1). In all the other patient groups (early disease nodal positive: T_{1-2}, N_1, M_0, large tumours: T_{3-4}, N_0, M_0 and advanced disease with distant metastases: T_{1-4}, N_{0-1}, M_1) there was no significant difference from the control subjects and the benign breast disease group (Table 1). The highest mean plasma 7α-hydroxy DHA concentration of the breast cancer patient groups was in those patients presenting with distant metastases. There was also a tendency for increasing plasma 7α-hydroxy DHA with more advanced disease at presentation but the differences between the disease stage groups were not significant. Although the mean plasma concentration of 7α-hydroxy DHA was greater in the benign breast disease group than in the control group, there was no significant difference.

The effect of mastectomy or radiotherapy on plasma 7α-hydroxy DHA

A comparison of the mean plasma levels of 7α-hydroxy DHA in early breast cancer patients (T_{1-2}, N_0, M_0 and T_{1-2}, N_1, M_0 combined) before and after mastectomy indicates there was no change after the removal of small primary tumours. The treatment of patients with advanced breast cancer (T_{3-4}, N_0, M_0 and T_{1-4}, N_{0-1}, M_1 combined) by mastectomy or radiotherapy was accompanied by a significant decrease in the mean plasma 7α-hydroxy DHA ($P<0.05$) to about half the pretherapy concentration. This decrease was not, however, observed consistently in matched pre- and post-therapy samples and may require further investigation.

The effect of age on plasma 7α-hydroxy DHA

Although there was a tendency for plasma 7α-hydroxy DHA to decrease with increasing age there was no significant correlation, using linear regression analysis, in any of the disease or control groups.

A comparison of 7α-hydroxy DHA with DHA and DHAS in the plasma of patients and control groups

There was a strong positive correlation between plasma 7α-hydroxy DHA and DHA in all the breast cancer patient groups regardless of the stage of disease at presentation (Table 2). There was also a strong correlation between these two plasma steroids in the control group but the correlation in the benign disease group was not significant ($P<0.10$). The correlation between 7α-hydroxy DHA and

DHA in the combined breast cancer group (Table 2c) was highly significant ($P<0.001$) and was much stronger than the correlation between 7α-hydroxy DHA and DHAS. This tendency for a stronger correlation between plasma 7α-hydroxy DHA and DHA than between 7α-hydroxy DHA and DHAS was consistent throughout all the patient and control groups (Table 2).

On comparing the differences between the slopes of the regression lines of 7α-hydroxy DHA on DHA (Fig. 1a), by analysis of covariance, there was a significant difference between the patients with distant metastases (T_{1-4}, N_{0-1}, M_1) at presentation and either the control and benign (combined) group ($t=4.27$, $P<0.01$) or the early disease nodal negative and nodal positive (combined: T_{1-2}, N_{0-1}, M_0) group ($t=4.02$, $P<0.01$). There was also a difference between patients with large tumours (T_{3-4}, N_0, M_0) and the control and benign (combined) group ($t=2.08$, $P<0.05$) but not compared to the combined early disease group ($t=1.85$, $P<0.10$).

In comparing differences between the slopes of the 7α-hydroxy DHA on DHAS regression lines (Fig. 1b) there was a similarity between the slopes of all the regression lines except for the group presenting with distant metastases which, when compared to the control group, was significantly different ($t=2.71$, $P<0.02$).

DISCUSSION

The concentrations of DHA and DHAS in human plasma fluctuate, particularly during the early morning hours, due to episodic

Table 1. The concentration of 7α-hydroxy DHA in the plasma of breast cancer patients

Subject group with stage of disease at presentation	*N*	7α-hydroxy DHA (pg/ml plasma) ±S.D.	Age ±S.D.
Control	24	195.8 ±76.1	45.2 ±12.1
Benign breast disease	12	224.5 ±80.0	37.7 ±10.1
Early disease, nodal negative (T_{1-2}, N_0, M_0)	25	149.0 ±83.2	57.0 ±10.2
Early disease, nodal positive (T_{1-2}, N_1, M_0)	12	173.9 ±117.8	57.2 ±12.0
Advanced disease, large tumours (T_{3-4}, N_0, M_0)	15	167.1 ±140.4	51.0 ±13.6
Advanced disease, distant metastases (T_{1-4}, N_{0-1}, M_1)	15	207.5 ±160.2	59.3 ±8.2

