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Epidermal Growth Factor and Oestradiol in Human Breast Cyst Fluid

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Gross cystic breast disease is a common condition in women. Women with apocrine breast cysts (breast cyst fluid $Na^+/K^+ < 3$) may be at higher risk of breast cancer than women who have cysts lined by flattened epithelium ($Na^+/K^+ \geq 3$). Breast cyst fluid concentrations of epidermal growth factor were significantly higher in the low electrolyte ratio group than in the high electrolyte ratio group (356.2 ng/ml *vs* 104.1 ng/ml, $P < 0.0003$). A negative correlation was obtained between intracystic epidermal growth factor concentrations and Na^+/K^+ ($r_s = -0.666$, $P < 0.001$). No significant difference was found between the total oestradiol concentrations in the two cyst groups. However, the unbound oestradiol concentrations on a limited number of samples were significantly higher in the low electrolyte ratio group than in the high electrolyte ratio group ($P < 0.05$). The higher concentrations of EGF in cyst fluid with $Na^+/K^+ < 3$ may explain why women with apocrine breast cysts may be at increased risk of developing breast cancer.
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INTRODUCTION

WOMEN with gross cystic breast disease (cysts > 3 mm in diameter) may be at increased risk of subsequently developing breast cancer [1, 2]. These cysts are lined either by apocrine or flattened epithelium. The ratios of breast cyst fluid concentrations of sodium to potassium for cysts lined by flattened epithelium (≥3) have been shown to be higher in all cases than the values obtained for cysts lined by apocrine epithelium (<3) [3]. Women with apocrine cysts may be at higher risk of developing breast cancer than women with cysts which are lined by flattened epithelium [2, 4].

Wide ranging concentrations of epidermal growth factor (EGF) are present in breast cyst fluid [5, 6]. EGF has been shown to promote the proliferation of mammary epithelial cells [7, 8] as well as human breast cancer cells in culture [9]. Evidence that EGF plays a role in carcinogenesis is accumulating [10, 11].

In view of the possible role which EGF may have in the development of breast cancer, the distribution of EGF concentrations in the two cyst groups was compared to see if differences between EGF concentrations in the two groups existed and, if so, whether the differences could help explain why women with apocrine cysts may be at higher risk of breast cancer. In addition, breast cyst fluid concentrations of total and unbound, biologically active oestradiol (E_2) between the two groups were compared in view of the 'necessary but insufficient' role which E_2 plays in the development of breast cancer.

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MATERIALS AND METHODS

Patient samples

Breast cyst fluid samples were obtained by needle aspiration from patients attending the breast clinic at St. Mary's Hospital, London. Approval for this study was obtained from the local District Ethics Committee. Samples were immediately centrifuged at 1500 *g* for 10 min and the supernatant stored at −20°C until assayed. Sodium and potassium concentrations were measured in 102 samples. EGF concentrations were measured in 98 samples. As cyst fluid samples had also been used in other experiments it was possible to measure total E_2 in only 39 samples and the unbound E_2 fraction in only 25 samples. Cytological examination of cyst fluid was not carried out.

Materials

Anti-human EGF antiserum raised in Dutch male rabbits and pure urogastrone (EGF isolated from human urine) were gifts from Dr H. Gregory, ICI plc, Macclesfield, U.K. Recombinant human EGF, iodine-125 and [2,4,6,7-^{3}H]E_2 (93 Ci/mmol) were obtained from Amersham International plc, Amersham, U.K. Anti-E_2 antiserum raised against oestradiol-6(*O*-carboxymethyl)-oxime-bovine serum albumin in goats was a gift from Dr G. Knaggs. Human transforming growth factor-α was purchased from Peninsula Laboratories Europe Ltd, St. Helens, Merseyside, U.K.

Measurement of electrolytes

Sodium and potassium concentrations in breast cyst fluid were measured by an indirect ion-selective electrode (Beckman Electrolyte 2 Analyser).

Measurement of EGF

EGF was measured by radioimmunoassay. This methodology has been described [12].

