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Papers

Oestrogen and Progesterone Receptor Status in Bone Biopsy Specimens from Patients with Breast Cancer

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Of 16 breast cancer patients with histologically proven, tumour-infiltrating biopsy specimens most had low ER and PR values; the ER and PR contents varied between 0 and 135 and 0 and 44 fmol/mg protein, respectively. With the conventional clinical threshold of 10 fmol/mg protein, 8 specimens (50%) were ER−PR−, 4 (25%) ER−PR+, 3 (19%) ER+PR+ and 1 (6%) ER+PR−. ER levels were significantly lower in the tumoral bone lesion compared with the primary tumour. For 15 patients with negative biopsies and without endocrine treatment, ER and PR concentrations were quantifiable (2 fmol/mg protein or more) in 9 (60%) and 11 cases (73%), respectively. 8 of 9 patients over 55 (89%) were ER+ (2 fmol/mg protein or more). Conversely, for patients under 55, 1 of 6 (17%) was ER+ ($P < 0.001$). Results for PR were similar. These data strongly suggest that steroid receptors are present in healthy bone tissue.
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INTRODUCTION

THE RESPONSE rate to endocrine therapy in breast cancer is higher for oestradiol (ER) and progesterone receptor (PR) positive tumours than for receptor negative tumours [1, 2]. Analysis of the response rates in 51 clinical trials in which tamoxifen was the major endocrine treatment revealed that bone metastases are among the least responsive sites [3]. This can be partly explained by the difficulty of evaluating the response of bone lesions. Moreover, the steroid receptor content tends to differ in the primary tumour and in synchronous [4, 5] and asynchronous distant sites [6–11]. Most information about ER and PR concentrations in metastatic sites concerns soft tissue or nodal metastases [4–7, 9]. Overall, the percentage of receptor positive estimations is lower in metastatic tissue than in primary tumours. Although bone metastases are one of the most frequent secondary sites of breast cancer, we are aware of only two reports on steroid receptor measurements in bone metastases of breast carcinoma [12, 13]. Furthermore, because of the limited quantity of biological material generally available from bone biopsies, only ERs were measured in these studies. We have used the micromethod we developed for measurement of both ER and PR in small tumour fragments [14] to obtain additional data on these hormone receptors in bone biopsy samples from 38 female patients.

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MATERIAL AND METHODS

The characteristics of the 38 female patients summarised in Tables 1 and 2 were obtained from the clinical records (age, TNM classification [15], ER and PR levels in primary tumours and bone biopsies, initial and palliative treatments, time between ER and PR measurement in primary tumour and bone sites, response to treatment). Bone metastasis was suspected on the basis of clinical, radiological and/or scintigraphic findings. Patients were separated into two groups: those with histologically proven tumour-infiltrating bone biopsy specimens (positive biopsies) and those with tumour-free bone biopsy specimens as proven by histological examination (negative biopsies). The

Table 1. Patients with histologically proven tumour-infiltrating bone biopsy specimens (positive biopsies)

Patient (age)	TNM	Receptors in primary (fmol/mg protein) ER	PR	Initial treatment*	Receptors in bone (fmol/mg protein) ER	PR	Interval (yr)	Protein concentration (mg/ml)	DNA concentration (mg/g tissue)	Palliative treatment	Response
Not receiving endocrine therapy											
1 (82)	—	—	—	—	4	14	—	1.6	3,54	H	NR
2 (62)	T4N0M1	10	18	—	0	9	0	1.0	1.25	CH, H	PR (14 mo)
3 (61)	T2N0M0	1	4	S, CH	0	4	0.75	1.4	ND	CH	NR
4 (40)	T3N1M0	113	115	CH, H	135	12	5	2.9	ND	H	NR
5 (46)	T2N1M0	5	6	S, CH	0	17	2	1.5	2.56	H	NR
6 (58)	T4N0M1	95	10	CH	35	0	1.5	1.5	1.67	H	NR
7 (54)	—	—	—	CH, H	5	5	—	5.0	ND	H	NE
8 (77)	T2N1M0	24	0	S, RT	4	11	2	3.2	ND	RT	NE
9 (64)	T2N1M1	5	293	S, CH, H	3	1	3	4.7	1.96	RT	NE
10 (47)	T2N0M0	16	170	S, CH, H, RT	0	0	5	2.3	1.05	H	PR (15 mo)
11 (69)	—	23	36	S, CH, RT	0	0	4	5.7	3.64	CH, RT	NR
12 (71)	T2N1M0	335	30	S, H	0	0	7	4.6	ND	CH, H	NR
13 (63)	—	—	—	S, H, RT	0	3	—	4.8	ND	RT	PR (13 mo)
14 (76)	T4N0M0	—	—	CH, RT	18	44	—	0.5	2.33	CH	CR (24 mo)
Receiving endocrine therapy											
15 (71)	—	—	—	H*	0	44	—	1.4	8.86	H, RT	NE
16 (73)	T4N0M0	20	—	S, H*	24	10	—	1.0	2.08	H	PR (24 mo)

