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ESTRONE AND ESTRADIOL CONTENT IN HUMAN BREAST TUMORS: RELATIONSHIP TO ESTRADIOL RECEPTORS

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SUMMARY

We have measured the estradiol receptor content together with the endogenous estrone and estradiol concentrations in human breast tissue cytosols. There was no evidence for a relationship between high estrogen levels and low receptor measurements. Receptor positive tumor cytosols contained a statistically significant greater estradiol concentration than those of receptor negative tumors or of normal tissue. This was confirmed in a study of sixteen pairs of tumor and normal tissue from the same breast in which the estradiol but not estrone concentration of the receptor positive tumors exceeded that of its normal partner. The results indicate that (a) false negative receptor assays due to the presence of endogenous estrogens are not likely; (b) receptor positive tissues retain greater amounts of estradiol than receptor negative tumors.

INTRODUCTION

Evidence of the clinical usefulness of estrogen receptor determinations in guiding the management of advanced breast cancer has provoked widespread interest in such measurements [1]. The methods available for receptor assay are all based on the specific uptake of radioactive estradiol by the receptor. The receptor–estrogen complex dissociates only slowly [2] under the conditions of the assay so that if the endogenous estrogens are present the methodology as presently practiced permits the detection of only vacant receptor molecules. This has created the potential for false negative results since the receptors may be present but they may be saturated with endogenous ligands and hence are not detectable. Such false negative conclusions would constitute a serious problem since they would result in the diagnosis of the tumor as not hormone dependent and lead to the failure to institute treatment which could have been of benefit. The significance of this problem was realized by many investigators who have considered the impact of endogenous estrogen levels on measured receptor content. Most of the studies found that the incidence of receptor positive tumors is comparable in pre and post menopausal women, but that the receptor content in the latter tends to be higher [1, 3–6], although the relationship is not always statistically significant. Considering the much larger plasma estradiol concentrations in premenopausal women a more clear cut difference in the receptor content between the two categories would have been expected. In another study plasma estradiol concentrations were measured in conjunction with tumor receptor assays [7]. No reciprocal relationship between tissue receptor content and estradiol concentration below 200 pg per ml were observed. Only at rare very high estradiol levels of 200–400 pg per ml was there a lack of measured receptors suggesting possible false negatives. Similarly, Sakai and Saez[8] have found little consistent relationship between plasma estrogens and the degree of occupancy of both cytoplasmic and total cellular estrogen receptors. These results therefore suggest that plasma estrogens may not represent an accurate index of tissue receptor saturation. The reason for this may be because the plasma–tissue gradient of estradiol is quite variable with tissue concentrations exceeding plasma levels by different factors in different individuals and in different tissues [9]. A more valid appraisal, therefore, of the influence of endogenous estrogen content on the detection and measurement of estrogen receptors would be available from an assay of the estrogens contained in the tissue being assayed for receptor content. The very limited number of such simultaneous receptor and estrogen tissue assays that have been reported [10] permit no clear cut conclusion to be established. More recently the steroid hormone content of human breast tumors has been investigated but without simultaneous estradiol receptor determinations [11]. In this communication we present our results in measuring the estrone and estradiol content of a large number of tumor and normal breast tissue cytosol samples which have also been assayed for receptor content. Significant differences in the relationships between these values in normal and tumor tissues are reported, and evidence is presented that high tissue concentrations of estradiol or estrone do not preclude the detection of receptors in the same samples.

MATERIALS AND METHODS

Breast tissue cytosols—preparation and receptor assay. The minced tissue, freed of fat as much as possible, is homogenized in 4 vol. of TES-Thio buffer (10 mM N-Tris hydroxymethyl-2-aminomethane sulfonic acid; 12 mM monothioglycerol) pH 8 containing 250 mM sucrose, with a Polytron homogenizer at 0°. The cytosol is obtained by centrifugation at 105,000 *g* for 60 min at 4° [12].

