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Steroid Receptor Enhancement by Natural Interferon-β in Advanced Breast Cancer

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In the current study we investigated the effect of two different doses of natural interferon-beta (IFN-β) on steroid hormone receptors in 45 patients with advanced breast cancer. IFN-β seems to regulate the receptor mechanisms, inducing in cutaneous metastases an increase of oestrogen and progesterone receptors. Moreover, using IFN-β and tamoxifen as a combined therapy in 23 receptor-positive patients, no negative interference of the two drugs was observed and no relevant side-effects due to the treatment were noticed. The moduation of steroid receptor content by IFN-β in advanced breast cancer might represent an interesting way to ameliorate the clinical responsiveness to anti-oestrogens.
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INTRODUCTION

BOTH QUALITATIVE and quantitative determinations of oestrogen and progesterone receptors (ER, PR) have been shown to be strongly related to hormonal responsiveness in breast cancer [1–3]. In particular, it is well established that response rate to endocrine treatment in ER/PR positive patients is improved with increasing receptor concentrations [4]. As far as anti-oestrogen therapy is concerned, it is known that in patients with tumours containing ER and PR the response rate to tamoxifen in advanced disease is about 10-fold higher than in those without receptors [5], even if other mechanisms influencing tumour growth regulation cannot be excluded. Loss of receptor activity may be associated with the progression from hormone-sensitive to insensitive status [6]. Moreover, receptor expression is linked to cell differentiation. In fact, in poorly differentiated breast cancer cells receptor content is lower and this is related to a more aggressive course of the disease [7]. Interferon-alpha (IFN-α) was reported to increase ER when added directly to breast or uterine cell homogenates or to human breast cancer cell cultures [8–10]. Both natural IFN-β and recombinant IFN-α have been shown to enhance ER and PR, evaluated by a whole cell assay, in CG-5 mammary cancer cells [11, 12]. Nevertheless, some authors claimed to find no variation of receptor expression in mammary cancer cells, treated with IFN [13–16]. Different culture conditions, types and concentrations of IFN used could explain these controversial data. On the contrary, there is a general agreement on the IFN capability of sensitising a variety of ER-positive human breast cancer cell lines to the antiproliferative effect of tamoxifen [9, 10, 13, 15, 16]. IFN was also reported to increase ER and PR in primary breast cancer [17, 18] and in cutaneous metastases of few patients affected by advanced mammary tumours [19].

In this paper, we investigated the action of IFN-β on ER and PR level in 45 postmenopausal patients with metastatic breast cancer. Moreover, we report some preliminary clinical data obtained using a combination of IFN-β with tamoxifen.

PATIENTS AND METHODS

A total number of 45 postmenopausal patients with metastatic breast carcinoma and soft tissue metastases were entered into the study. Admission criteria included: (1) multiple soft tissue biopsiable, histologically-proven metastatic breast cancer; (2) ECOG performance status <3; (3) life expectance > 12 weeks; (4) age ≥45 ≤75 years; (5) ER and PR present or absent for admission to IFN-β treatment, positive (≥10 fmol/mg protein) for admission to tamoxifen treatment; (6) informed consent. The exclusion criteria were: (1) previous treatment for advanced disease; (2) adjuvant treatment not stopped at least 1 year before; (3) history of a second tumour except basal cell skin cancer radically treated; (4) brain metastases prior to the entry; (5) impaired renal function (creatinine >150 μmol/l) and liver function (bilirubin >20 μmol/l); (6) impaired haematological function (white blood cells $<4 \times 10^9$/l; granulocytes $<2 \times 10^9$/l; platelets $<100 \times 10^9$/l); (7) history or current evidence of clinically significant cardiovascular and neurological disease. During IFN-β treatment acetyl salicylic acid, non steroidal anti-inflammatory drugs and corticosteroids were avoided.

