CLINICAL RESEARCH

Oestrogen receptor content of normal breast cells and breast carcinomas throughout the menstrual cycle

CHRISTOS MARKOPOULOS, UTA BERGER, PATRICIA WILSON, JEAN-CLAUDE GAZET, R CHARLES COOMBES

Abstract

To examine the presence and distribution of oestrogen receptors in the normal breast during the menstrual cycle cytological samples obtained by fine needle aspiration from 69 premenopausal women with normal breasts were analysed immunocytochemically with a monoclonal antibody to oestrogen receptor; samples from 15 postmenopausal women were also analysed. The receptor content of breast cancers from 83 premenopausal women was also determined in relation to when during the menstrual cycle excision was performed. In the normal premenopausal women oestrogen receptors were detected in the nuclei of epithelial cells in 21 out of 68 (31%) assessable samples. All 21 of these samples were obtained from the 35 women who were studied during the first half of their menstrual cycle (days 28 to 14). None of the 33 samples obtained during the second half of the cycle contained oestrogen receptors. Samples were assessable in eight of the postmenopausal women, six giving a positive result for oestrogen receptor. Fifty one of the 83 carcinomas were positive for oestrogen receptor, 24 having been excised during the first half of the cycle and 27 during the second half.

Production of oestrogen receptor protein is suppressed at the time of ovulation in the normal breast epithelium of premenopausal women. In contrast, breast carcinoma cells either synthesise this protein continuously throughout the cycle or fail to express it despite fluctuations of serum hormone concentrations.

Introduction

The production of monoclonal antibodies against oestrogen receptors by Greene *et al* has permitted the development of oestrogen receptor assays based on direct antigenic recognition rather than steroid binding activity.^{1,2} Immunocytochemical staining is a powerful tool for detecting and localising antigens in tissue sections or cell preparations. Various studies have described immunocytochemical localisation of oestrogen receptors in the nuclei of several target tissues, including cultured human breast cancer cells,³ human breast cancer tissue,⁴ and human endometrium.^{5,6}

In a previous study we assayed oestrogen receptors in neoplastic breast tissue and samples of breast tissue obtained by fine needle aspiration with an immunocytochemical assay; the results correlated well with those from the steroid binding (dextran coated charcoal) assay.^{7,8} Therefore, in this study we examined the presence of oestrogen receptors in cytological samples obtained by fine needle aspiration from women with normal breasts and studied the distribution of these receptors in normal breast epithelial cells throughout the menstrual cycle as the distribution of oestrogen receptors during the cycle has already been established in human endometrium.⁶ Furthermore, we compared our results in normal breasts with those obtained by measuring oestrogen receptors biochemically in carcinomas removed at different times during the menstrual cycle.

Patients and methods

Approval for this study was obtained from the hospital's ethical committee.

Patients with normal breasts—Altogether 84 women were studied; 69 were premenopausal and 15 postmenopausal. Women were considered to be premenopausal only if they had regular menstrual periods and were not taking the contraceptive pill or other preparations containing hormones. Most (94%) had a 28 day menstrual cycle. The women were attending the breast clinic (a) because of vague breast symptoms but no abnormality on clinical examination; (b) for screening because of a family history of breast cancer; (c) because of a fibroadenoma in one breast; or (d) after having had an ipsilateral biopsy, which had shown a benign lesion. All 15 postmenopausal women were at least five years postmenopausal. The mean age of the

St George's Hospital and Ludwig Institute for Cancer Research, St George's Hospital Medical School, London SW17 0RE

CHRISTOS MARKOPOULOS, MD, research registrar

UTA BERGER, MD, cytopathologist

PATRICIA WILSON, BSC, senior technician

JEAN-CLAUDE GAZET, MS, FRCS, consultant surgeon

R CHARLES COOMBES, MD, PHD, consultant in medical oncology

Correspondence to: Dr R C Coombes, Ludwig Institute for Cancer Research, St George's Hospital Medical School, London SW17 0RE.

premenopausal women was 37.9 (SD 8.5) years (range 20-55) and that of the postmenopausal women 55.3 (4) years (range 47-63). Patients from whom assessable samples were obtained in the first half of the menstrual cycle were of similar age to those from whom samples were obtained in the second half (mean 39 (SE 1) and 40 (2), respectively). The breast that was aspirated was normal on clinical examination. In 52 (62%) patients mammography was also performed and showed no evidence of breast abnormality.