S = surgery, RT = radiotherapy, CH = chemotherapy, H = hormonotherapy and H* = tamoxifen-containing hormonotherapy.
NR = no response, PR = partial response, CR = complete response and NE = not evaluable.
ND = not determined.

Table 2. Patients with tumour-free bone biopsy specimens (negative biopsies)

Patient (age)	TNM	Receptors in primary (fmol/mg protein) ER	PR	Initial treatment	Receptors in bone (fmol/mg protein) ER	PR	Interval (yr)	Protein concentration (mg/ml)	DNA concentration (mg/g tissue)
Not receiving endocrine therapy									
17 (73)	T3N1AM0	—	—	S, RT	50	4	—	1.4	1.09
18 (77)	RENAL CA	—	—	S, RT	7	11	—	1.6	5.63
19 (55)	T3N1M0	—	—	S, RT	1	6	—	2.5	3.00
20 (61)	T2N0M0	93	14	S, RT	8	25	2	1.2	3.78
21 (72)	—	—	—	S, H, RT	0	4	—	2.3	5.50
22 (57)	—	150	125	S, H, RT	4	6	1	1.6	2.50
23 (70)	—	—	—	S, RT	4	2	—	3.6	2.47
24 (58)	—	7	12	RT	2	4	0	2.2	4.19
25 (45)	—	—	—	CH, H, RT	0	0	—	3.0	2.38
26 (77)	—	—	—	S, H	11	4	—	1.4	15.08
27 (52)	T3N0M0	—	—	S, CH, RT	4	0	—	1.1	2.40
28 (52)	T2N1M0	55	305	S, CH, H	0	0	2.5	1.9	5.60
29 (38)	—	13	15	S	0	0	2	1.3	6.07
30 (62)	T2N0M0	—	—	S	16	6	—	1.3	4.14
31 (40)	T2N1M0	—	—	S, CH, H, RT	0	16	—	1.1	1.00
Receiving endocrine therapy									
32 (68)	—	90	70	S, CH, H*, RT	5	0	3	1.3	9.60
33 (63)	T2N0M0	70	80	S, CH, H*, RT	6	0	4	1.2	10.30
34 (72)	—	45	24	H*, RT	0	0	1.5	1.1	2.33
35 (60)	—	—	—	S, CH, RT, H*	2	36	—	2.1	ND
36 (63)	—	—	—	CH, RT, H*	0	4	—	1.1	ND
37 (56)	T1N0M0	—	—	S, H*	8	15	—	1.8	1.58
38 (63)	—	80	46	S, H*	5	0	—	1.4	4.33

mean age of the 16 patients in the positive biopsy group was 63.4 (range 40–82). The negative biopsy group had 22 patients with a mean age of 61.0 (40–77); the negative biopsy group included 21 cases of breast carcinoma and 1 renal carcinoma.

Because various investigators [6, 8, 11, 16] have suggested that endocrine therapy modifies ER and PR levels, we analysed separately those patients who had been on endocrine treatment for at least 2 weeks before bone biopsy and those patients who had never received any hormone therapy or who had stopped such treatment at least 4 weeks before the biopsy. Bone biopsies for all patients were done on the anterior iliac crest; specimens were cut into two parts, one for histological examination and the other for assay of ER and PR. The average weight of the biopsy material used to measure steroid receptors was about 40 mg (10–100).