Eligible patients were randomly assigned to the following treatment groups:

A = 2×10^6 U of IFN-β (Frone®, Industria Farmaceutica Serono, Italy) intramuscularly three times/week for 2 weeks

B = 6×10^6 U of IFN-β (Frone®,) intramuscularly 3 times/week for 2 weeks

ER and PR determination was performed immediately before

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and the day after this course of IFN-β treatment on tissue taken from cutaneous metastases.

Patients whose receptor level was ≥10 fmol/mg protein were enrolled in a pilot study to verify the safety and the effectiveness of a combination of IFN-β/tamoxifen. Tamoxifen (Nolvadex®, ICI-Pharma, Italy) was administered after the second biopsy at a dose of 30 mg/day by mouth for 12 weeks. IFN-β was given three times a week during the 7th and the 11th week from the beginning of the study at the dose of 2×10^6 U and 6×10^6 U, respectively for groups A and B.

At the end of the administration of IFN-β/tamoxifen combination clinical response was assessed according to WHO criteria. Through the duration of the treatment each patient was monitored for possible clinical adverse reactions.

ER and PR determination was carried out at the laboratory of histology and embryology of the Catholic University of Rome, which is included in the Italian Committee for Standardization of Methods of Receptor Measurements in Endocrine Tumours.

Steroid receptor assay was performed using a single point saturation analysis; due to the small amount of tissue available only in a few cases was a Scatchard analysis carried out.

Briefly, aliquots of cytosol obtained from metastatic tissue (histologically confirmed) were incubated with 5 nmol/l [^{3}H]oestradiol in the presence or absence of a 200-fold excess of diethylstilbestrol for ER determination and with 5 nmol/l [^{3}H]ORG 2058 in the presence or absence of a 200-fold excess of unlabelled ORG 2058 for PR determination. If Scatchard analysis was carried out, the concentration of labelled hormones used ranged from 0.1 to 5 nmol/l.

The unbound fraction of ligands was separated by dextran-charcoal technique.

Protein content of the cytosol was evaluated by the Bradford's method. Results were expressed as fmol/mg protein and no cut-off of positivity was chosen.

Statistical analysis

To assess the homogeneity of the clinical features of the patients enrolled in the two groups of treatment, the χ^2 test for contingency tables was employed. When frequencies were ≤1, the frequency data were grouped in a smaller number of categories. The 95% confidence interval (C.I.) was reported to indicate the true interval of observed proportions.

Comparison of receptor levels before and after IFN-β administration was performed by the Wilcoxon's signed rank test; the difference in basal and post-treatment receptor content between the two groups of treatment was assessed by the Mann–Whitney U test.

The analysis of correlation between ER and PR before and after IFN-β was carried out by the Spearman rank correlation test. Linear regression analysis (least squares method) was performed on both log transformed and untransformed data. The statistical significance of the regression was determined by the analysis of variance to separate the variability due to the regression from the residual variability. The determination

