Report

Influence of the menstrual cycle on the concentrations of estrogen and progesterone receptors in primary breast cancer biopsies

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Key words: estrogen receptor, menstrual cycle, progesterone receptor

Summary

There is controversy in the literature regarding the effects of endogenous hormones on estrogen receptors (ER) and progesterone receptors (PR) in young women with breast cancer.

We studied 117 young women with primary breast cancer and assessed their breast biopsies for ER and PR. The women had a record of their last menstrual period prior to breast biopsy. The menstrual cycle was divided into four phases – early proliferative (days 1–7), late proliferative (days 8–15), early secretory (days 16–22), and late secretory (days 23–30). There were lower levels of both ER and PR in biopsies excised during the early secretory phase than in other phases of the cycle; early proliferative phase receptor positive medians of ER = 77 fmol/mg protein and PR = 467 fmol/mg protein fell to ER = 28 fmol/mg and PR = 128 fmol/mg protein in the early secretory phase.

Introduction

The importance of the estrogen receptor (ER) in the clinical management of patients with breast cancer is now established. Clark and McGuire [1] showed that the progesterone receptor (PR) provided additional information to aid in selecting those patients more likely to benefit from endocrine manipulation when their disease recurred. Fisher et al. [2] and Rose et al. [3] suggested that receptor quantitation may be of value in predicting the effectiveness of adjuvant therapy.

There is controversy in the literature regarding the effects of endogenous hormones on ER and PR in young women with breast cancer. It has been noted that ER levels are lower in women younger than 50 years. Clark et al. [4] showed that this appeared to be primarily a function of age rather than of menopausal status. If ER and PR are to be used as clinical markers, it is imperative that the confounding factors are documented so that the clinical ‘cut off’ threshold at which the efficacy of treatment can be predicted may be established.

We examined receptor levels in young patients with breast cancer in relation to the day of their menstrual cycle when breast biopsy was performed. Our results suggest that the concentrations of ER and PR are influenced by the phase of the menstrual cycle.

Method

One hundred and seventeen women under 50 years
of age had their primary breast carcinoma biopsied for histopathological diagnosis and estrogen and progesterone receptor analyses. These women also had a record of the date of the menstrual period prior to breast biopsy. The age range was 28 to 50 years.

The menstrual cycle was divided into four phases, early proliferative (days 1–7), late proliferative (days 8–15), early secretory (days 16–22) and late secretory (days 23–30).

Breast cancer biopsies were snap-frozen as soon as possible after excision. Specimens from outlying centres were transported to the laboratory in small test tubes on dry ice, or frozen in a block of storage buffer and similarly transported on dry ice.

Patients in this study had their biopsies between June 1983 and June 1986. Information regarding menstrual history was obtained at the time of the breast biopsy with the cooperation of surgeons who referred their patients to this laboratory for receptor analyses.

The receptors were assessed on freshly prepared cytosols using standard ligand binding techniques as described by us [5] and later adapted to a micro-method [6]. In brief, aliquots of the cytosol (2–3 mg protein/ml cytosol) were immediately incubated in microtitre plates, in parallel, with six different concentrations of tritiated ligand, either with or without excess unlabelled competitor (to assess low affinity binding). Dextran-coated charcoal was used to adsorb unbound ligand, and aliquots of the supernatant were counted in scintillant. Scatchard plots were developed and specifically bound receptor protein was calculated by the least squares methods and reported as fmol/mg cytosol protein.

Generally, specimens were large enough for full saturation analyses for both receptors, each using six ligand concentrations. New England Nuclear’s tritiated estradiol (0.1 nM to 5 nM) was used for the ER assay and tritiated Promegestone for the PR assay. In January 1985 Amersham’s tritiated Organon 2058 (0.2 nM to 10 nM) replaced the Promegestone for PR measurement. The laboratory has been a member of the Australian National Quality Assurance Programme since its inception in 1981.

Results

In our experience approximately 61% of breast cancer tumours contain ER (>8 fmol/mg protein) and only 42% exhibit significant PR protein (>10 fmol/mg protein).

Four hundred and four primary breast cancers assessed in our laboratory during 1983 to 1985, used here as historical controls, showed that the PR was seen more frequently in women under 50 years (50%) than in older women (38%), while the ER was more prevalent in women over 50 years of age (66% vs 48%) and set at higher quantitative levels. In Table 1 the mean ER+ value was 68 fmol/mg protein in younger women and rose to 194 fmol/mg protein in older women. A t-test resulted in a t = -7.5, df = 301 and P = 0.0000. The means of the PR+ values were similar in both populations.

Of the 117 women whose biopsies were assessed for ER and who had a record of their last menstrual period prior to this biopsy, 55 (47%) were ER+. Fig. 1 illustrates the receptor concentrations recorded on receptor positive biopsies excised at different times during the menstrual cycle. The median ER+ level fell from a high of 77 fmol/mg protein in the early proliferative phase, to a low of 28 fmol/mg protein in the early secretory phase. The Kruskall-Wallis one-way analysis of variance by ranks was used to compare the medians at each of the four phases, and gave H = 8.6, df = 3 and P<0.05.

There were 109 patients who had both receptors assayed on their biopsies, 42 ER+ PR+ (38%), 10

| Table 1. Receptor combinations in young women compared to older women. |
|----------------------------------------|--------|--------|
|                                       | <50 years n = 122 | >50 years n = 282 |
| ER+PR+                                 | 40%    | 36%    |
| ER+PR−                                 | 8%     | 30%    |
| ER−PR+                                 | 10%    | 2%     |
| ER−PR−                                 | 42%    | 32%    |
| ER+ Mean*                              | 68 ± 86 s.d. | 194 ± 213 s.d. |
| PR+ Mean*                              | 294 ± 358 s.d. | 267 ± 283 s.d. |

* fmol/mg protein.

s.d. = standard deviation.