ER and PR levels in the primary tumours were measured by the dextran-coated charcoal (DCC) technique [17]. The ER and PR contents of the bone biopsy specimens were assayed as described [14]. In brief, this method involves cytosol incubation with the DCC method in the simultaneous presence of 3H-estradiol and 3H-ORG-2058, extraction of the steroids bound to the receptor by precipitation with ethanol/trichloracetic acid and high-pressure liquid chromatography of the eluted fractions collected and counting of the radioactivity. A highly significant correlation was obtained between the two methods for ER, $r = 0.966$ ($P < 0.001$) and for PR, $r = 0.975$ ($P < 0.001$).

Two thresholds were used in the study. The analytical threshold for positivity was set at 2 fmol/mg protein while the clinical threshold was 10 fmol/mg protein (when performed on the primary tumour).

RESULTS

Patients with positive biopsies are shown in Table 1. The cystol protein content varied between 0.5 and 5.7 mg/ml (median = 1.95); DNA content varied between 1.05 and 8.86 mg/g tissue (2.20). Most ER and PR values were low; ER and PR contents varied between 0 and 135 and 0 and 44 fmol/mg protein, respectively. With the classical therapeutic threshold of 10 fmol/mg protein, 3 biopsies (19%) were ER+PR+, 1 (6%) was ER+PR−, 4 (25%) were ER−PR+ and 8 (50%) were ER−PR−.

Steroid receptor levels in the primary tumour and in the positive bone biopsy specimens were compared in 10 cases; in all cases except 1, the interval between the two successive measurements ranged between 9 months and 7 years. As shown by Fig. 1, ER levels were significantly lower in tumoral bone lesions than in the primary tumours. For PR, only a trend was noted ($P = 0.07$). A similar drop in the ER concentration was observed in patients whether treated with hormonotherapy or not during this time. Hormonotherapy greatly affected the course of PR levels.

Table 2 presents data for patients whose bone biopsies revealed no histological evidence of tumoral involvement. Considering only those patients whose biopsies were not done during endocrine treatment ($n = 15$), ER and PR levels ranged between 0 and 50 and 0 and 25 fmol/mg protein, respectively. ER and PR concentrations were quantifiable (2 fmol/mg protein or higher) in 9 cases (60%) and 11 cases (73%), respectively. Taking patient age into account when analysing the steroid receptor distribution in bone, 8 of 9 patients over 55 (89%) were ER+. For patients under 55, 1 of 6 (17%) was ER+ (χ^2 test, $P < 0.001$). Observations for PR were similar: 9 of 9 patients over 55 (100%) were PR+; 2 of 6 patients under 55 (34%) were PR+ ($P < 0.001$).

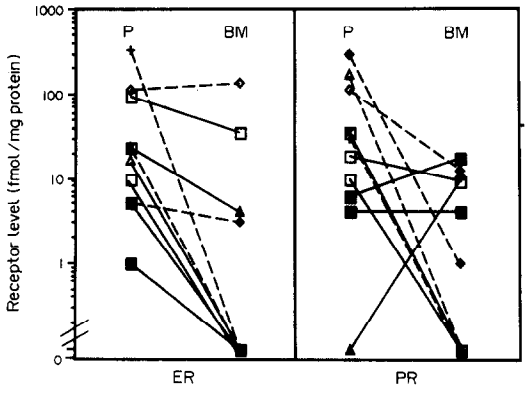

Fig. 1. Changes in ER and PR levels between primary tumour (P) and bone metastases (BM). — = interval therapy not including hormonotherapy and ---- = interval therapy including hormonotherapy. Wilcoxon matched pairs sign-rank test: ER, $P = 0.04$ and PR, $P = 0.07$.

In those patients ($n = 7$) whose bone biopsies were done while they were on hormone therapy (tamoxifen), ER and PR were assayable in 5 cases (71%) and 3 cases (43%), respectively. There was no significant difference in the distribution of ER+PR+ biopsy specimens between the two subsets of patients (treated or not by endocrine therapy).