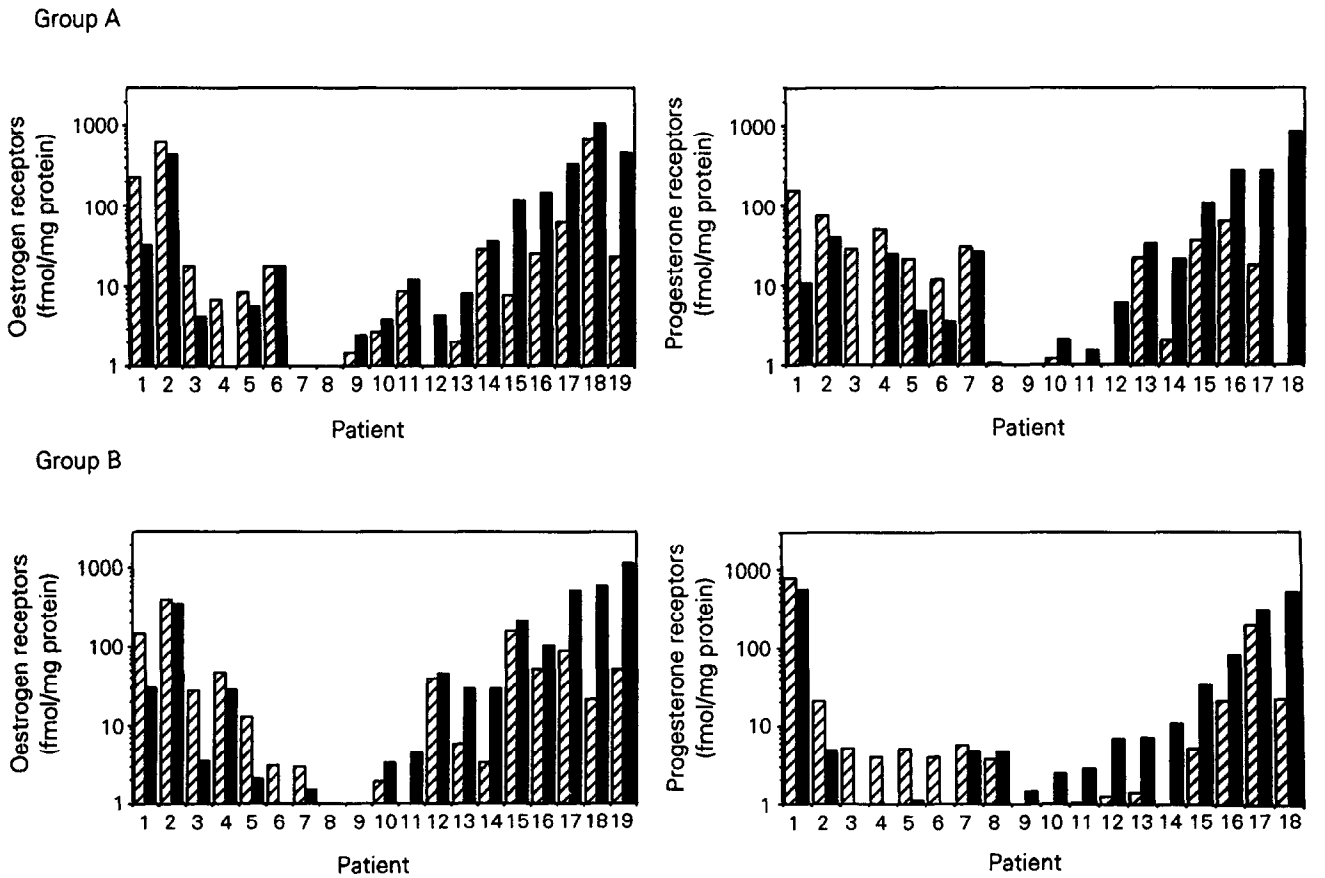

Fig. 1. Oestrogen and progesterone receptor content (fmol/mg protein) in cutaneous metastases of breast cancer patients before (▨) and after (■) IFN-β treatment (upper panels: group A; lower panels: group B). Receptor values <1 fmol/mg protein are plotted as 1. Values from each patient are ordered by the differences due to the treatment.

coefficient (r^2) indicates the proportion of variance of y explained by linear regression on x.

RESULTS

45 postmenopausal patients with advanced breast cancer were entered in our study. Nevertheless, 7 cases were not evaluable because of the following reasons: patient refusing a second biopsy, sample insufficient for receptor assay or kept at inadequate temperature during the transportation to the laboratory. The patients randomised to each of the two groups were comparable with regard to age, performance status, disease-free interval, previous treatment and dominant metastatic site (χ^2 test).

In Fig. 1 ER and PR levels before and after treatment with the two different doses of IFN-β are reported. There is a large variability of basal receptor levels, which are in the ranges described for metastatic tissue [20, 21]. The same variability in ER and PR content after IFN-β treatment was found.