The sensitivity of the assay was 0.04 ng/ml. The intra-assay and interassay coefficients of variation were less than 10%. Parallelism was obtained between the competitive binding

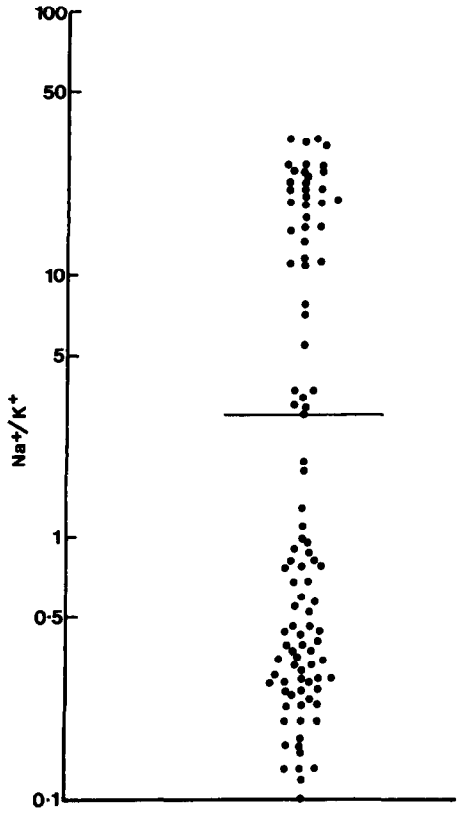

Fig. 1. Distribution of sodium to potassium ratios in breast cyst fluid. The line at $Na^+/K^+ = 3$ divides the two groups of breast cysts.

curves generated by dilutions of samples of breast cyst fluid and the standard curve.

No cross-reaction was found with transforming growth factor-α, which exerts all of its biological effects via the EGF receptor, up to concentrations of 1 μg/ml. Cross-reactivity of this antiserum with a large variety of other substances has been tested by other investigators [5, 13].

Measurement of total E_2

The methodology for the measurement of total E_2 has been described [14]. The sensitivity of the assay was 18 pmol/l. The intra-assay and interassay coefficients of variation were 6.5% and 10.7% respectively.

Measurement of unbound E_2 fraction

The percentage of unbound E_2 in breast cyst fluid was measured using a Dianorm equilibrium dialysis machine [15]. The principle of the technique is to dialyse breast cyst fluid against [2,4,6,7-^{3}H]E_2 (approximately 10,000 cpm) until equilibrium is reached. The unbound E_2 concentration was calculated by multiplying the percentage of unbound E_2 by the total E_2 concentration. The intra-assay and interassay coefficients of variation were 3.7% and 12.9% respectively.

Statistical analyses

The concentrations of the various analytes were not normally distributed. Logarithmic transformations did not normalize the distribution of the data. Non-parametric statistics were, therefore, used. Wilcoxon's rank sum test was used to compare distributions of EGF and E_2 between the two cyst groups as defined by their sodium to potassium ratios ($Na^+/K^+ < 3$ and $Na^+/K^+ \geq 3$). Correlation coefficients were calculated using Spearman's rank correlation method (Spearman's rank correlation = r_s). Results were considered to be statistically significant when $P < 0.05$.

RESULTS

The distribution of sodium to potassium ratios in breast cyst fluid is shown in Fig. 1 ($n = 102$). Two groups of breast cysts are apparent and a cut-off of 3, as was found by Dixon *et al.* [3], was considered to distinguish between the two cyst groups satisfactorily.

The concentrations of EGF in breast cyst fluid ranged widely from 0.4 to 861 ng/ml ($n = 98$). The distribution of EGF concentrations in the two groups of breast cysts is shown in Fig. 2. The EGF concentrations in the low electrolyte ratio group (median = 309 ng/ml, $n = 62$) were significantly higher than the concentrations in the high electrolyte ratio group (median = 68 ng/ml, $n = 36$), $P < 0.0003$. A negative correlation was obtained between EGF concentrations and sodium to potassium ratios ($r_s = -0.666, P < 0.001$).

Figure 3 shows the distributions of total E_2 and unbound E_2 concentrations in the two cyst groups. No significant difference was found between the total E_2 concentrations in the low electrolyte ratio group (median = 120 pmol/l, $n = 22$) and the high electrolyte ratio group (mean = 121 pmol/l, $n = 17$). The unbound E_2 concentrations were significantly higher ($P < 0.05$) in the low electrolyte ratio group (median = 12.7 pmol/l, $n = 10$) than in the high electrolyte ratio group (median = 8.1 pmol/l, $n = 15$). No correlation was found between total or unbound E_2 concentrations and sodium to potassium ratios in breast cyst fluid or between intracystic concentrations of EGF and total or unbound E_2.

