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Effect of Recombinant Human Interferon-alpha$_{2b}$ on Receptors for Steroid Hormones and Epidermal Growth Factor in Patients with Endometrial Cancer

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Interferons (IFNs) may modulate oestrogen (ER), progesterone (PR) and epidermal growth factor (EGFR) receptor expression *in vitro*. ER, PR and EGFR levels in tumour specimens taken from 13 patients with endometrial adenocarcinomas before and after 5 days' intramuscular treatment with 5 × 10^6 U per recombinant human leucocyte interferon-alpha$_{2b}$ (rh IFN-α_{2b}). After treatment, ER ($P < 0.01$) and PR ($P < 0.05$) levels were significantly increased with a simultaneous reduction of EGFR content ($P < 0.05$). Since the expression of ER and PR characterises more differentiated hormono-sensitive tumours, while EGFR are preferentially expressed in less differentiated tumours, the increase of steroid hormone receptor levels with the reduction of EGFR expression suggests that rh IFN-α_{2b} may induce endometrial cancer cell differentiation. Moreover, the decrease of EGFR levels may explain the antiproliferative effect of IFNs.
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INTRODUCTION

THE MECHANISM by which interferons (IFNs) inhibit the growth of malignant cells is not clear. Their antiproliferative activity could be the result of the prevention of DNA replication [1]; on the other hand the cytostatic effects could be derived from the cell-cycle changes produced by IFNs [2, 3]. In addition, IFNs might antagonise the cellular response to positive effectors, through an interaction with the biochemical pathways by which hormones or other growth factors control cell proliferation [1, 4]. IFNs can modulate the synthesis of proteins involved in growth regulation and differentiation [1, 5]. In hormone-sensitive tumours, natural beta-IFN increases the *in vitro* expression of oestrogen (ER) and progesterone (PR) receptors in human breast cancer cells [6] and in human endometrial explants [7]. Moreover, IFN-β enhances ER and PR in cutaneous metastases of patients with advanced disease [8, 9]. Recombinant humans (rh) alpha IFN has similar effects on human breast cancer cells *in vitro* [10] and in primary breast cancer [11]. Zoon *et al.* [12] demonstrated that IFN-α_2 modulates *in vitro* epidermal growth factor receptor (EGFR) expression and its binding affinity. This may be extremely relevant since the growth of breast and endometrial cancer cells is regulated by EGF through the interaction with a specific transmembrane receptor [13, 14].

In this study we looked for *in vivo* modifications of ER, PR and EGFR in patients with endometrial cancer undergoing a short period of systemic treatment with rh IFN-α_{2b}.

PATIENTS AND METHODS

This study included 13 postmenopausal women with endometrial adenocarcinomas. None of the patients had heart or metabolic disease that might contraindicate rh IFN-α_{2b} treatment. Tumours were well, moderately or poorly differentiated in 7, 4 and 2 patients, respectively.

7 days before surgery, patients underwent hysteroscopy and endometrial biopsy and were subsequently treated intramuscularly with 5 × 10^6 U per day leucocyte rh IFN-α_{2b} (Essex, Milan) for 5 consecutive days. At the time of surgery a representative tumour specimen from the same area of the biopsy was taken.

Tumour specimens, frozen on dry ice shortly after surgical or biopsy removal, were stored at −80°C. Tumour cytosol and membrane fractions were prepared [13]. Tumour specimens were minced and homogenised in ice-cold buffer, 25 mmol/l Tris, 1.5 mmol/l EDTA, 5 mmol/l NaN_3, 0.1% monothioglycerol and 20% glycerol (TENMG) with several bursts in an Ultra-Turrax. The homogenate was centrifuged at 7000 *g* for 20 min at 0°C. The supernatant containing the cytosol and membrane fraction was then centrifuged at 105 000 *g* for 75 min at 0°C. The resulting supernatant was drawn off for ER and PR analysis, and the pellet was assayed for EGFR. ER and PR were measured with a single-point saturation assay with 5 nmol/l of [2, 4, 6, 7-3H]oestradiol (3.4 GBq/mmol), and 5 nmol/l [3H]ORG-2058 (3.2 GBq/mmol) (Amersham International) as ligands, in the presence or absence of a 100 fold excess of diethylstiboestrol and ORG-2058 (SIGMA). Membrane pellets were resuspended in 25 mmol/l Tris, 1.5 mmol/l EDTA, 5 mmol NaN_3, 20% glycerol and 10 mmol/l $MgCl_2$ (TENG + $MgCl_2$). Aliquots of membrane (100 μl containing 300–500 μg protein) were incubated with ^{125}I-EGF (2.6 nmol/l)