Table 2. Correlation of 7α-hydroxy DHA with DHA and DHAS in control subjects and different disease stage groups

Subject group	Steroid	*N*	Correlation coefficient (*r*)	Significance (*P*)
Control	DHA	24	0.530	<0.01
	DHAS	18	0.587	<0.025
Benign breast disease	DHA	12	0.522	n.s.
	DHAS	10	−0.411	n.s.
All breast cancer patients	DHA	102	0.721	<0.001
	DHAS	90	0.464	<0.001
Early disease nodal negative (T_{1-2}, N_0, M_0)	DHA	25	0.683	<0.001
	DHAS	25	0.296	n.s.
Early disease nodal positive (T_{1-2}, N_1, M_0)	DHA	12	0.800	<0.01
	DHAS	12	0.644	<0.025
Advanced disease, large tumours (T_{3-4}, N_0, M_0)	DHA	15	0.694	<0.01
	DHAS	14	0.004	n.s.
Advanced disease, distant metastases (T_{1-4}, N_{0-1}, M_1)	DHA	15	0.863	<0.001
	DHAS	15	0.743	<0.01

adrenocortical activity stimulated by corticotrophin [12]. In any study comparing baseline or unstimulated concentrations of these steroids in human plasma it is, therefore, desirable that samples be collected outside the main period (01.00–12.00 hr) of episodic secretion. There may also be acute periods of adrenocortical activity, provoked by mild stress, which are largely unavoidable. Blood

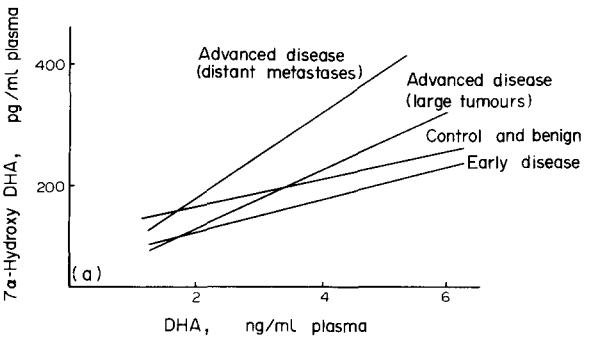

Fig. 1 (a). Relationship of 7α-hydroxy DHA to DHA.

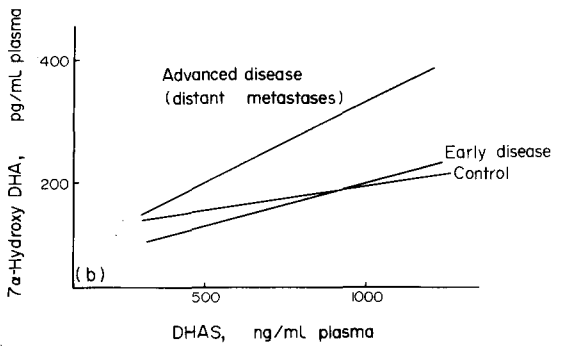

Fig. 1 (b) Relationship of 7α-hydroxy DHA to DHAS.

sample collection for this study was initially specified for 12.00–17.00 hr but, because of procedural difficulties, this requirement was relaxed. Consequently a small proportion (20–30%) of the data is derived from samples collected between 09.00–12.00 hr. Episodic activity of the adrenal cortex is, however, considerably decreased by this time and some investigators have presented evidence to suggest that, for DHAS, there is no significant variation in plasma concentration between samples collected at 10.00 and 17.00 hr [13]. It would also seem that the associations between the steroids studied here were found to be significant despite any variation caused by sample collection.