DISCUSSION

ER and PR assays in primary breast cancer have proven their clinical utility [18, 19]. Like other investigators [4–11], we [16] have studied the course of ER and PR status in the interval between diagnosis of the primary tumour and diagnosis of distant sites of involvement. Most studies have concerned lymph nodes or cutaneous metastases; very few have dealt with steroid receptor content in bone lesions [12, 13]. These two studies only included ER assays. Data have not been available on the quantitative measurement of both ER and PR in bone metastases of breast cancer.

We used a micromethod developed in our laboratory to measure ER and PR in bone biopsy samples from 38 patients with clinical, radiological and/or scintigraphic suspicion of bone metastases. Enough material was available in all cases for measurement of both receptors. Another analytical approach would have been immunochemistry which allows identification of the nature of cells presenting steroid receptors but gives only a semiquantitive evaluation of these receptors. Systematic histological examination of each bone biopsy specimen allowed identification of two groups: specimens with and without histological evidence of tumoral involvement. Both the ER and PR levels of tumour-infiltrating biopsy samples were low: only 17% were ER+PR+ (threshold 10 fmol/mg protein), and only 25% were positive based only on the ER level. This last frequency is similar to that reported by other investigators: 32% by Manegold *et al.* [12] and 39% by Wortman *et al.* [13]. Our results were not significantly changed when the 2 patients who were under endocrine therapy were excluded from analysis. Comparison of the initial steroid receptor values in the primary tumour and those in the bone biopsy specimen revealed a significant decrease in both ER and PR. This difference between the primary and distant tumour concurs with the findings of several other groups [4–11]. It is unlikely that an analytical factor could explain our observations, because the two methods correlate well for both high and low ER and PR values [14]. Controversy still exists about the influence of hormone therapy on the modification in

ER/PR status when administered in the time between diagnosis of the primary and metastatic tumours. For Harland *et al.* [7], exclusion of patients who had received some form of hormone treatment in the interval between biopsies did not reduce the proportion of cases in which variation occurred. By contrast, others have reported that endocrine therapy had a strong influence on modification of the ER [6, 8] and PR [11] status between detection of the primary tumour and subsequent locoregional or distant tumours. In this study, the administration of hormone therapy between measurements did not modify the decrease observed in ER and PR levels between the primary breast tumour and secondary bone metastasis (Fig. 1). Our limited number of cases precluded analysis of the relation between the ER and PR levels in bone lesions and response to palliative hormonal treatment. However, the low ER and PR levels in bone metastases are compatible with their low response rate to endocrine therapy [3] and the adverse prognostic value of low steroid receptor levels [19].

Although both oestradiol and progesterone are intimately involved in the regulation of bone metabolism [20, 21], bone cells were generally not thought to contain steroid receptors until recently [22, 23]. In 1988, Kaplan *et al.* [24] described ERs in bone from a patient with McCune–Albright syndrome. More convincing evidence was provided by Eriksen *et al.* [25], who reported human osteoblast-like cells exhibiting steroid-specific saturable and temperature-dependent nuclear binding. We thus felt it interesting to analyse separately those patients whose biopsy specimens were histologically tumour-free. Particular attention was paid to patients who were not under endocrine treatment, the bone of this population being considered physiologically normal. In this group, positive ER and PR levels were obtained in 60% and 73%, respectively. This finding complements the previous work [24, 25]. In our study, the distribution of steroid receptors in bone differed as a function of patient age: ER and PR levels were significantly lower in women under 55, and *vice versa*. This finding may be compatible with a direct physiological role of steroids in bone via specific endogenous receptors and the existence of a regulatory compensatory mechanism of these levels as a function of age (physiological oestradiol levels are lower after the menopause). This point warrants investigation in a larger set of patients. Administration of tamoxifen has been reported to preserve bone mass during treatment [26]. The possibility that tamoxifen may act as a partial agonist of oestrogen [27] makes this effect pharmacologically coherent in the light of our results.

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