Short Communication

Plasma oestrogens and oestrogen receptors in breast cancer patients

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The concentration of oestrogen receptors (ER) in a breast cancer is now well established as an index for predicting the subsequent response of the disease to endocrine therapy (Hawkins *et al.*, 1980). Oestrogen receptor concentration is influenced by menstrual status, tumour cellularity and tumour grade (Steele *et al.*, 1982), and also rises with increasing age (e.g., Clark & McGuire, 1983; Mercer *et al.*, 1983; Daxenbichler *et al.*, 1982). The influence of tissue and plasma levels of oestrogen on ER concentration, however, is as yet less clearly understood: whilst high levels of circulating oestrogen have been found to be associated with low/absent tumour ER in premenopausal women (Maass *et al.*, 1972; Nagai *et al.*, 1979; Hawkins *et al.*, 1979), less is known about this relationship in postmenopausal women.

In the present study, we have examined the quantitative relationship between plasma oestrogens and ER in the tumours from 50 postmenopausal women.

Plasma for the assay of oestrone and oestradiol-17β and tumour for the assay of ER were obtained from 50 postmenopausal women with primary cancer of the breast. Plasma samples were obtained betweem 9 a.m. and 11 a.m. in the week prior to operation, and at operation, the tumour was placed on ice for immediate transfer to the laboratory.

Oestrogen receptor activity was determined by by saturation analysis (Hawkins *et al.*, 1975) with minor modifications. A portion of tumour (300 mg) was homogenised (1:10 w/v) in Tris buffer (10 mM Tris, 0.25 M sucrose, 1 mM ethylene diamine tetracetate, containing 10% v/v glycerol and 1% monothioglycerol) and centrifuged at 2040 g for 20 min. Aliquots of the resulting cytosol were incubated overnight at 4°C with 0.031 nM [2,3,6,7-³H] oestradiol and varying amounts of competing non-radioactive oestradiol (0, 0.03, 0.09, 0.15, 0.21, 0.28, 3.06 and 61.2 nM). Free and bound steroid

fractions were separated by the addition of dextran-coated charcoal suspension, adsorption and centrifugation, and the radioactivity in the bound fraction was determined by liquid scintillation counting. The concentration of receptor was calculated by 6-point Scatchard analysis (Scatchard, 1949). Cytosolic protein concentration was determined by the method of Bradford (1976) and receptor levels in excess of 5 fmol mg⁻¹ tumour cytosol protein were regarded as positive.

Radioimmunoassay of plasma oestrone Plasma samples (1 ml), diluted 1:1 (v/v) with water plus 7.4 fmol tracer [2,4,6,7-³H], oestrone, were extracted with 10 ml of Analar ether. The extract was backwashed with 0.2 ml of 8% (w/v) sodium bicarbonate solution, evaporated to dryness and dissolved in Tris buffer. A portion (1/3) was removed and counted to assess manipulative losses and the remainder was used for radioimmunoassay. After overnight incubation at 4°C with 0.1 ml of antioestrone antibody (1:62,750 v/v in buffer), 0.1 ml of [2,4,6,7-³H] oestrone solution (~10,000 cpm was added). Bound hormone was separated from free by the addition of dextran-coated charcoal and adsorption: after centrifugation, the radioactivity in the bound fraction was measured by liquid scintillation counting. The amount of oestrone present was determined by reference to a standard curve derived from known amounts of radioinert oestrone (0.003–0.617 pmol ml⁻¹) and was corrected for manipulative losses. Values were expressed as nmol oestrone l⁻¹ plasma.

Radioimmunoassay of oestradiol-17β Similarly, 2 ml samples of plasma, after the addition of 0.2 ml of 0.3 M sodium hydroxide solution and 7.4 fmol [³H] oestradiol-17β tracer, were extracted with ether. The extract was backwashed with 0.2 ml of water and the ether extract was evaporated to dryness. After removal of a portion (1/3) for counting to monitor losses, the remainder was subjected to radioimmunoassay using an antibody against oestradiol-17β (1:83,000 v/v) and [³H] oestradiol as radioligand. The mass of oestradiol-17β present was read from a standard curve prepared using radioinert oestradiol-17β and after

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correction for manipulative losses, the results were expressed as nmol oestradiol l^{-1} plasma.

Of the 50 tumours investigated, 36 (72%) were ER-positive and 14 (28%) ER-negative. The concentrations of oestrone and oestradiol-17β in the plasmas from patients with ER-positive and -negative tumours are shown in Figure 1. No significant difference in plasma oestrogen concentration was evident between these two groups of tumours ($P>0.1$ Wilcoxon Rank sum test) for either oestrogen.
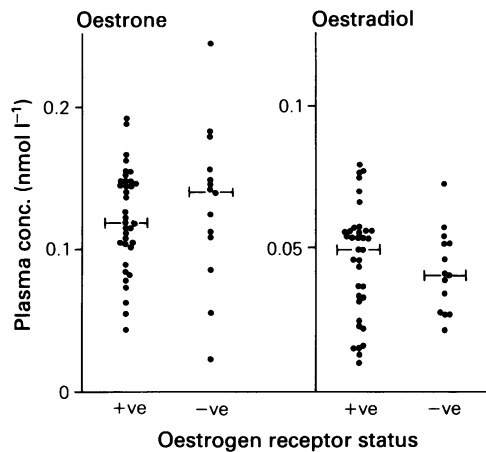

Figure 1 Levels of plasma oestrogen in women with ER-positive and negative tumours. Horizontal lines indicate median values.

