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LEVELS OF ANDROGEN CONJUGATES AND OESTRONE SULPHATE IN PATIENTS WITH BREAST CYSTS

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Summary—Plasma and cyst fluid were obtained from patients with palpable breast cysts and analysed for androgen conjugates and oestrone sulphate content by radioimmunoassay. Concentrations of androgen conjugates in cyst fluids varied from 15.6 to 475.5 μmol/l. These levels were much greater than those in plasma (1.3–5.2 μmol/l) and there was no association between values in cyst aspirates and plasmas obtained from the same individuals. Levels of oestrone sulphate in breast cyst fluids (1.5–744.0 nmol/l) were also generally in excess of those in plasma (2.0–59.9 nmol/l) and again no relationship was evident between concentrations in cyst fluid and the circulation. Neither was there a relationship between levels of androgen conjugate and oestrone sulphate in plasma. In contrast, a highly significant correlation ($P < 0.001$) was identified between the androgen conjugate and oestrone sulphate content of cyst fluids. Levels of both androgen conjugates and oestrone sulphate were also significantly different in groups of cysts subdivided according to electrolyte classification, cysts with low $Na^+:K^+$ ratios having higher steroid concentrations than those with high $Na^+:K^+$ ratios. The biological significance of the relationship between the two conjugates in cyst fluids remains unclear but it is suggested that the accumulation of these steroids involves a common mechanism.

INTRODUCTION

Approximately 7% of women in Western populations develop breast cysts [1] and this may predispose for breast cancer [1, 2]. Despite the frequency of occurrence and possible association with breast cancer, comparatively little is known of the derivation or content of breast cysts. However, since endocrine influences may affect the incidence of both diseases [1] there has been considerable interest in the hormonal content of breast cysts. High levels of conjugated 17-ketosteroids have been detected in cyst fluids [3–8]. Oestrogen conjugates may also be present in concentrations markedly in excess of those normally found in the circulation [3, 9–12].

It was therefore of interest to determine the relationship between androgen and oestrogen conjugates in breast cyst fluid.

EXPERIMENTAL

Materials

Cyst fluids were aspirated from each of 53 breast cysts in 36 patients attending the breast clinic in the University Department of Surgery. In 19 of the patients peripheral blood (20 ml) was collected into heparinized tubes immediately before aspiration of breast cyst fluids. Plasma was separated within 1 h by centrifugation (600 *g* for 20 min). Aspirates and plasma were stored at −20°C until hormone analysis.

Measurement of androgen conjugates

Androgen conjugates were measured by radioimmunoassay using the method of Buster and Abraham[13] and an antibody against dehydroepiandrosterone (DHA) conjugated to ovalbumin whose specificity has been described previously [14]. The antibody cross-reacts primarily with DHA and its sulphate but also shows significant cross-reactivity with DHA glucuronide and epiandrosterone conjugates. The results therefore reflect levels of androgen conjugates although for simplicity, values are reported as units of DHA-sulphate (DHA-S) equivalents.

Assay for oestrone sulphate

Oestrone sulphate (OE_1-S) was measured in breast cyst fluid and plasma by radioimmunoassay [12]. Samples were extracted with diethyl ether to remove unconjugated oestrogens and the aqueous phase subjected to enzymic hydrolysis with sulphatase. The steroids released were then extracted with diethyl ether and assayed for oestrone using a highly specific antibody generated against 6-oxo-oestrone conjugated to bovine serum albumin.

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Table 1. Concentrations of androgen conjugates and oestrogen sulphate in breast cyst fluids and plasma

	Androgen conjugates		Oestrone sulphate	
	median	(range)	median	(range)
Cyst fluid (53)[a]	85.6	(15.6–475.5 μmol/l)	76.0	(1.5–744.0 nmol/l)
Plasma (19)[b]	3.3	(1.3–5.2 μmol/l)	4.7	(2.0–59.9 nmol/l)
Cyst fluid:plasma (28)[c]	33	(6–366)	29	(0.3–66)

[a]Number of cysts aspirated. [b]Number of patients studied. [c]Cyst fluid:plasma ratio, where more than one cyst was aspirated from an individual patient separate ratios have been calculated.

Determination of the electrolyte content of cyst fluid

Sodium (Na^+) and potassium (K^+) levels were analysed in all cyst fluids using an EEL model 150 flame photometer. Fluid samples were diluted 1 in 400 in distilled water. Standard solutions containing known concentrations of Na^+ and K^+ were routinely used to calibrate and confirm accuracy of the machine during a series of measurements.