In a relevant percentage of patients there was an increase in ER (group A: 68.4%, 95% C.I. 43.4–87.4; group B: 52.6%, 95% C.I. 26.9–75.6) and PR (group A: 50.0%, 95% C.I. 26.0–74.0; group B: 61.1%, C.I. 35.7–82.7). Both ER and PR increased in 38.9% (95% C.I. 17.3–64.3) in group A and in 33.3% of patients (95% C.I. 13.3–59.0) in group B. In both group A and B a trend in favour of receptor enhancement after IFN-β administration has been observed. Nevertheless, probably due to the limited number of patients, statistical significance was not reached (Wilcoxon's signed rank test).

On the other hand, the presence of a correlation between ER and PR after IFN-β treatment both in group A ($P<0.01$) and in group B ($P<0.001$) was observed using the Spearman correlation analysis. No correlation was present before IFN-β administration.

In addition, linear regression analysis of the log transformed data yielded interesting results. A strong correlation was noted between ER and PR levels only after IFN-β treatment in both groups A and B. This relationship is also confirmed by a highly significant F-ratio (Fig. 2).

On the other hand, the correlation was still present if the analysis was carried out on untransformed data (not shown).

As no statistically significant difference was demonstrable between the two groups either in basal receptor level, or in receptor response to the two different doses of IFN-β employed (Mann–Whitney U test), pooling data obtained from both dosage groups appeared justified. After that, a statistical significance was found between receptor levels before and after IFN-β treatment (ER: $P<0.01$; PR: $P<0.05$, Wilcoxon's signed rank test).

Linear regression analysis performed on pooled receptor data confirmed the trend observed in separated groups (not shown).

When Scatchard analysis was feasible (five assays) the K_d of ER were in the range described in the literature [22] both before (0.08–0.147 nmol/l) and after (0.08–0.84 nmol/l) IFN-β treatment, suggesting that the drug did not modify receptor affinity.

