Europ. J. Cancer Vol. 17, No. 6, pp. 659–664, 1981
Printed in Great Britain

0014-2964/81/060659-06 $02.00/0

Effect of Presurgical Radiotherapy on the Steroid Receptor Concentrations in Primary Breast Carcinoma

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Abstract—*With age, oestradiol receptor concentrations increased in primary breast carcinoma while age did not seem to affect the progesterone receptor levels. Above the age of* 70, *all tumours examined proved to be hormone-dependent. Analysis by light microscope did not allow correlation of the receptor-positive tumours to any specific or predominant cellular structure. Presurgical radiotherapy of* 20 gray *significantly reduced the oestradiol and to an even greater extent the progesterone receptor concentrations in the tumours. Prebioptic irradiation with* 8 gray *accentuated the inhibition of steroid receptor proteins. This reduction in receptor concentration after radiotherapy should be taken into account when interpreting steroid receptor values.*

INTRODUCTION

ALTHOUGH hormone treatment of breast cancer remains a valuable therapy, the general tumour response rate is low. Slightly more than 50% of oestradiol-receptor-positive tumours adequately respond to hormone therapy, while less than 10% of oestradiol-receptor-negative breast cancer can still be considered hormone-dependent [1–7]. Simultaneous determination of progesterone receptor levels and the quantitative evaluation of the receptor concentrations increase the precision of prediction of results of hormone therapy to more than 80% [8–15]. As the oestradiol receptor concentrations in tumours of postmenopausal patients prove generally to be higher than during the premenopausal period [3, 5, 6, 11, 16–19], presurgical treatment and possibly radiotherapy may well affect the receptor binding sites. If unknowingly, presurgical radiotherapy should considerably modify the receptor protein concentrations, the receptor assay results could be wrongly interpreted to predict the results of subsequent hormonal therapy. In order to improve the interpretation of steroid receptor concentrations in mammary carcinoma, the oestradiol and progesterone receptor proteins concentrations are analyzed and compared with the patient's age and menopausal status, the histological characteristics and the size of the tumour concerned, but particularly considering any radiotherapy carried out before surgery.

MATERIALS AND METHODS

A total of ninety-seven patients with breast cancer, from three Medical Centres† (CA with 44, CB with 30 and CC with 23 patients) were examined. All were eventually treated with surgical ablation, either tumourectomy or mastectomy. Before surgery no chemotherapy was applied. However, presurgical radiotherapy was carried out on all patients from CA. Radiotherapy of the whole breast during five consecutive days amounted to 20 gray (Philips cobalt therapy source) while surgery took place not later than one week after the last irradiation. Twenty-nine

Accepted 24 December 1980.
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CA patients received a prebioptic supplementary radiation of 8 gray applied in two sessions of 4 gray each directly applied on the tumour (Betatron, Brown-Boveri, 35 F-Asclepitron). This presurgical and prebioptic radiotherapy, although not widely practiced, was used at CA as a routine precaution to reduce the tumour volume and possibly to avoid metastases during surgical intervention [20]. Immediately after surgery, part of the tumour was frozen and transported in liquid nitrogen. Oestradiol and progesterone receptor concentrations were determined as described before [21].