RESULTS

High concentrations of androgen conjugates were found in all cyst fluids and, as is shown in Table 1, there was a large range of vaues from 15.6 to 475.5 μmol/l. This contrasts with levels in plasma which were substantially lower and displayed a much narrower range (1.3–5.2 μmol/l). A comparison of concentrations in cyst fluid and plasma from the same individuals showed that in each case cyst fluid contained a higher level of androgen conjugates than plasma. Whilst the median value in cyst fluids was 33-fold higher than that in plasma, the relative excess varied between 6 and 366. No significant relationship was evident between concentrations in cyst fluid and plasma.

Concentrations of oestrone sulphate in the same cyst fluids varied from 1.5 to 744.0 nmol/l with a median value of 76.0 nmol/l (Table 1). As with androgen conjugates, plasma levels were substantially lower (median value 4.7 nmol/l) and displayed a narrower range (2.0–59.9 nmol/l). In subjects from whom both cyst fluid and plasma were obtained the relative excess of oestrone sulphate in cyst fluid varied from 0.3 to 66. Although the median value for the ratio (29) was similar to that for androgen conjugates, oestrone sulphate tended to be present in relatively less excess than androgen conjugates and in some individuals concentration of oestrone sulphate was lower in cyst fluid than plasma.

Although there was not a significant correlation between concentrations of oestrogen sulphate and androgen conjugates in plasma (Fig. 1) there was a highly significant correlation between the steroids in cyst fluids (Fig. 2).

Cyst fluids may be divided into sub-groups on the basis of their electrolyte composition, Group A having a $[Na^+]:[K^+] < 4$ and Group B possessing a $[Na^+]:[K^+] > 4$ [15]. As is shown in Fig. 3, this classification also produces subgroups of fluids which differ markedly in their levels of steroid conjugates, levels of both androgen conjugates and oestrone sulphate being significantly higher in Group A cysts as compared with Group B ($P < 0.001$ in each case).

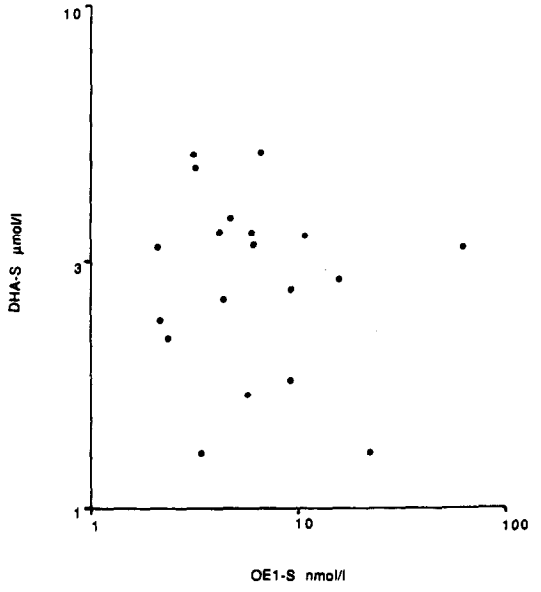

Fig. 1. Relationship between concentrations of androgen conjugates (DHAS) and oestrone sulphate (OE_1-S) in plasma from women with breast cysts. No significant correlation between the steroid levels.

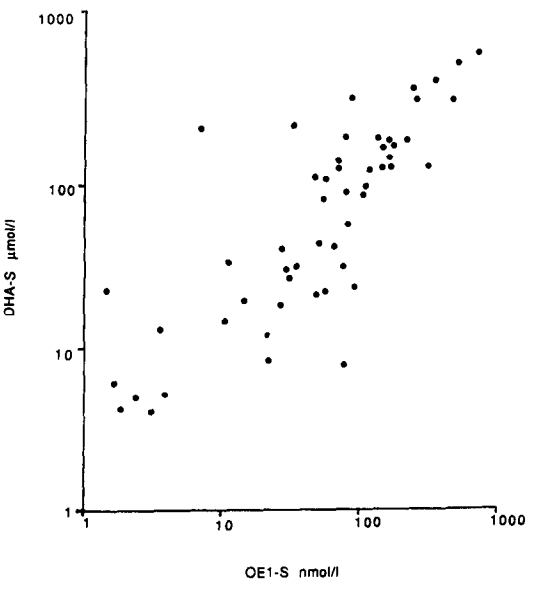

Fig. 2. Relationship between concentrations of androgen conjugates (DHAS) and oestrone sulphate (OE_1-S) in breast cyst fluids. Correlation coefficient, $r = 0.832$, $P < 0.001$.