Previous reports have discussed the ability of mammary tumour tissues to convert plasma androgens to other steroids, particularly oestrogens [14–17]. It has commonly been found that conversions to physiologically active steroids, such as oestradiol, testosterone and dihydrotestosterone, are small. The report of the conversion of DHAS and DHA to 7α-hydroxy DHA in high yields by mammary tumour tissues *in vitro* (Couch *et al.*, 1975 [8] and unpublished results) suggested that malignant mammary tissues may secrete sufficient amounts of this steroid to cause an elevation of the plasma concentration. The observation that the plasma concentrations of 7α-hydroxy DHA in control subjects and patients with benign breast disease were not different to, and sometimes greater than, the concentrations in patients presenting with breast cancer (Table 1) is anomalous in the light of the *in vitro* metabolism studies [8].

There was, however, a strong correlation between plasma 7α-hydroxy DHA and DHA suggesting that the availability of the primary substrate, DHA, to the 7α-hydroxylase may largely control the 7α-hydroxy DHA production. Since breast cancer is associated with decreased plasma DHA compared to control subjects [18] the lower 7α-hydroxy DHA in the majority of breast cancer patients may be explained by a tendency for lower plasma DHA in these patients. A secondary role for plasma DHAS in determining plasma 7α-hydroxy DHA was suggested by the lower correlation between these two steroids (Table 2). Since the main differences in the production of 7α-hydroxy DHA by human mammary tumours *in vitro* was with [^{3}H] DHAS as the substrate [8], the minimal effect of small, malignant mammary tumours *in vivo* on plasma 7α-hydroxy DHA may be rationalised.

Differences between patients presenting with metastases or with large tumours compared to control subjects and patients with benign breast disease were apparent in the slopes of the 7α-hydroxy DHA on DHA regression lines (Fig. 1a). The divergence of the regression lines at 1–3 ng DHA/ml plasma suggests that it is only as the plasma DHA increases above this level that the plasma 7α-hydroxy DHA is clearly elevated in patients with advanced disease. If this elevation is indeed the result of the metabolism of DHA by malignant mammary tissues it may be possible to exploit this phenomenon using a DHA 'loading' test [19] or by stimulation of adrenal DHA secretion with ACTH and measuring plasma 7α-hydroxy DHA in blood samples collected after a suitable time period. If plasma DHA is sufficiently increased by these procedures there should be a greater increase in plasma 7α-hydroxy DHA in patients with metastases than in those with

small, localised tumours and this procedure may assist in the selection of patients for additional therapy.

The suggestion that severe illness and consequent medication may cause decreased androgen excretion (measured as 11-deoxy-17-oxosteroids) by increasing the hydroxylation of DHA and testosterone to 'polar metabolites' in breast cancer patients [20] deserves some comment. No medication was prescribed or suggested for these patients before the pre-therapy samples were obtained. It therefore seems unlikely that medication may influence the plasma 7α-hydroxy DHA data presented in Table 1 or Fig. 1. Although the differences observed between patient groups in the slopes of the 7α-hydroxy DHA on DHA regression lines (Fig. 1a) may be caused by the severity of illness this is questionable since overt signs of illness do not commonly differentiate patients presenting with metastases or large tumours from patients with small or benign tumours. It would also seem likely that, if medication or severity of illness caused a sufficient increase in androgen hydroxylation to decrease urinary 11-deoxy-17-ketosteroid excretion, high plasma 7α-hydroxy DHA should be associated with low plasma DHA. There should also be a decrease in the slopes of the 7α-hydroxy DHA on DHA regression lines in the two advanced disease groups compared to the early disease, benign disease and control groups (Fig. 1a). Since the opposite is true, our data cannot support the interpretation of increased plasma 7α-hydroxy DHA as an effect of medication or of the severity of the illness. A simpler, although unproven, interpretation is that the greater the tumour mass, whether as a large primary tumour or as a number of small metastatic deposits, the greater is the production of 7α-hydroxy DHA if a sufficient concentration of the 7α-hydroxylase substrate, DHA, is available.

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