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Steroid Hormones in Lymph and Blood from Women with Early Breast Cancer

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Postoperative levels of steroid hormones have been measured in lymph and blood of 28 patients undergoing iridium implants as part of conservative treatment for operable breast cancer. The aim was to establish whether there were higher levels of hormones in lymph draining from necrosing tumour cells compared with peripheral blood levels. No differences were found in the ratios of oestradiol, dehydroxyepiandrosterone sulphate or sex hormone-binding globulin in those patients with complete compared with incomplete excision of the primary tumour. However, premenopausal patients who had an incompletely excised primary tumour had increased levels of free testosterone in lymph draining from the tumour site on the third and fourth postoperative days. Thus androgens may have an important role in the intracellular metabolism of some breast cancers.
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INTRODUCTION

THERE IS an abundance of epidemiological and experimental data from rodent models to suggest a central role for steroid hormones in the development of breast cancer[1, 2]. Ovarian hormones probably have a promotional effect after a carcinogenic "first hit" [3, 4]. However, studies of women with established breast cancer, or of those who subsequently develop the disease, have shown at best only a weak association between steroid hormones and risk [5]. In part this may have arisen because most studies examined total levels of steroids in urine and blood. However, much of the measured steroids is protein bound and thus unavailable to target cells [6]. Women at risk of breast cancer, those who subsequently develop the disease and those with clinical carcinoma have increased proportions of free oestradiol compared with controls [7, 8]. However, others were unable to confirm these findings [9].

Notwithstanding, it is not plasma or serum that bathes cells but interstitial fluid, and the endocrine content of this compartment has not been measured because of the difficulties in obtaining adequate samples. One approach is to analyse lymphatic fluid draining from the breast. Such an opportunity arises from a new combined technique at Guy's Hospital for the conservative treatment of early breast cancer. The surgical and radiotherapy procedure has been tested in a prospective controlled trial of women with unifocal carcinomas up to 4 cm in diameter [10]. Patients are treated with tumourectomy, axillary clearance, iridium implant and external radiotherapy. The aim of the iridium implant is to destroy residual malignant cells at the biopsy site which are present in about 50% of patients. During the period of the implant, the axilla, which has been cleared surgically, is drained via a vacuum drain that collects lymphatic fluid from both arm and breast. This enables the collection of interstitial fluid and in addition allows a comparison of that draining from residual cancer cells at the biopsy site or from an area cleared of malignancy. The likely presence or absence of cancer cells at the original tumour site can be determined by subsequent histological examination of the margins of the tumourectomy specimen.

We have analysed the steroid hormone profile of both lymph and blood from patients undergoing iridium implants. We included both premenopausal and postmenopausal patients who had had complete or incomplete excision of their primary tumours.

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PATIENTS AND METHODS

Patients

The study comprised 28 women all of whom had histologically confirmed breast carcinoma and who were treated with breast conservation, with an interstitial boost dose of radiation (20 Gy) to the tumour site. This boost was given as an iridium implant, in the immediate postoperative period (within 48 h). All patients had axillary clearance. The axillary drain remained *in situ* for at least 5 days postoperatively. 10 ml venous blood was collected on the morning of surgery and subsequently for the next 5 days between 0900 and 1000 h (bloods 1–5). The lymphatic fluid was collected in vacuum containers that were replaced every 24 h. 20 ml was collected each day and the total drainage volume was measured.

Both blood and lymph were centrifuged and the serum and lymph were frozen at −30°C. All the samples were assayed together.

Hormone assays

Sex hormone binding globulin (SHBG). Immunoradiometric kits were purchased from Farmos Diagnostics. Samples and standards were diluted 1:100 with assay buffer. A 200 μl mixture of ^{125}I labelled SHBG antibody and anti-SHBG antiserum was added to 100 μl of diluted sample serum. After incubation at 37°C for 1 h, a solid-phase donkey-anti-rabbit IgG antiserum was added, followed 15 min later by 2 ml 0.9% NaCl. After pelleting, the supernatant was aspirated and the pellet was counted in a Packard PRIAS gamma counter. Results were calculated by curve fitting to a spline equation.

Total oestradiol. The ER-150 kit from EIR Wurenlinger was used. 0.5 ml serum was added to ground-glass stoppered tubes to which 4 ml anaesthetic grade diethyl ether was added. After

Table 1. Comparative features of patients with complete and incomplete excision of primary tumour

	Complete	Incomplete
Number	11	17
Mean age (yr)	48.8	47.6
Mean weight (S.D.) (kg)	62.9 (10.9)	68.7 (10.5)
Premenopausal	7 (64%)	8 (47%)
Postmenopausal	4 (36%)	9 (53%)
T_1	2	3
T_2	9	14

stoppering and shaking, the serum layer was frozen by placing the tube into dry ice and acetone. The ether was decanted and evaporated, and the extract was redissolved in 0.5 ml assay buffer. Duplicate assays were done and free counts were separated from bound counts with dextran-coated charcoal. Tubes were incubated at 4°C for 20 min and then spun at 2000 *g* for 15 min. The supernatant was decanted and counted for 1 min in the gamma counter. Oestradiol concentrations were calculated directly from the spline fit programme.