Patients with breast cancer—We also examined the receptor state of 83 sequential premenopausal patients (mean age 43 (SD 5.4), range 31-53) who presented to this unit from 1984 to 1986 with a tumour graded as T1 or T2 (less than 5 cm) and determined when in the menstrual cycle surgery had been carried out. Fifty one of them were found to have tumours positive for oestrogen receptors (>15 fmol/mg cytosol protein), the 32 others having tumours without oestrogen receptors. We measured the receptor state in these tumours by the dextran coated charcoal technique.⁷ Thirteen (25%) of these tumours were also analysed by the oestrogen receptor immunocytochemical assay, and the results correlated well with those by the dextran coated charcoal technique.

Samples—Fine needle aspiration was performed with a 21 gauge needle. Aspirates were smeared on to two microscope slides treated by tissue adhesive (poly-L-lysine) and were immediately frozen in a container with dry ice or liquid nitrogen. All samples were stored in vapour phase liquid nitrogen for one to four weeks until being stained immunocytochemically. In general, only two smears were available from each aspirate. In some cases the material was sufficient for more slides, but these slides were provided for other purposes. In a pilot group of 24 patients with normal breasts two additional slides were sent for routine cytological examination, and the results confirmed the presence of epithelial cells in the smear.

Immunocytochemical oestrogen receptor assay—The monoclonal antibody used was a rat antibody against oestrogen receptor provided by Abbott Laboratories, Chicago. A sensitive peroxidase-antiperoxidase technique was used to visualise the oestrogen receptor.⁷ Briefly, the samples were fixed in formalin, methanol, and acetone in phosphate buffered saline and treated with a blocking reagent to prevent non-specific binding. One of the two slides from each patient was incubated with the primary antibody, a monoclonal (rat) antibody to human oestrogen receptor, and the other was incubated with the control antibody, normal rat IgG. The slides were then incubated with the bridging antibody, goat antirat IgG_{2b} immunoglobulin, which binds to the rat antibody against human oestrogen receptor in the specimen treated with the primary antibody and to any non-specifically bound normal rat IgG bound to the sample treated with control antibody. The rat peroxidase-antiperoxidase complex was added to each slide to bind to the bridging antibody. A chromogen-substrate solution containing substrate reagent (hydrogen peroxide) and diaminobenzidine hydrochloride was added to each sample. The reaction of peroxidase with hydrogen peroxide converts diaminobenzidine hydrochloride to an insoluble reddish brown product. Monoclonal antibody complexes with oestrogen receptors are thus visualised because of the amplification from the bridging antibody, the peroxidase-antiperoxidase complex, and the products of the final enzyme reaction.

Evaluation of samples—We considered samples obtained by fine needle aspiration to be assessable only if they showed grouped single cells or clusters

of epithelial cells, or both, when smeared on the slide. As we found it difficult to identify the epithelial origin of isolated single cells we excluded samples with only a few single epithelial cells scattered over the surface of the slide. All assessable samples containing any number of epithelial cells with oestrogen receptors were considered to be positive for the receptors (figs 1 and 2). The stained smears were compared with their control slide, which contained a comparable number of cells. The control slides showed only the weak grey-blue staining with the counterstain, haematoxylin. Each staining run also included a control slide of MCF-7 cells (a breast cancer cell line), which showed a strong positive reaction. On each occasion no staining was seen in the negative control slide when the first antibody was omitted.

FIG 1—(a) Fine needle aspirate of normal breast during first part of menstrual cycle incubated with monoclonal antibody to oestrogen receptor. (b) Control sample from same woman incubated with normal rat IgG and counterstained with haematoxylin.

FIG 2—(a) Breast cancer cells incubated with monoclonal antibody to oestrogen showing heterogeneous, moderate to strong, positive nuclear reaction. (b) Control sample incubated with normal rat IgG and counterstained with haematoxylin.