For the 36 ER-positive tissues, the quantitative relationships between the concentration of ER in the tumour and of those oestrone or oestradiol in the plasma are shown in Figures 2 and 3. A significant positive correlation was found between the level of ER in the tumour and the level of oestrone in the plasma ($r=0.50$, $P<0.01$, Spearman's rank correlation test). There was also a tendency for the levels of tumour ER to rise with increasing plasma oestradiol concentration but this did not attain significance ($r=0.26$, $P>0.10$, Spearman's rank correlation test).

These results demonstrate that in postmenopausal women, in contrast to our earlier findings in premenopausal subjects (Hawkins *et al.*, 1979), the level of oestrogen receptor activity detected in a breast cancer, which has prognostic and predictive value, is correlated with the concentration of the major circulating, unconjugated oestrogen, oestrone. The plasma levels of oestradiol-17β were much lower, and showed a similar, but non-significant relationship with tumour ER activity.

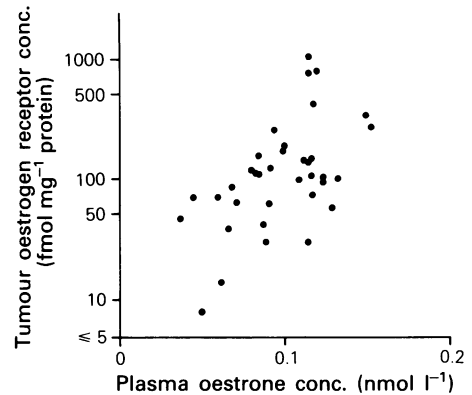Our findings are in agreement with those of Saez and her colleagues (1978) who found that tumour

Figure 2 The quantitative relationship between the concentrations of oestrone in plasma and oestrogen receptors in the tumour. By Spearman's rank correlation test, $r=0.50$, $P<0.01$.
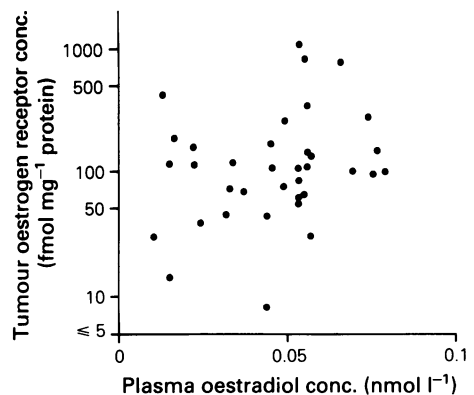

Figure 3 The quantitative relationship between the concentrations of oestradiol-17β in the plasma and oestrogen receptors in the tumour. By Spearman's rank correlation test, $r=0.26$, $P<0.10$.

ER levels were significantly correlated with plasma oestrogen level in a group of postmenopausal patients with tumours positive for both oestrogen and progestogen receptors. Drafta *et al.* (1983), on the other hand, found that the plasma oestradiol concentration was higher in patients with receptor-positive tumours than in those with receptor-negative tumours.

The present study implies that the levels of circulating oestrogens, (~0.02–0.08 nmol l^{-1} for oestradiol, ~0.04–0.20 nmol l^{-1} for oestrone) are sufficient to half-saturate the oestrogen receptor (~0.03 nmol l^{-1} for oestradiol, ~0.09 nmol l^{-1} for oestrone at 4°C) and may thus influence the rate of its synthesis or degradation in the tumour. However, it seems likely that it is the level of

oestrogen in the tissue, rather than that in the plasma which ultimately influences ER levels. In tumour tissue, the endogenous oestrogens derive not only from the plasma, but also from biosynthesis within the tumour (Miller & Forrest, 1974; Miller et al., 1981; Mason et al., 1981) and hydrolysis of oestrone sulphate (Santner et al., 1984); oestradiol rather than oestrone appears to be the major free oestrogen (Poortman et al., 1983). In general, in tumour tissue, oestrogen concentrations are likely to be higher when ER are present than when they are absent (Fishman et al., 1977; Edery et al., 1981; Thorson et al., 1982; Drafta et al., 1983), and in one study (Edery et al., 1981), tumour oestradiol content rose with increasing ER level.

Oestrogens can affect receptor levels in at least two ways: (i) they fill empty receptor sites and (ii) they may influence the rate of synthesis or degradation of receptor (see e.g. Hawkins et al., 1977; Bayard et al., 1978). This study suggests that whilst the former effect may predominate in premenopausal women, where progesterone levels may determine oestrogen receptor synthesis (Saez et al., 1978), the latter effect may occur, to some extent, in postmenopausal subjects.

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