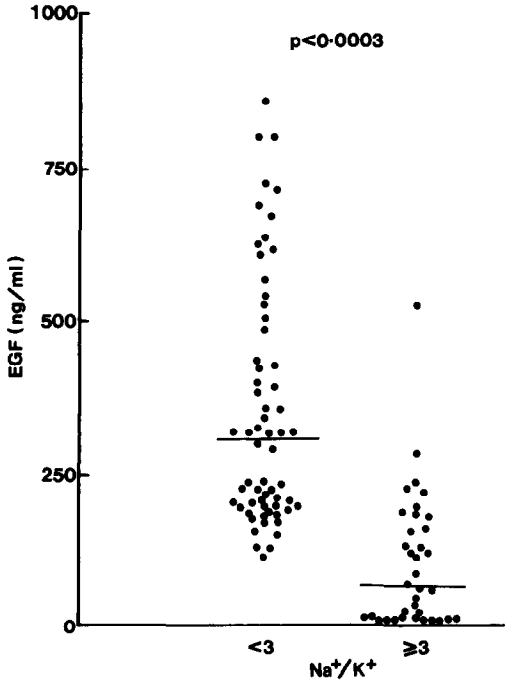

Fig. 2. Distribution of concentrations of EGF in the two groups of breast cysts. The horizontal lines represent median EGF concentrations.

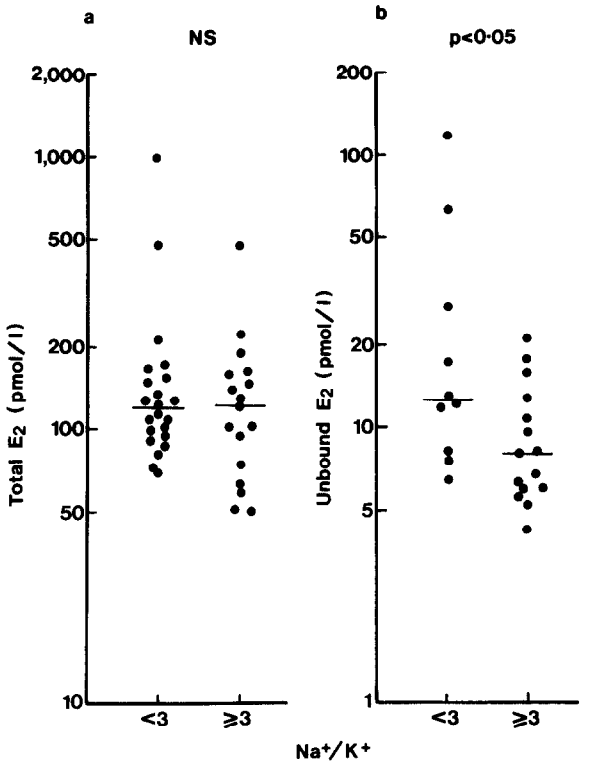

Fig. 3. Distribution of concentrations of total E_2 (a) and unbound E_2 (b) in the two groups of breast cysts. The horizontal lines represent median concentrations.

DISCUSSION

EGF may act as a tumour promoter in carcinogenesis and has been shown to enhance the carcinogenic potential of methylcholanthrene in skin [16, 17]. Increased numbers of EGF receptors have been demonstrated in certain malignancies, e.g. squamous cell carcinomas and gliomas [18, 19]. EGF may play an important role in mouse mammary tumorigenesis. Kurachi *et al.* [20] found that removal of the submandibular salivary glands, a rich source of EGF in mice, from virgin female mice of the C3H/HeN strain, which have a high incidence of mammary tumours shown to be related to a virus transmitted from females to their young through milk, between 14 and 22 weeks of age reduced the tumour incidence significantly from 62.5% to 12.8% at the age of 52 weeks. The finding of significantly higher concentrations of EGF in the low electrolyte ratio cyst group is, therefore, important and may explain, in part, why women with apocrine breast cysts may be at increased risk of breast cancer, assuming that the strict relationship between intracystic electrolyte ratios and cyst morphology as was shown by Dixon *et al.* [3] exists. The finding of significantly higher intracystic concentrations of unbound, biologically active E_2 in the low electrolyte ratio group than in the high electrolyte ratio group is interesting in view of the possible role which oestrogens may have in the development of breast cancer [21]. The sample size was, however, small and no conclusions were, therefore, possible. Preliminary findings of this study have been published in abstract form [22, 23]. The distribution of EGF concentrations in the low and high electrolyte ratio groups fully support the findings of Boccardo *et al.* [24]. The negative correlation between EGF concentrations and sodium to potassium ratios suggests that the epithelium lining cysts with low electrolyte ratios may be secreting EGF more actively than the epithelium lining cysts with high electrolye ratios.