To 0.15 ml aliquots of the cytosol are added 0.05 ml of TES-Thio buffer, pH 8 containing egg albumin (1 g/dl). Then [2,4,6,7-^{3}H]-estradiol to yield final concentrations of 1, 3 and 5 nM radioactive estradiol in separate tubes is added to permit construction of a 3 point Scatchard plot and calculation of the dissociation constant. A fourth tube is prepared as above but the buffer solution contains 1 μM cold estradiol and 1 nM radioactive estradiol. Incubations are carried out for 60 min at ambient temperature. Charcoal–Dextran suspension, 0.05 ml (2.5 g charcoal and 0.026 g Dextran/dl) is added and the tubes are shaken at 0° for 20 min and centrifuged at 1220 *g* for 20 min after which 0.15 ml of the supernatant is removed for counting. Concentration of receptor is expressed as fmol per mg of protein based on the amount of [^{3}H]-estradiol bound when its concentration in the incubation mixture is 1 nm, and on the protein content of the cytosol. The specificity is related to the extent of inhibition of specific binding in the presence of 1 μm cold estradiol. The use of diethylstilbesterol instead of estradiol did not confer any advantages on this determination.

Assay of estrone and estradiol in breast tissue cytosols. Following the receptor measurement, aliquots of the remaining cytosol, which ranged from 0.4 to 1 ml were extracted with 3 × 5 ml of ethyl ether. When sufficient material was available duplicate samples were assayed. [6,7-^{3}H]-Estradiol or [6,7-^{3}H]-estrone were added to alternate samples prior to extraction to monitor recovery. The organic extract was then reduced to dryness under a stream of nitrogen in a warm water bath. The residue was taken up in 0.6 ml of phosphate buffer pH 7.2. From this solution 0.05 ml was removed to monitor recovery, 0.25 ml was used for estradiol assay and 0.25 for estrone assay. The estradiol and estrone assays were carried out in the conventional manner using highly specific antiestradiol and antiestrone antisera. Cross-reaction of estrone with antiestradiol antiserum was less than 4%, and that of estradiol with the antiestrone antiserum was less than 3%. Dextran coated charcoal was used to separate the bound and unbound ligands. Recoveries of estrone and estradiol averaged 88 and 82% respectively and the duplicates agreed to within ±12%.

RESULTS AND DISCUSSION

The estradiol, estrone and estrogen receptor content was determined in 129 human breast tissue cytosols. The receptor content is expressed as fmol per mg of protein, and the values are corrected for the specificity of the binding as determined by inhibition with excess estradiol. The concentration of estrone and estradiol is given in terms of pg of steroid per mg of protein content. These can readily be converted to fmol per mg of protein using the relationship of 1 pg ≅ 4 fmol. It needs to be emphasized that the actual amounts of estrogens measured were invariably much larger since all cytosol samples contained more than 1 mg/ml of protein. To detect whether there is any either direct or inverse relationship between estradiol content and measured receptor, these two values are plotted in Fig. 1, and a similar plot combining estrone and receptor content is shown in Fig. 2. It is evident from these figures that neither high estradiol nor high estrone values are related to low or negative receptor measurements. In actuality most of the receptor negative samples are clustered in the very low estrogen region. Only one very high estrogen containing

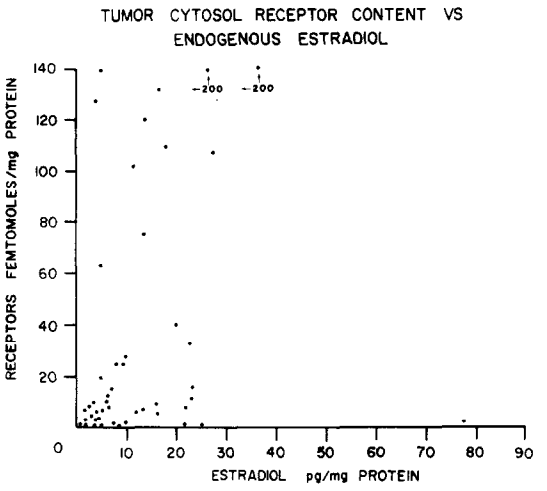

Fig. 1. Tumor cytosol receptor content vs endogenous estradiol.