Tumour tissue was pulverized at −196°C in a steel mortar and homogenized (Potter–Elvehjem) with three volumes TED buffer, pH 7.4 [10 mmol Tris–HCl (Merck)/1.5 mmol EDTA (Calbiochem)/0.5 mmol 1,4-dithio-DL-threitol (Fluka)] for the oestradiol receptor determination. For the progesterone receptor homogenization took place in TEG buffer (TED buffer containing 10% glycerol v/v). The homogenate was centrifuged at 105,000 *g* (International Ultracentrifuge B 60, swinging-bucket SB 405) for 70 min. The supernatant, freed from its lipid layer, was used for receptor assay. The procedure in duplicate took place at 0–4°C in Cooke microtiterplates (Greiner V-shaped) with 8 × 12 cuvettes of 0.3 ml. Six times four cuvettes were used in duplicate for each receptor determination, as four cuvettes were needed for each of the six different concentrations of labelled steroids used, varying from 0.1 to 10 nmol [2, 4, 6, 7-^{3}H(N)]-oestradiol-17 β (specific radioactivity: 94 Ci/mmol, New England Nuclear) for oestradiol receptor assay or 0.2–12 nmol (17α-methyl-^{3}H) promegestone (R 5020 specific radioactivity: 87 Ci/mmol New England Nuclear) for progesterone receptor assay. Apart from 5 μl of the labelled steroid solution which was added to each cuvette, the following additions were made: to cuvettes numbered 1, 55 μl TED or TEG buffer, to cuvettes 2, 205 μl TED or TEG buffer, to cuvettes 3, 5 μl TED or TEG buffer as well as 50 μl cytosol, and to cuvettes 4, 50 μl cytosol and 5 μl of a 100-fold excess of unlabelled steroid. The labelled steroid solutions also contained a 100-fold excess of unlabelled dihydrotestosterone (Sigma Co.) or cortisol (Sigma Co.) to avoid the oestradiol or progesterone, respectively, binding to plasma. The titerplates were covered with parafilm and incubated overnight for 15 hr at 4°C on a vibrating microshaker (Dynatech). After incubation the cuvettes numbered 1, 3 and 4 received 150 μl of DCC suspension [TED buffer/0.25% charcoal/0.025% dextran (grade C, BDH Biochemical]. After precipitation no more than 2% of the initial radioactivity could be recovered from the supernatants in cuvettes 1, which were considered to be DCC blanks. Cuvettes 2 served to measure the total amount of added tracer. Cuvettes 3 represented the total bound tracer and cuvettes 4 the aspecific binding. The microtiterplates were shaken for 10 min, followed by centrifugation at 1000 *g* (International Centrifuge, Size 2, Mod K, Rotor 240 with microtiterplate carrier). Subsequently 100 μl was transferred to microtiter vials (Lumac System AG), which received 1 ml scintillation liquid Aqua Luma (Lumac Systems AG). Counting took place in a Liquid Scintillation Spectrometer (Rack Beta, LKB, Wallac) [22]. After corrections for background, DCC blanks and quenching the disintegrations per minute were expressed in nmol per liter incubate. Scatchard analysis [23] allowed the calculation of the binding constants, K_d, and the receptor concentrations (fmol per mg of protein or per mg of tissue). Tissue protein concentrations were determined by the Folin–phenol method [24]. Statistical analysis was carried out according to the variance analysis, the Wilcoxon–Mann–Whitney rank-sum test, the linear regression or the Chi-squared test with the Student's t distribution, depending on the characteristics of the comparison and of the populations examined [25].

RESULTS

The patients treated with radiotherapy before surgery were generally younger than the untreated patients; thus the number of premenopausal patients in the irradiated group amounted to 48% while in the non-treated group only 28% were premenopausal. Only 4% of the whole population could be considered perimenopausal and the age of menopausal onset was identical in all three Centres. With increasing age, the number of oestradiol-positive tumours increased. Above the age of 70, all tumours examined contained oestradiol receptor proteins with concentrations higher than 5 fmol/mg protein. The progesterone receptor positive tumours were not related to age. The oestradiol receptor levels in tumours of postmenopausal patients (175 ± 70 fmol/mg protein) reached higher levels ($P = 0.01$) than in non-irradiated premenopausal patients (35 ± 15 fmol/mg protein). However, when only the oestradiol-receptor-positive tu-

mours were take into account, these differences disappeared while the progesterone receptor values remained unaffected. The light microscopical analysis of the tumours showed a distribution into poorly, moderate and well differentiated structures as well as a squirrous group which occurred in a relative higher proportion after the menopause ($P=0.08$). These different anatomo-pathological structures did not seem to affect the steroid receptor concentrations. The average tumour size during presurgical evaluation was similar with approximately the same staging interpretation for the three Centres. The degree of tumour invasion did not affect the oestradiol or the progesterone receptor levels (Table 1).

Table 1. Morphological characteristics of the mammary carcinoma

Differentiation		Staging-TNM classification			
Poor	31	T_1	5	N_0	51
Moderate	27	T_2	34	N_1	41
Well	2	T_3	40	N_2	3
Squirrous	31	T_4	18	N_3	2
Other	6				

All 97 tumours were histologically examined and classified according to differentiation and TNM staging. Each value gives the number of patients belonging to one of these groups.

Although 16% of all the examined patients equally distributed between the Centres had received hormonal contraception with progestagens, from 3 to 1 month before surgery, no effect on the steroid receptor concentrations could be detected due to this treatment.

In the radiotherapy-treated tumours, the concentrations of oestradiol and progesterone receptor proteins were consistently lower than in non-irradiated tumours. These differences became even more pronounced when only postmenopausal patients were considered, thus avoiding a possibly interfering age factor as the menopausal age was similar for all patients.

In these postmenopausal irradiated tumours, the oestradiol receptor concentrations amounted to 28.27 ± 7.10 fmol/mg protein compared with the previously non-treated patients with receptor levels amounting to 61.87 ± 12.30 fmol/mg protein ($P=0.01$) (Fig. 1). The progesterone receptor concentrations in the non-irradiated patients reached 29.90 ± 10.35 fmol/mg protein, while in irradiated tumours, the concentrations decreased to 9.14 ± 6.70 fmol/mg protein ($P=0.001$). These differences were also confirmed when only the steroid-receptor-positive tumours were compared (Fig. 2).

