Interferon-β Can Induce Progesterone Receptors in Human Endometrial Adenocarcinoma

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BACKGROUND. The induction of estrogen and progesterone receptors (ER and PGR) has been reported in breast and endometrial cancer cells exposed to human fibroblast interferon-β (hIFN-β). Clinical verification of this finding might provide the rationale for new therapeutic approaches. This study was designed to evaluate whether clinical treatment with high doses of hIFN-β induced ER and PGR in patients with endometrial adenocarcinoma.

METHODS. Two biopsies were obtained, 1 before and 1 after hIFN-β treatment (3×10^6 i.m. every other day for 3 weeks) from 36 patients with endometrial adenocarcinoma. ER and PGR were determined with standard procedures using radiolabeled ligands.

RESULTS. hIFN-β treatment did not affect the proportion of ER-positive (i.e., >15 fmol/mg protein) or PGR-positive (i.e., >20 fmol/mg protein) cases. However, in patients with detectable ER and PGR at baseline, hIFN-β raised the levels. Using a 35% difference before and after therapy as a cut-off, 72 and 79% of cases had increases in ER and PGR, respectively. The difference was highly significant for PGR.

CONCLUSIONS. In patients with endometrial adenocarcinoma with undetectable ER or PGR, hIFN-β did not induce the expression of these receptors. When the receptors were present they were upregulated by hIFN-β. Whether this increase in receptor levels, particularly PGR, has therapeutic applications remains to be established. *Cancer* **1996; 78:448–53.** © *1996 American Cancer Society.*

KEYWORDS: hIFN-β, progesterone receptors, estrogen receptors, endometrial adenocarcinoma.

Estrogen (E) and progesterone (PG) receptors (R) in patients with breast and endometrial cancer predict survival and response to hormonal therapy.[1–8] Human leukocyte interferon-β (hIFN-β) raises ER and PGR levels in growing breast cancer cells in vitro and in vivo in biopsies from patients with advanced breast cancer.[9–11] An enhancement of PGR was reported in AE-7 endometrial cancer cells and an enhancement of both PGR and ER in human endometrial adenocarcinoma explants exposed to hIFN-β in vitro.[12,13] The effects of hIFN-β on ER and PGR may have therapeutic implications, particularly if induction is observed in tumors which do not otherwise express these receptors. These considerations prompted us to investigate whether hIFN-β increases ER and PGR in vivo in tumor biopsies from endometrial cancer patients.

PATIENTS AND METHODS

Patients and Treatment

Thirty-six newly diagnosed patients with histologically proven endometrial adenocarcinoma previously not treated with hormones or

TABLE 1
Clinical Stage and Histologic Grade of 36 Patients with Endometrial Adenocarcinoma

Histologic grade	No. of patients	%
1	9	25
2	17	47
3	10	28
Clinical stage		
I	26	72
II	4	11
III	6	17

chemotherapy entered the study. Tumor specimens were taken before and within 2 weeks of the end of hIFN-β treatment, frozen, and stored in liquid nitrogen until receptor assay.

All patients received 3×10^6 IU per day of hIFN-β i.m. (Frone, Serono, Italy) every other day for 3 weeks. Some biopsies were obtained from three additional patients (Nos. 37, 38 and 39) before hIFN-β but were not examined after treatment because they had received different doses from the others.

Table 1 shows the patients' clinical stage and histologic grade. Tumors were staged according to International Federation of Gynecology and Obstetrics (FIGO) criteria[14] and histologically graded as well (G_1), moderately (G_2), and poorly differentiated (G_3). The grade in the table was recorded by the pathologist examining the specimen obtained at hysterectomy. Comparison of the assessments of the grade of tumor differentiation at the first and second biopsy and after hIFN-β treatment showed good consistency; in approximately 70% of the cases the tumor grade of the two biopsies was the same. In about 30% of the cases the grade assessed after the first and second biopsies differed by one unit—lower in about 11% and higher in 19%.

The median age of the female patients was 71 years (range: 40–93). Approval was obtained from the local ethical committee and informed consent was obtained from all of the patients.

Receptor Assay

Cytoplasmic ER and PGR were assayed as previously described.[15] When the bioptic material was sufficient, Scatchard analysis was carried out, otherwise assays were made at a single saturating concentration. Tritiated 17-beta-estradiol (Amersham, Buckinghamshire, U.K.) or synthetic progestin ORG 2058 (Amersham, Buckinghamshire, U.K.) were used as labeled ligands. Nonspecific binding was determined by adding a 200-fold molar excess of unlabeled diethylstilbestrol for ER or ORG 2058 for PGR.

The free ligand was separated by the dextran-charcoal technique. ER and PGR were expressed as fmoles of specifically bound ligand per mg of cytosol proteins. Cytosol proteins were measured according to the method of Lowry et al.[16] The receptor concentrations used as criteria for receptor positivity were >15 fmol/mg protein for ER and >20 fmol/mg protein for PGR.

RESULTS

Table 2 shows the number and percentage of ER and PGR positive cases before and after hIFN-β therapy, according to histologic grade and clinical stage. As previously reported,[6–8] the percentage of ER and PGR positive cases significantly differed between Grades 1 or 2 and 3 ($P < 0.01$ chi-square test). The percentages of ER and PGR positive cases were the same before and after therapy.