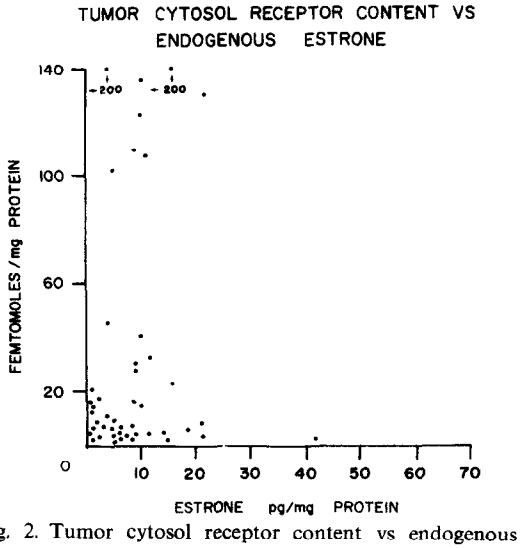

Fig. 2. Tumor cytosol receptor content vs endogenous estrone.

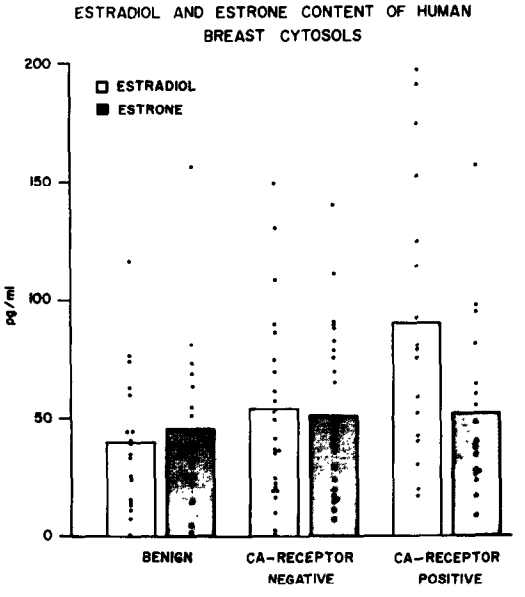

Fig. 3. Estradiol and estrone content of human breast cytosols.

sample (78 pg estradiol, 42 pg estrone per mg of protein) was devoid of receptors and could be classified as a possible "false" negative. However, this possibility was not tested by attempting to displace estrogen from a putative receptor.

To determine whether the nature of the tissue cytosol sample has any bearing on its estrone or estradiol content the tissue samples were divided into benign, receptor negative and receptor positive tumor categories. The designation of positive was arbitrarily assigned to those cytosols which contained more than 10 fmol of receptor per mg cytosol protein. The results obtained are presented graphically in Fig. 3 in which the estrone and estradiol content is given in terms of pg per ml of cytosol solution. The estrone concentration of all three types of tissue showed considerable variation between individual samples, but there was no difference between the three categories. In contrast the average estradiol concentration and standard deviation of the normal tissue cytosols was 38 ± 29 pg/ml cytosol, that of the receptor negative tumor cytosols was 51 ± 39 pg/ml cytosol and that of the receptor positive tumor cytosols was 91 ± 59 pg/ml cytosol. The mean estradiol content of receptor positive tumor tissue was therefore greater than that of normal tissue with a significance of $P < 0.001$, and also greater than that of the receptor negative cancer tissue with $P < 0.02$. There was no significant difference between the estradiol content of normal and receptor negative tumor tissues.

The above averages are composed of samples obtained from different subjects. Since differences between the individual from whom the tissues were obtained could have influenced the estradiol content changes, we also examined the estrogen content of breast tissue samples, normal and tumor, both derived from the same breast. Sixteen such pairs were studied, seven of which contained receptor negative and 9 receptor positive tumors. The results of the receptor negative pairs are recorded in Table 1 and those for the receptor positive pairs in Table 2. There is no consistent trend in the differences in the estrone content between the normal and receptor negative tumor pairs, estrone being higher in the normal tissue in 2 cases, lower in 4 cases and equal in one. There is also no pattern to the difference of estradiol content of the normal and receptor negative tumor pairs with 4 normal tissues being higher and 3 lower. In the receptor positive tumor pairs there is again no consistency in estrone content variation with normal tissues in 4 of the pairs containing less estrone, 3 more and two being equal to the corresponding tumor samples. In contrast, however, there is a clear trend to higher estradiol content in the receptor positive tumors compared to the corresponding normal tissues with 8 of the 9 sample pairs exhibiting such a difference. These results further confirm that tumor tissue containing receptors has a higher endogenous estradiol content than either receptor negative tumors or normal tissue. No such differences in estrone content have been found. It must also be stressed that in the present study the estrogen content of cytosols only was determined. Clearly translocation of an endogenous estradiol–cytosol receptor complex into the nucleus is an ongoing process in the presence of functional cytosol receptors, and therefore the estrogen content of the nuclei would also be of interest. Unfor-