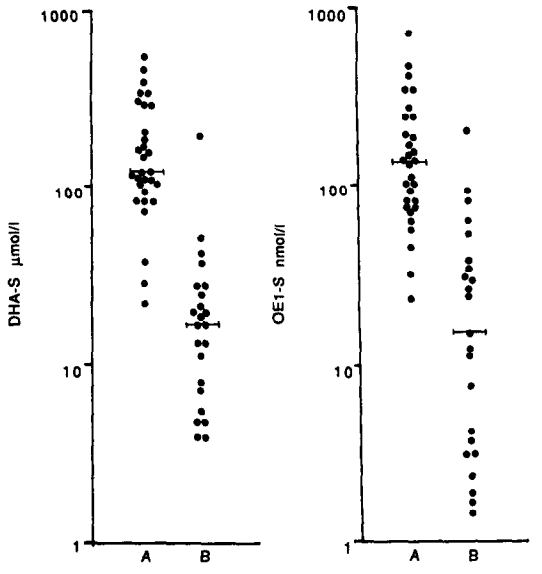

Fig. 3. Concentration of androgen conjugates (DHA-S) and oestrone sulphate (OE_1-S) in groups of cyst fluids divided according to electrolyte composition, Group A having $[Na^+:K^+] < 4$ and B having $[Na^+:K^+] > 4$. Bars represent median values. There is a significant difference between the groups by Wilcoxon Rank test ($P < 0.001$ in each case).

DISCUSSION

This study confirms previous reports that high levels of both androgen and oestrogen conjugates are present in breast cyst fluids. Concentrations reported in the present series compare well with values reported in previous investigations [3–12]. In comparison with levels measured by the same methods in plasma, values for androgen conjugates in cyst fluids were always higher and, in general, levels of OE_1-S were also higher in cyst fluid. The range of values for both androgen conjugates and OE_1-S was much wider in cyst fluids than those in the circulation. Furthermore, there was no obvious relationship between the values of these steroid conjugates in cyst fluids and plasma. This was reflected in the wide variation of the cyst fluid:plasma ratio. As a result, it is not possible to predict levels of these hormones in cyst fluids from circulating levels. This observation may explain the difficulties in relating endocrine activity, as determined by measurements of circulating hormones, to events occurring within breast cysts.

An important observation in this study was the strong correlation between the androgen conjugates and OE_1-S in breast cyst fluids. As radioimmunoassay techniques were used to measure these hormones, it is possible that the relationship reflects cross-reaction of steroids in the assay systems. However, this is unlikely for two reasons, (a) the use of column chromatography which separated androgens from oestrone prior to radioimmunoassay, produced results for oestrone sulphate which were similar to those achieved with radioimmunoassay alone and (b) no correlation was evident between androgen conjugates and oestrone sulphate in plasma.

The lack of correlation between androgen and oestrogen conjugates in plasma would also suggest a common mechanism by which these steroids accumulate in cyst fluids. The high levels in cyst fluid compared to plasma points to the involvement of an active transport process (either concentration against a gradient or active synthesis within the breast). However, transport studies [4] indicated that, following intravenous administration, DHA-S but not OE_1-S enters cyst fluid in significant amounts.

Whilst there is evidence that androgens may be transformed into oestrogens by breast tumours and fat [16, 17] no convincing data exists for the presence of activity within cyst fluids or their linings. Neither is there evidence that cysts can synthesise androgens from either cholesterol or C21 steroid precursors. The source and mechanism by which high concentrations of steroids conjugates accumulate in cyst fluids is therefore still a matter for conjecture. It is also unclear whether these high concentrations of steroid conjugates have a biological action in cyst fluids and have a role in the natural history of cysts. Whilst androgen and oestrogen conjugates have less biological activity than unconjugated metabolites, the potential exists within the breast for these conjugates to be converted into more potent products [12, 18, 19] although again, these interconversions have never been convincingly shown in breast cysts. It is of interest however, that the levels of both androgen conjugates and OE_1-S are significantly higher in Group A as compared with Group B cyst fluid. Insofar as Group A fluids have a composition more akin to intracellular fluids (for example having high concentrations of K^+ [15]), it seems likely that the androgen and oestrogen conjugates found in cyst fluids have been derived from cells associated with breast cysts. The observation that Group A fluids come from cysts lined by active apocrine epithelium [15, 20] would support this concept and suggest that apocrine budding of cellular constituents is responsible, at least in part, for the contents of these fluids. In view of the suggestion that sub-populations of cysts have different behaviour [20] and the further possibility that a subgroup of cysts may be associated with increased risk of breast cancer [20], the observation of high concentrations of steroid conjugates in cyst fluids warrants further investigation.

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