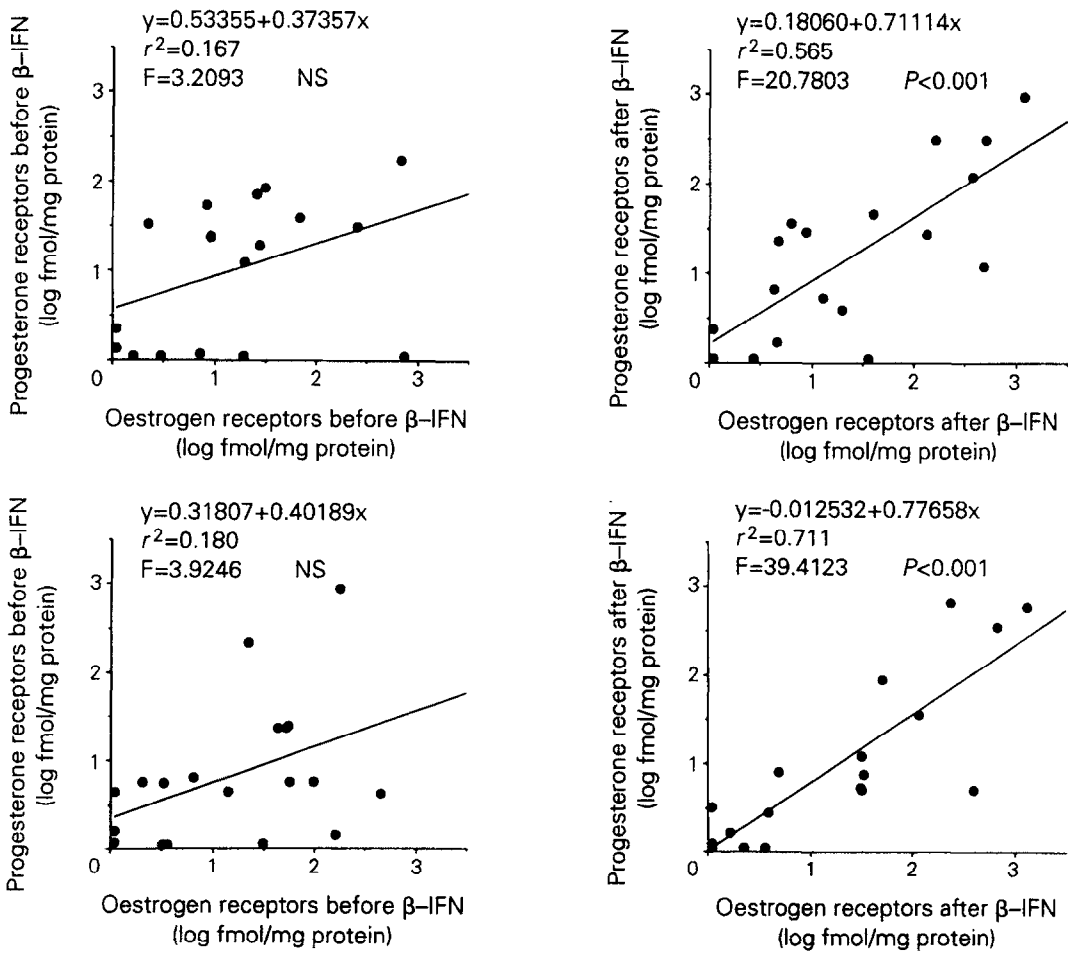

Fig. 2. Relationship between ER and PR concentration in group A (upper panels) and B (lower panels), before (left) and after (right) IFN-β administration. Receptor values <1 fmol/mg protein were considered equal to 1. Data were log transformed.

Table 1. Clinical features of evaluable patients treated with IFN-β and tamoxifen

Characteristic		Group A No. of patients 12	Group B 11
Age (years)	Median	71.5	64
	Range	49–75	50–74
Performance status (ECOG)			
0		6	4
1		5	6
2		1	1
Menopausal status			
Natural/surgery		11/1	9/2
from (years)	Median	22	14
	Range	3–33	1–33
Disease-free interval (months)	Median	49.59	41.03
	Range	13.53–133.67	0–92.17
Prior therapy			
A–Surgery		12	11
B–Radiotherapy		3	3
C–Chemotherapy		3	7
D–Hormonotherapy		2	2
Involved organ sites			
Soft tissues		8	7
Soft tissues + bone		2	3
Soft tissues + viscera ± bone		2	1

Table 3. Side effects recorded during treatment

Side-effect		Group A No. of patients 22	Group B (% of cases)* 23
Fever	<38°C	3 (13.64)	8 (34.78)
	>38°C	1 (4.54)	1 (4.35)
Shiver	Grade I	3 (13.64)	2 (8.6)
Asthenia	Grade I	3 (13.64)	2 (8.69)
	Grade II	1 (4.54)	2 (8.69)
Arthralgia	Grade I	1 (4.76)	1 (4.35)
Leukopenia	Grade I	1 (4.54)	1 (4.35)
	Grade II	1 (4.54)	1 (4.35)
Somnolence	Grade II	0	1 (4.35)
Itching	Grade II	0	1 (4.35)
Hypertension	Grade II	1 (4.54)	0
Transaminases	Grade I	0	1 (4.35)
	Grade II	0	1 (4.35)
	Grade IV	0	1 (4.35)
γ-GT	Grade I	0	2 (8.69)
	Grade IV	0	1 (4.35)
Lactate dehydrogenase	Grade III	0	1 (4.35)
Alkaline phosphatase	Grade I	2 (9.09)	2 (8.69)

*Treated with IFN-β for at least 2 weeks.

The number of evaluable cases did not allow any kind of stratification of receptor values according to usually employed histopathological and clinical features of patients.

24 out of 38 evaluable patients, showing ER and PR levels ≥10 fmol/mg protein, were treated with IFN-β/tamoxifen combination after the first IFN-β course. 1 patient in group B refused to continue the tamoxifen treatment. No significant differences in clinical features of evaluable patients in the two groups were found (Table 1, χ^2 test).