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*Table 1. Effect of rh IFN-α_{2b} on receptors for steroid hormones and EGF in patients with endometrial cancer**

Case	Before treatment			After treatment		
	EGFR	ER	PR	EGFR	ER	PR
1	13.60	12.90	0	4.30	35.90	65.80
2	15.10	32.00	5.00	6.68	32.00	53.10
3	11.80	5.13	10.11	4.97	38.30	84.70
4	5.54	0	0	1.63	0	0
5	12.01	13.12	0	4.74	31.20	2.85
6	0	0	34.20	3.62	10.29	41.23
7	5.18	0	0	0.69	0	0
8	15.30	17.60	43.70	7.40	43.00	655.56
9	4.27	34.53	8.20	3.29	74.51	89.44
10	4.25	0	8.74	2.28	0	0
11	8.67	0	16.35	3.96	9.70	28.70
12	14.70	19.80	27.78	4.10	35.60	47.16
13	7.80	28.01	36.60	3.56	42.30	56.48
Median	8.67	12.90	8.74	3.96	32.00	47.16

*All fmol/mg protein.

in the presence (non-specific bound) or absence (total binding) of unlabelled EGF (1 mmol/l), for 16 h at room temperature in a final volume of 400 µl. Binding was stopped by the addition of 3 ml of 25 mmol/l Tris, 20% glycerol, 5 mmol/l NaN_3 and 0.1% bovine serum albumin. Pellets, obtained by centrifugation 2000 *g* for 20 min at 0°C, were counted in a gamma counter for 1 min. Results were expressed as fmol/mg membrane protein. The value of 10 fmol/mg protein and 1.5 fmol/mg protein were chosen as the cut-off of positivity for ER and PR and for EGFR respectively.

RESULTS

Overall, before treatment, ER ranged from 0 to 34.53 fmol/mg protein (median 12.90) and 7 out of 13 cases (54%) were positive (Table 1). PR ranged from 0 to 43.70 fmol/mg protein (median 8.74) and 6 cases (46%) were positive. After rh IFN-α_{2b} treatment, we observed a significant increase of ER median levels (32.00 fmol/mg protein), with values ranging from 0 to 74.51 fmol/mg protein ($P < 0.01$, Wilcoxon matched-pairs signed-rank test). The median increase of ER levels was 14.29 fmol/mg protein (range from 0 to 39.98). In particular, 2 out of 6 ER negative tumours became positive after rh IFN-α_{2b} exposure. After treatment we found significantly higher PR levels (median 47.16, range 0 to 655.56) ($P < 0.01$), with a median increase of 19.35 fmol/mg protein (−8.74 to 611.86), and 3 out of 7 PR negative tumours became positive.

All tumour specimens contained appreciable amounts of EGFR (median 8.67 fmol/mg protein; range 0 to 15.30). A significant decrease of EGFR levels was observed after rh IFN-α_{2b} treatment (median 3.96; range 0.69 to 7.40) ($P < 0.01$). We observed a significant decrease (from −1.97 to −10.26 fmoles/mg protein, median −7.05) in all but 1 case.

DISCUSSION

In human endometrial adenocarcinoma *in vivo* rh IFN-α_{2b} increases steroid hormone receptor content with a simultaneous decrease of EGFR expression. The increase of ER and PR expression in endometrial cancer is in agreement with results obtained *in vitro* on primary human endometrial cancer explants exposed to beta-IFN [7]. An increase of steroid hormone receptor content after IFN treatment has also been reported in human breast cancer cells both *in vivo* [8, 9] and *in vitro* [6, 10, 15] although some investigations failed to confirm these results [16, 17]. These conflicting data could be explained by the fact that different cell lines, culture conditions and concentrations of IFNs were used.