Positive correlations have been found between intracystic concentrations of EGF and dehydroepiandrosterone [12] and between intracystic concentrations of EGF and dehydroepiandrosterone sulphate [12, 24] suggesting that EGF concentrations in breast cyst fluid may be modulated by androgens, although a common stimulus elevating the levels of EGF, dehydroepiandrosterone and dehydroepiandrosterone sulphate is a possibility. No evidence was found in this study to suggest that intracystic concentrations of EGF may be modulated by E_2.

In conclusion, the higher levels of EGF in the low electrolyte ratio group may provide an explanation for the higher risk of breast cancer observed in patients with apocrine breast cysts provided that a strict relationship exists between intracystic electrolyte ratios and cyst morphology.

1. Azzopardi JG. *Problems in Breast Pathology*. Philadelphia, Saunders, 1979, 92–112.
2. Haagensen CD, Bodian C, Haagensen DE. *Breast Carcinoma: Risk and Detection*. Philadelphia, Saunders, 1981.
3. Dixon JM, Miller WR, Scott WN, Forrest APM. The morphological basis of human breast cyst populations. *Br J Surg* 1983, **70**, 604–606.
4. Dixon JM, Lumsden AB, Miller WR. The relationship of cyst type to risk factors for breast cancer and the subsequent development of breast cancer in patients with breast cystic disease. *Eur J Cancer Clin Oncol* 1985, **21**, 1047–1050.
5. Jaspar JM, Franchimont P. Radioimmunoassay of human epidermal growth factor in human breast cyst fluid. *Eur J Cancer Clin Oncol* 1985, **21**, 1343–1348.
6. Collete J, Hendrick JC, Jaspar JM, Franchimont P. Presence of α-lactalbumin, epidermal growth factor, epithelial membrane antigen, and gross cystic disease fluid protein (15,000 Daltons) in breast cyst fluid. *Cancer Res* 1986, **46**, 3728–3733.
7. Tonelli QJ, Sorof S. Epidermal growth factor requirement for development of cultured mammary gland. *Nature* 1980, **285**, 250–252.
8. Taketani Y, Oka T. Epidermal growth factor stimulates cell proliferation and inhibits functional differentiation of mouse mammary epithelial cells in culture. *Endocrinology* 1983, **113**, 871–877.
9. Osborne K, Hamilton B, Titus G, Livingston RB. Epidermal growth factor stimulation of human breast cancer cells in culture. *Cancer Res* 1980, **40**, 2361–2366.
10. Goustin AS, Leof EB, Shipley GD, Moses HL. Growth factors and cancer. *Cancer Res* 1986, **46**, 1015–1029.
11. Stoscheck CM, King LE Jr. Role of epidermal growth factor in carcinogenesis. *Cancer Res* 1986, **46**, 1030–1037.
12. Lai LC, Ghilchik MW, Shaikh NA, Reed MJ, James VHT. Relationship between epidermal growth factor and dehydroepiandrosterone and its sulphate in breast cyst fluid. *Br J Cancer* 1989, **60**, 320–323.
13. Gregory H, Holmes JE, Willshire IR. Urogastrone levels in the urine of normal adult humans. *J Clin Endocrinol Metab* 1977, **45**, 668–672.
14. Braunsberg H, Reed MJ, Short F, Dias VO, Baxendale PM. Changes in plasma concentrations of oestrogens and progesterone in women during anaesthesia and gynaecological operations. *J Steroid Biochem* 1981, **14**, 749–755.
15. James VHT, Reed MJ, Folkerd EF. Studies of oestrogen metabolism in postmenopausal women with cancer. *J Steroid Biochem* 1981, **15**, 235–246.
16. Reynolds VH, Boehm FH, Cohen S. Enhancement of chemical carcinogenesis by an epidermal growth factor. *Surg Forum* 1965, **16**, 108–109.
17. Rose SP, Stahn R, Passovoy DS, Herschman H. Epidermal growth factor enhancement of skin tumor induction in mice. *Experientia (Basel)* 1976, **32**, 913–915.
18. Cowley G, Smith JA, Gusterson B, Hendler F, Ozanne B. The amount of EGF receptor is elevated in squamous cell carcinomas. *Cancer Cells* 1984, **1**, 5–10.