Androgens. Free testosterone and dehydroxyepiandrosterone sulphate (DHS) hormones were measured without extraction of the sample. "Coat-a-count" kits were obtained from Diagnostic Products Corporation. 25 μl serum and 1 ml ^{125}I labelled steroid were mixed and incubated at 37°C for 3 h in tubes with antibody-coated internal walls. After decanting, the bound radioactivity was measured in a gamma counter. Steroid concentrations were calculated from the standard curve set up in parallel.

RESULTS

The clinical characteristics of the patients are shown in Table 1. 11 patients had a complete excision of the primary tumour and 17 had an incomplete excision. There were 15 premenopausal and 13 postmenopausal women.

The lymph/blood ratios of free testosterone during the 5 postoperative days among the 15 premenopausal cases are shown in Fig. 1. These ratios are subdivided on the basis of complete

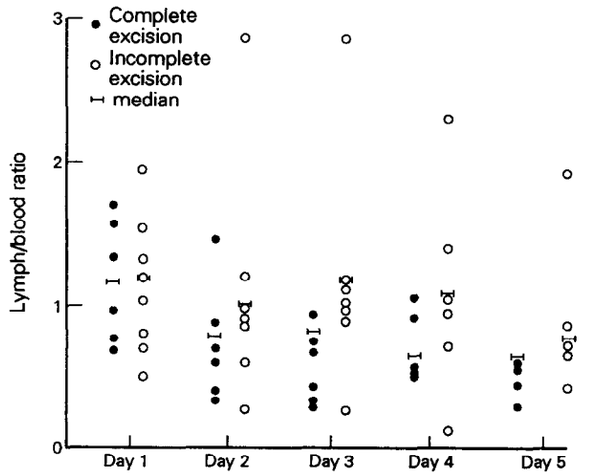

Fig. 1. Ratio of lymph/blood free testosterone in premenopausal women with breast cancer during postoperative days 1–5.

Table 2. Median values of ratios of hormone levels in lymph and blood

Hormone	Menopausal status	Complete excision	Incomplete excision
Total estradiol	Pre	0.73	0.75
	Post	0.28	0.79
SHBG	Pre	0.44	0.36
	Post	0.41	0.49
DHA sulphate	Pre	0.5	0.6
	Post	0.62	0.5
Free testosterone	Pre	0.76*	1.15
	Post	0.44	0.75

$P = 0.05$ (Wilcoxon rank-sum test)

or incomplete excision. Although there were no significant differences between the blood levels of testosterone in either group, there was an emerging difference between the lymph/blood testosterone ratios. By day 2 there was a slight elevation of free testosterone ratio in those with incompletely excised tumours compared with the completely excised group. By the third day, when the after-loaded iridium implant was in place, there was a significant elevation in ratio among the group with incompletely excised tumours (median 1.15 vs. 0.76, $P = 0.05$ Wilcoxon rank-sum test). This continued on day 4 but by the 5th postoperative day there was no statistical difference between the ratios in the two groups.

There were no significant differences on day 3 in the ratios of total oestradiol, SHBG or DHA sulphate in any of the subgroups (Table 2).

DISCUSSION

This has been the first attempt to measure levels of steroid hormones in interstitial fluid draining from primary breast tumours. Others have found increased levels of steroid hormones compared in homogenised tumour tissue with serum, but similar concentrations of total testosterone [11]. Malarkey *et al.* [12] reported elevated levels of testosterone in premenopausal patients with breast cancer. Similarly, Secreto *et al.* [13] found that premenopausal breast cancer cases had significantly higher blood androgen concentrations than controls, and suggested that androgens might have a role in both the induction and development of breast cancer.

Our results revealed no measurable release of oestradiol by the primary tumour. However, there were higher levels of free testosterone within the lymph of patients who had an incomplete excision of tumour, which suggests that as a sequel of necrosis of tumour cells there was a release of intracellular androgen. Our data will almost certainly represent an underestimate of this effect because of lymphatic dilution from non-tumour bearing areas of the breast and normal lymph from the ipsilateral arm. These findings suggest that testosterone may have a central role in the internal economy of established breast cancer cells.