Table 1. Estradiol and estrone content of human normal and receptor negative tumor tissue cytosols from the same breast

Sample pair	Estradiol (pg/ml Cytosol)			Estrone (pg/ml Cytosol)		
	Normal breast	Tumor	Tumor / Normal	Normal breast	Tumor	Tumor / Normal
1	138	80	0.6	8	48	6.0
2	116	66	0.6	41	78	1.9
3	104	200	1.9	45	78	1.7
4	76	40	0.5	48	12	0.3
5	32	32	1.0	62	21	0.3
6	28	32	1.1	32	31	1.0
7	16	6	0.4	14	60	4.3

Table 2. Estradiol and estrone content of human normal and receptor positive tumor tissue cytosols from the same breast

Sample pair	Estradiol (pg/ml cytosol)			Estrone (pg/ml cytosol)		
	Normal breast	Tumor	Tumor / Normal	Normal breast	Tumor	Tumor / Normal
1	8	60	7.5	30	44	1.5
2	7	18	2.6	34	34	1.0
3	44	80	1.8	20	58	2.9
4	60	58	1.0	74	56	0.8
5	64	120	1.9	54	12	0.2
6	70	190	2.7	50	90	1.8
7	74	108	1.5	150	150	1.0
8	74	112	1.5	78	8	0.1
9	72	188	2.6	46	98	2.1

tunately these were no longer available for analysis in this study. Nevertheless, in a dynamic *in vivo* situation such as reflected in these tissues the estrogen concentration in the cytosol and nuclei would be in an equilibrium relationship. Therefore, the estradiol concentration in the cytosol, the system of principal interest, will provide information on the whole tissue endogenous estrogen receptor relationship.

The present results indicate that high cytosol concentrations of estradiol or estrone are not inconsistent with the presence of vacant, measureable specific estradiol receptors. Therefore, failing to detect receptors is not likely to be a consequence of endogenous steroid saturation. The estrogen measurement method does not distinguish between free and bound estrogens and hence cannot shed light whether the estrogens in the cytosol were actually bound to the receptors. The greater estradiol content of the receptor positive tumors compared to the receptor negative tissues, both tumor and normal, present in the averages of the multiple samples and also in the specific paired tissues from the same breast, supports the conclusion that the estradiol in the tissues is at least in part receptor bound. Confirmation that receptor binding is involved in the greater concentration of estradiol follows from the fact that there is no similar increase in estrone concentration which has a much lesser affinity for the binding protein. The existence of unfilled receptors even in the presence of considerable endogenous estrogens is not surprising in view of the evidence that the hormone can control the synthesis of its own receptors [13, 14], and hence can be expected to stimulate synthesis of the binding protein. Sakai and Saez[8] have also clearly demonstrated the fractional saturation of estrogen receptors even in the presence of considerable plasma estrogens.

The presence of estradiol receptors is associated with positive response to endocrine therapy in only about 65% of breast cancer patients [1] and clearly additional criteria of selection are necessary for more precise prediction. It is possible that the presence of a definitie quantity of endogenous estrogen in the tumor tissue is a required criterion for remission. Theoretically, treatment which reduces an already minimal hormone content in a hormone dependent tumor should be of little value. Further work will be necessary to evaluate whether endogenous estradiol concentrations in conjunction with receptor assays can provide a better prognostic evaluation for endocrine therapy in breast cancer than that provided by the receptor alone.

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