19. Libermann TA, Nusbaum HR, Razon N et al. Amplification, enhanced expression and possible rearrangement of EGF receptor gene in primary human brain tumours of glial origin. *Nature* 1985, **313**, 144–147.
20. Kurachi H, Okamoto S, Oka T. Evidence for the involvement of the submandibular gland epidermal growth factor in mouse mammary tumorigenesis. *Proc Natl Acad Sci USA* 1985, **82**, 5940–5943.
21. James VHT, Reed MJ. Steroid hormones and human cancer. In: Iacobelli S, King RJB, Lindner HR, Lippman ME, eds. *Hormones and Cancer*. New York, Raven Press, 1980, 471–487.
22. Dunkley SA, Reed MJ, Lai LC, Ghilchik MW, Shaikh NA, James VHT. Free, biologically active, oestradiol and sex hormone binding globulin in breast cyst fluid. *J Endocrinol* 1988, **117** (Suppl), 75.
23. Ghilchik MW, Lai LC, Dunkley SA, Reed MJ, James VHT. Free oestradiol, epidermal growth factor and electrolytes in breast cyst fluid. Proceedings of the 8th International Congress of Endocrinology, Kyoto, Japan, 1988, 16-19-118.
24. Boccardo F, Guglielmo V, Zanardi S et al. Epidermal growth factor in breast cyst fluid: relationship with intracystic cation and androgen conjugate content. *Cancer Res* 1988, **48**, 5860–5863.

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Quality of Life During Chemotherapy for Small Cell Lung Cancer: Assessment and Use of a Daily Diary Card in a Randomized Trial

Duncan M. Geddes, Leanne Dones, Elizabeth Hill, Kate Law, Peter G. Harper, Stephen G. Spiro, Jeffrey S. Tobias and Robert L. Souhami

Fifty-three patients who were taking part in a randomized trial of chemotherapy in small cell lung cancer (SCLC) were entered into a study of quality of life measurement using a daily diary card. Patients received either four or eight cycles of initial chemotherapy and daily records were scored, using a four point scale of nausea, sickness, appetite, sleep, mood, pain, activity and general well being. Two hundred and fifty-six of a possible 379 cards were returned (68% compliance). The first 31 patients took part in an assessment of the diary card where comparison was made with nurse ratings using the card, the EORTC questionnaire and the Spitzer quality of life index. These comparisons showed appropriate convergent and divergent validity and demonstrated the sensitivity of the diary card to short term changes compared with the other measures.

In the randomized trial the diary card demonstrated a worsening of sickness and related variables as treatment continued. This spilled over into mood and general well being although physical variables of pain, sleep and activity were largely unaffected. Prophylactic cranial irradiation was associated with a transient increase in sickness and vomiting.

The study shows that the diary card is an instrument sensitive to short term changes in quality of life and thus especially useful for comparing effects during the period of treatment.
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INTRODUCTION

THERE IS increasing recognition that the measurement of quality of life is an essential part of trials in the treatment of cancer. This is especially important when survival is likely to be short and the treatment administered has appreciable toxicity. The problems involved in measuring quality of life in cancer have been well reviewed by Fayers and Jones [1] and while a number of instruments have been proposed, none has yet become generally accepted as the best. Comprehensive health status questionnaires such as the sickness impact profile [2] and Index of Wellbeing [3] scales have been well validated and tested in a range of different diseases but these instruments are complex to administer, require dedicated staff and are not well suited to repeated use over a short period. In contrast the simplest measures of all such as the Karnovsky [4] and ECOG scores are very easy to administer but are seriously limited in scope. Between these two extremes a number of other instruments have been tried such as the EORTC questionnaire [5] and the Spitzer quality of life index [6].

None of these instruments is ideally suited to the chemotherapy of small cell lung cancer (SCLC) as in this condition median survival is usually less than 1 year, in spite of a good chemotherapy response, and drug toxicity can be considerable. The EORTC questionnaire contains 47 questions and cannot be administered frequently. The Spitzer index assesses five variables only. In evaluating two different treatments consideration has to be given to the symptoms of the disease, the side effects of treatment and the effect of both on the patient's

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