1. MacMahon B, Cole P, Brown J. Etiology of human breast cancer: a review. *J Natl Cancer Inst* 1973, **50**, 21–42.
2. Miller AB, Bulbrook RD. The epidemiology and etiology of breast cancer. *N Engl J Med* 1980, **303**, 1246–1248.
3. Moolgavkar SH, Day NE, Stevens RG. Two-stage model for

carcinogenesis: epidemiology of breast cancer in females. *J Natl Cancer Inst* 1980, **65**, 559–569.
4. Pike MC, Krail MD, Henderson BE, *et al.* Hormonal risk factors, breast tissue, age and the age-incidence of breast cancer. *Nature* 1983, **363**, 767–770.
5. Moore JW, Thomas BS, Wang DY. Endocrine status and the epidemiology and clinical course of breast cancer. *Cancer Surv* 1986, 5, 537–559.
6. Westphal U. Steroid-protein interaction. *Monogr Endocrinol* **4**. Springer, New York, 1971.
7. Siiteri P, Simberg N, Murai J. Estrogens and breast cancer. *Ann NY Acad Sci* 1984, **464**, 100–105.
8. Moore JW, Clark GMG, Bulbrook RD, *et al.* Serum concentrations of total and non-bound estradiol in patients with breast cancer and in normal controls. *Int J Cancer* 1982, **29**, 17–21.
9. Langley MS, Hammond GL, Bardsley A, *et al.* Serum steroid binding proteins and the bioavailability of oestradiol in relation to breast cancer. *J Natl Cancer Inst* 1985, 75, 823–829.
10. Habibollahi F, Fentiman IS. Breast conservation techniques for early breast cancer. *Cancer Treat Rev* 1989, **16**, 177–191.
11. Vermeulen A, Deslypere JP, Paridaens R, *et al.* Aromatase, 17 beta hydroxysteroid dehydrogenase and intratissular sex hormone concentrations in cancerous and normal glandular breast tissue in post-menopausal women. *Eur J Cancer Clin Oncol* 1986, **22**, 515–523.
12. Malarkey WB, Schroeder LL, Stevens VC, *et al.* Twenty four hour pre-operative endocrine profiles in women with benign and malignant breast disease. *Cancer Res* 1977, **37**, 4655–4659.
13. Secreto G, Recchione C, Fariselli G, *et al.* High testosterone and low progesterone circulating levels in premenopausal patients with hyperplasia and cancer of the breast. *Cancer Res* 1984, **44**, 841–844.

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Accumulation of Active Androgens in Breast Cyst Fluids

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80 breast cyst fluids (BCF) from 57 patients were divided by K^+/Na^+ ratio: 56 with ratio over 1 (type I) and 24 with ratio less than 1 (type II). Significantly higher amounts of testosterone, dihydrotestosterone and dehydroepiandrosterone sulphate (DHAS) were found in type I than in type II cysts. A positive relation was found between testosterone and dihydrotestosterone in both types. DHAS was significantly correlated with testosterone and dihydrotestosterone in type I cysts only. In 52 patients, blood was sampled after cyst evacuation. Testosterone was significantly higher in blood than in BCF while dihydrotestosterone and androstenedione were significantly higher in BCF. No relation was observed between circulating levels of androgens and their intracystic concentrations. Women bearing type I cysts may be at increased risk of developing cancer. These findings support the hypothesis that androgens play a role in the hormonal aetiology of breast cancer.
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INTRODUCTION

EXAMINATION OF human breast cyst fluid (BCF) reveals two major subgroups of cyst: type I with a high K^+/Na^+ ratio, resembling the intracellular milieu, and large amounts of dehydroepiandrosterone sulphate (DHAS); and type II with low K^+/Na^+ ratio and lower amounts of DHAS. A third population with intermediate K^+ and Na^+ concentrations has also been reported [1–3]. Type I cysts are lined with apocrine epithelium [4, 5] and their ion and hormonal content reflects the activity of the lining cells. Type II cysts are lined with flattened epithelium [4], although a few, less active apocrine cells have been found in their fluids [5]. Apocrine epithelium is under the influence of androgens and is an active site for conversion of weak androgen precursors into more active substances [6, 7]. Accordingly, an androgenic milieu may exist inside breast tissue bearing type I cysts.

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Apocrine metaplasia of breast epithelium is frequently present in breast cancer [8, 9] and in populations at increased risk of breast cancer [10–12]. Dixon *et al.* [12] reported the appearance of cancer in 11 out of 80 patients who had apocrine cysts vs. 1 of 30 who had flattened cysts. These findings fit the original hypothesis from our laboratory pointing to increased androgenic activity in the hormonal aetiology of breast cancer [13].

In this study we have explored the relation between ion and androgen concentrations in BCF. We also correlated BCF contents with circulating levels of testosterone, dihydrotestosterone and androstenedione.

MATERIAL AND METHODS

Biochemical and hormonal examinations were done in 80 BCF specimens drawn by needle aspiration from 57 women with gross cystic breast disease. Simulataneous evacuation of multiple cysts was achieved in 14 patients: 8 with two cysts, 4 with three cysts, 1 with four and 1 with five cysts. The patients were aged 24 to 55 (mean 45.9 [S.D. 5.6]).

49 patients were still menstruating. 5 had their last menstrual cycle 3–9 months before entering the study. The other 3 had