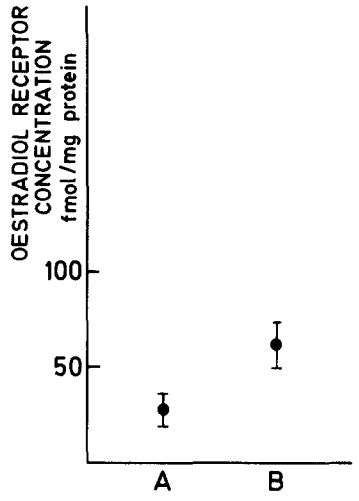

Fig. 1. Oestradiol receptor concentrations in tumours of (A) radiotherapy-pretreated postmenopausal patients (n=22) *and (B) non-irradiated postmenopausal patients* (n=32). P *values are calculated according to the rank-sum test.*

Irradiation also reduced the number of oestradiol-receptor-positive tumours with titer values above 5 fmol/mg protein from 75 to 54% ($P=0.05$).

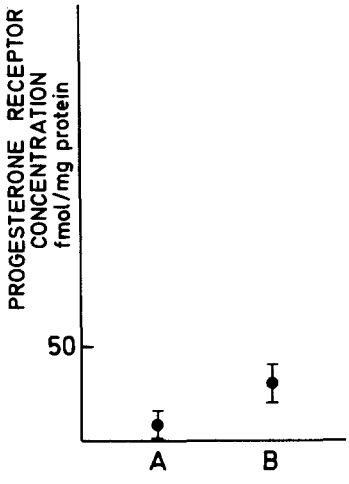

Fig. 2. Progesterone receptor concentrations in tumours of (A) radiotherapy-pretreated postmenopausal patients (n=14). *and (B) non-irradiated postmenopausal patients* (n=27). P *values are calculated according to the rank-sum test.*

Prebioptic radiotherapy of 8 G ray added to a presurgical 20 G ray irradiation significantly reduced the oestradiol receptor concentrations from 48.33 ± 4.2 fmol/mg protein to 26.97 ± 7.50 fmol/mg protein ($P=0.01$) when receptor-positive tumours were compared. These effects were even more pronounced on the progesterone receptor concentrations

where application of an additional 1 gray of irradiation induced a reduction of 4.67 fmol/mg protein.

DISCUSSION

This study was initiated to analyse the effects of presurgical radiotherapy on the steroid receptor concentrations in mammary carcinoma. Radiation therapy was carried out on 44 out of a total of 97 tumours analyzed. The tumours were considered to contain steroid receptors if the concentrations measured exceeded 5 fmol/mg protein, which is considered as the lowest value limit at which significant differences can be observed. The number of oestrogen-receptor-positive tumours increase drastically above the age of 70, compared to the age groups from 50 to 70 and below 50. This seems to indicate that these differences may not only be attributed to the menopausal state but are also probably due to other yet unknown factors related to age [5, 11, 26]. The possibility is not excluded that although only the squirrous differentiated tumours seem to appear more frequently with increasing age, the tumour type somehow may change with age. Indeed it is known that the degree of elastosis which may be related to the oestradiol receptor levels influences the prognosis. This seems to be indicated by the fact that although the oestradiol receptor titers are generally unmodified with age, the number of oestrogen-positive tumours is significantly higher in the older age group. However, as age has no effect on the progesterone receptors, this argument loses its impact when considering the correlation between oestradiol receptor and progesterone receptor function during the priming effect [27]. Other possible parameters to be considered which affect the steroid receptor levels are the oestradiol concentration in blood plasma and in the tumour tissue as well as possible endogenous steroid production by the tumour cells [2, 17, 28, 29]. As the average age of the postmenopausal patients in the irradiated and non-irradiated groups are identical, the analysis of the receptor concentrations in tumours from these two groups may be considered to be unaffected by possible other age factors.

Although in some instances differences in steroid receptor concentration have been linked to specific histological structures, these data remain uncertain and conclusive evidence has not yet been obtained [3, 5, 15, 30–34]. Staging according to the TNM classification does not allow any correlation to be made between tumour size or invasion and steroid receptor levels [35].

Irradiation essentially reduces the progesterone receptor concentrations to an even greater extent than the oestradiol receptor concentrations [36]. With DMBA-induced mammary carcinomas in rats, analogous effects on the steroid receptor concentrations are observed with a 50% reduction in receptor concentrations at 5–10 days and a maximal reduction at 30 days after irradiation with 20 gray [37]. However, these inhibitory effects of irradiation have not been observed when *in vitro* irradiation effects are analyzed [20].

The prebioptic irradiation dose of 8 G ray amounting to 40% of the usual presurgical dose, unmistakably emphasizes the reduction of steroid receptor levels. As even discrete irradiation drastically affects the steroid receptor concentrations, the interpretation of the calculated receptor values should be made taking into account any possible previous exposures to ionizing irradiation.

Acknowledgement—The authors are indebted to the Belgian National Foundation for Medical Research (F.G.W.O.) for a grant to the laboratory.

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