Previous *in vitro* studies showed that the exposure of MDBK cells [12] and Daudi cells [18] to IFN-d is followed by a modulation of EGFR and insulin receptors, suggesting that the IFN induced decrease of growth factor receptor expression may mediate IFN's antiproliferative activity *in vitro* [18]. Our results suggest that the IFN antiproliferative effects could also occur by a negative modulation of EGFR *in vivo*. EGFR are expressed in a high percentage of human endometrial adenocarcinomas, suggesting that EGF may play a role in the growth regulation of this neoplasm [14]. Moreover, EGF affects the growth of established human endometrial cancer cell lines [19].

Our findings are also an indirect confirmation of IFN's differentiating activity [20–22]. There is general agreement that the persistence of ER and PR expression in hormone-sensitive tumours characterises biologically more differentiated tumours associated with a better prognosis. On the other hand, in endometrial cancer, higher EGFR levels were found in less differentiated than in more differentiated tumours [14]. Moreover, EGFR expression has been frequently associated with a situation of cellular escape from the growth regulatory mechanisms that are typical of a still differentiated status [23]. Therefore, the increase of steroid hormone receptor content and the simultaneous reduction of EGFR levels indicates that rh IFN-α_{2b} promotes tumour cell differentiation in endometrial cancer *in vivo*.

From a clinical point of view, our results suggest that rh IFN-α_{2b} might enhance the responsiveness of endometrial cancer to antioestrogen or progestin agents. This hypothesis is in agreement with the reports demonstrating that IFNs increase the antiproliferative effects of antioestrogens in human breast cancer cell lines [16, 17, 24–26].

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Potentiation of Retinoid-induced Differentiation of HL-60 and U937 Cell Lines by Cytokines

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Retinoids varied in their capacity to induce differentiation in HL-60 cells in this order: Ro 13-6307, tretinoin, isotretinoin, acitretin and Ro 13-7410 (high to low). In contrast, retinoids lacking a polar carboxylic acid, such as temarotene and Ro 14-6113, were inactive. Various cytokines had no differentiation-inducing effect by themselves. However, the addition of cytokines to retinoids increased differentiation. Combined with tretinoin, cytokines increased differentiation in this order: interferon (IFN) gamma, granulocyte colony-stimulating factor, interleukin-1α (IL-1α), IL-4, tumour necrosis factor alpha and IFN-α. Combination of cytokines with isotretinoin, acitretin, Ro 13-7410, and Ro 13-6307 showed a similar pattern of potentiation to that of tretinoin. Temarotene or Ro 14-6113 did not induce differentiation, alone or with cytokines. Combinations of cytokines were not synergistic in the presence of retinoids; antagonism was even observed. In U937 cells, lower levels of differentiation-induction were observed. Potentiation of the differentiation-inducing effect of retinoids by cytokines might indicate a clinical differentiation therapy of tumours.
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INTRODUCTION

RETINOIDS ARE structurally related to vitamin A and are active, experimentally as well as clinically, in prevention and therapy of a variety of neoplastic diseases [1–3]. The mechanism of action is not understood and includes: inhibition of proliferation, induction of differentiation or immune stimulation. Complete clinical remissions have been described in acute promyelocytic leukaemia after treatment with tretinoin (all-trans retinoic acid) [4, 5]. Since tretinoin induces differentiation, these results have aroused new interest in differentiation therapy. The differentiating effect of tretinoin on HL-60 cells, a human promyelocytic leukaemia cell line, was first reported by Breitman *et al.* [6]. Differentiation of tumour cells has been described in a variety of cell lineages, including U937, a human histiocytic lymphoma [7], murine embryonal carcinoma [8], murine teratocarcinoma [9] and human neuroblastoma [10]. Retinoids modulate tumour

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