Results

Fine needle aspirates from normal breasts—Sixty eight of the 81 samples taken from premenopausal women were assessable for determination of oestrogen receptors by immunocytochemical assay. Thirty five samples were obtained from women during the first half of the cycle and 33 during the second. Twenty one women showed positive nuclear staining with the assay; all the samples had been taken during the first half of the menstrual cycle (days 28 to 14). None of the 33 samples taken during days 15 to 27, the second part of the menstrual cycle, contained cells positive for oestrogen receptors (fig 3). Samples obtained in the first and second halves of the cycle were comparable in the number of assessable epithelial cells. Overall eight of the 15 (53%) postmenopausal women gave samples that were assessable by the immunocytochemical method. Six out of eight (75%) samples were found to be positive for oestrogen receptor compared with 21 out of 68 (31%) samples from premenopausal women irrespective of the day in the menstrual

cycle when the sample was obtained. The number of epithelial cells in smears ranged from about 10 to more than 100. All three groups included similar numbers of smears with a high (≥ 100) and low (about 10-99 grouped cells) number of cells.

FIG 3—Distribution of 68 assessable samples positive (■) and negative (□) for oestrogen receptor obtained by fine needle aspiration from premenopausal women with normal breasts on different days of menstrual cycle. Changes in serum concentrations of oestrogen and progesterone during typical 28 day cycle are shown for comparison.

Receptor content of breast cancers—The mean oestrogen receptor content in the 83 carcinomas determined by the biochemical technique was 32.1 (SE 7.7) fmol/mg cytosol protein in the first half of the menstrual cycle and 33.6 (6.1) fmol/mg in the second half. This difference was not significant. The 51 cancers positive for oestrogen receptor were also equally distributed between the first and second halves of the menstrual cycle, with 24 occurring in the first and 27 in the second half. Immunocytochemical results were available for 13 of these carcinomas and all were found to be positive. Ten were heterogeneously stained, as previously described. Six were obtained from women during the first half of the cycle and seven from women during the second half.

Discussion

Oestrogen receptors, as measured by the binding assay, have not previously been detected in normal breast tissue. Most tissue specimens in other studies were derived from breasts resected for malignancy or biopsy specimens of "normal" breast tissue near fibroadenomas, fibrocystic disease, or breast cancer.⁹⁻¹¹ Our results show that production of oestrogen receptor protein in normal mammary epithelial cells is cyclic in premenopausal women, being mainly produced during the early days of the menstrual cycle while plasma oestrogen and progesterone concentrations are still low (fig 3). In breast cancer that is positive for oestrogen receptors, however, this cyclic phenomenon is disturbed and the receptors seem to be produced throughout the menstrual cycle.

Several histological changes in normal breast during the menstrual cycle have been reported.¹²⁻¹³ Proliferative activity with mitotic figures has been identified in the early days of the cycle (days 3-14) but not during days 15-28 and 28-2 (secretory and menstrual phases).¹⁴ In contrast, other studies report a cyclic variation with mitoses occurring towards the end of the menstrual cycle and during menses.¹⁵ Breast epithelium in the first half of the menstrual cycle is characterised by small lobules and few terminal duct structures with condensation of the intralobular stroma; in contrast, in the second half of the cycle the normal breast shows an increase in size of lobules, duct epithelial cells, and terminal duct structures.¹³⁻¹⁵ Considerable controversy exists about the proliferative activity of normal breast epithelial cells throughout the menstrual cycle. Thus Vogel *et al* described mitotic figures during

the first half of the cycle,¹⁴ and Ferguson and Anderson, Anderson *et al*, and Longacre and Bartow found that most cell division occurred in the second half of the cycle.¹⁵⁻¹⁷

A similar cyclic variation in oestrogen receptor content has also been shown in human uterus. The receptors are present in the myometrium and the glands and stromal cells of the endometrium unlike in the normal breast, where only epithelial cells react. A further difference between breast and endometrial expression of oestrogen receptors is that the endometrium still expresses the receptors, although to a less extent, during the secretory phase of the cycle. We do not know the mechanism of synthesis of the receptors in normal breast cells of postmenopausal women. The regulation and control of oestrogen receptors may be related to locally produced oestrogen by the enzyme aromatase (oestrogen synthetase), but clear evidence for this is still lacking.

Our study shows that expression of oestrogen receptors in normal breast epithelial cells occurs mainly during the follicular phase of the menstrual cycle. In contrast, the receptor content of breast cancer does not seem to be cyclic, suggesting that the regulation of gene expression of oestrogen receptors is disturbed in cancer cells. If this disturbance occurred before transformation it could lead to increased susceptibility to carcinogens and abnormal proliferation as both animal¹⁸ and epidemiological studies of the incidence of breast cancer in women after ovariectomy¹⁹ have shown that oestrogen promotes the development of breast cancer. Further study of the oestrogen receptor content of premalignant lesions is needed to elucidate this mechanism fully.

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