The observed response to treatment is shown in Table 2. The overall response rate in the group A was 41.67% (95% C.I. 15.17–72.33) and 36.36% (95% C.I. 10.93–69.21) in the group B. The median duration of response was 31 weeks (range 29.7–43.3) in group A and 19.4 weeks (range 13.3–50.0) in group B. The median time to treatment failure was 15.5 weeks (range 6.1–56.9) in group A and 22 weeks (range 3.1–64.4) in group B. No relevant conclusions can be drawn from this small series of patients on the efficacy of the IFN-β/tamoxifen combination. However, the observed response rate is in the range of that described for tamoxifen-treated patients with ER-positive tumours [28].

Table 2. Clinical responses observed after the IFN-β/tamoxifen treatment

	Group A no. of patients 12	Group B 11
Complete response	1	1
Partial response	4	3
No change	3	4
Progressive disease	4	3

45 patients were evaluable for side-effects. In group A, 198 doses of IFN-β were administered to 22 patients. No side-effects were observed in 12/22 patients (54.55%), who received as a whole 102 doses of IFN-β (51.52%). 10/22 patients (45.45%) suffered mild side-effects (Table 3). In group B, 210 doses of IFN-β were administered to 23 patients. No side-effects were observed in 12/23 patients (52.17%), who received as a whole 108 doses of IFN-β (51.43%). 11/23 patients (47.83%) suffered mild side-effects (Table 3). In group B the only adverse event seen in a higher percentage of cases with respect to group A was a fever lower than 38°C.

No dose reduction or treatment interruption was required for the relevance of side-effects. In addition, other sporadic modifications in some haematological and/or biochemical as well as clinical parameters were observed, even if a correlation with IFN-β treatment was not clearly established (Table 3). No toxicity was attributable to tamoxifen.

DISCUSSION

On the basis of our data, it seems that IFN-β is able to modify ER and PR levels in cutaneous metastases of breast cancer patients. A trend in favour of receptor enhancement comes out at both the doses of IFN-β used. Pooling data obtained at two different dosage levels, a statistically significant difference due to the treatment was observed for both receptors.

The mechanism by which receptor enhancement is produced is, at present, unknown. The observed receptor increase is in agreement with IFN capability of inducing a number of genes and proteins, including some membrane receptor proteins [23–27] and could be due to an augmented synthesis. Moreover, in our opinion, IFN-β exerts a more complex influence on

receptor mechanism as suggested by the appearance of a correlation between ER and PR after IFN-β treatment. The observed reduction in ER and PR levels found in some cases in both groups could be explained on the basis of the heterogeneity of metastatic tissue [20], responsible for a different receptor expression and/or a different degree of sensitivity to interferon.

The augmented receptor content as well as other cellular modifications linked to the hormone-sensitive status, may be the result of the differentiative action of IFN. The percentage of clinical responses observed after the administration of IFN-β combined with tamoxifen is in agreement with the data reported in the literature on the effectiveness of hormonal therapy [28]. It is interesting to note that patients with objective response showed, at least in group B, a greater enhancement of both receptors due to IFN-β treatment with respect to patients in progression after tamoxifen treatment (data not shown).

Nevertheless, the limited number of patients who were treated in our study as well as in other studies [29] does not permit any conclusion concerning the clinical effectiveness of the combination IFN/tamoxifen. No relevant side-effects were induced by such a schedule of treatment. These findings are not in agreement with the data by Porzsolt *et al.* [14] and Macheledt *et al.* [29] who observed a greater toxicity using IFN-α plus tamoxifen in the treatment of advanced breast cancer, but reflect the toxicity profile shown by IFN-β in non-neoplastic disease (i.e. hepatitis).

Our results led us to start a randomised comparative trial, with the aim of evaluating the effectiveness and safety of IFN-β/tamoxifen combination vs. tamoxifen alone.

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