There was broad interindividual variability in the levels of ER and PGR before and after hIFN-β (Table 3). Seven of 36 cases were negative for ER (i.e., <15 fmol/mg protein) and 10 were negative for PGR (i.e., <20 fmol/mg protein). The quantitative changes in receptors before and after treatment could not be assessed in these cases. In two patients, Nos. 1 to 3 for ER and Nos. 1 and 2 for PGR, receptor levels were undetectable before therapy whereas posttherapy samples were positive; in these cases an arbitrary increase of 50% was assigned. For statistical analysis we estimated the increases as a percentage of the total change using the two-tailed Signed Rank Test. Using a 35% difference in pre and post therapy content of tumor tissue receptors as a cut-off, 72% of the cases for ER and 79% for PGR showed a significant increase (Table 4); with a cut-off of 50%, only PGR reached a significant increase after hIFN-β.

Figure 1 illustrates the hIFN-β induced changes of PGR in cases with low (Panel A), moderate (Panel B), and high (Panel C) basal values. Although we did everything possible to obtain the second biopsy from the same area as the first, some degree of heterogeneity could be expected and this might reduce the power of the statistical analysis. To clarify this point we examined two biopsies, a and b, taken simultaneously from the same patient. The results were similar in terms of positivity and negativity (Table 5). However, in Case No. 14, both ER and PGR were much lower in biopsy a than in biopsy b; in Case No. 7 (after hIFN-β therapy) PGR was higher in biopsy a than biopsy b and in Case No. 39 both ER and PGR were lower in biopsy a than biopsy b. The coefficient of variation of ER and PGR

TABLE 2
ER and PGR Positive Cases in Relation to Histologic Grade and Clinical Stage, before and after hIFN-β Treatment

		Before hIFN-β		After hIFN-β	
		ER+[a]	PGR+	ER+	PGR+
		No. (%)		No. (%)	
Histologic grade	No.				
1	9	10/10 (100)	9/10 (90)	10/10 (100)	10/10 (100)
2	17	13/17 (76.4)	12/17 (70.5)	14/17 (82.3)	15/17 (88.2)
3	10	6/9 (66.6)	4/9 (44.4)	4/9 (44.4)	3/9 (33.3)
Clinical stage					
I	26	22/26 (84.6)	19/26 (73)	22/26 (84.6)	21/26 (80.7)
II	4	3/4 (75)	3/4 (75)	3/4 (75)	4/4 (100)
III–IV	6	4/6 (66.6)	3/6 (50)	3/6 (50)	3/6 (50)
Total	36	29/36 (80.5)	25/36 (69.4)	28/36 (77.7)	28/36 (77.7)

ER: estrogen receptor; PGR: progesterone receptor.
[a] 15 and 20 fmol/mg cytosol protein were used as cut-off for positive ER and PGR, respectively.

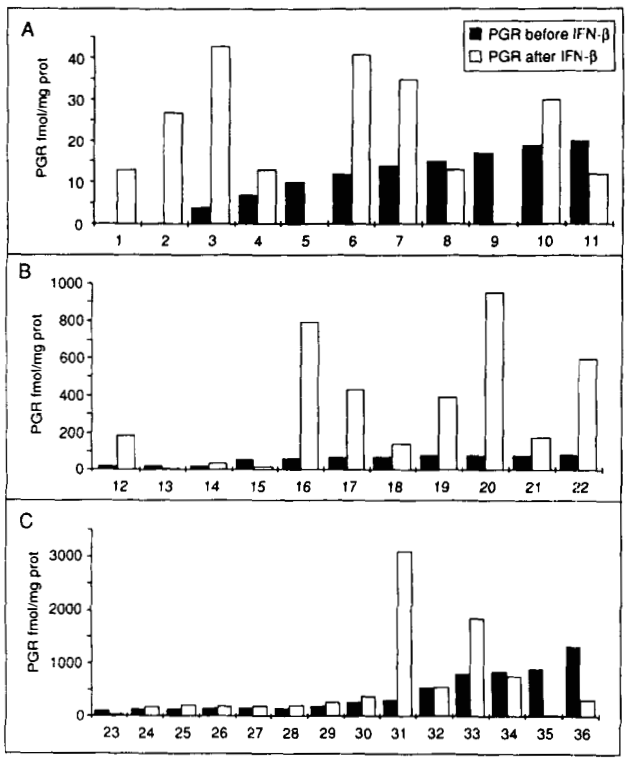

FIGURE 1. Change in PGR content before and after hIFN-β is shown. (A) Cases in which the basal receptor level was <20 fmol/mg prot. (B) Cases in which the basal receptor level was from 21 to 100 fmol/mg prot. (C) Cases in which the basal receptor level was >100 fmol/mg prot.

assayed in the same biopsy was <10%, indicating that the differences in receptor number in different biopsies cannot be attributed to the assay variability.

DISCUSSION

De Cicco et al.[13] found an increase in ER and PGR in fresh biopsies of endometrial carcinomas incubated for 48 hours in a medium containing hIFN-β at concentrations between 10 and 1000 IU/mL. At 10 IU/mL, which is closer to the concentrations achieved in vivo,[10] 60% and 42% of cases showed an increase in ER and PGR, respectively. Sica et al.[17] also reported an increase in ER and PGR in endometrial adenocarcinoma patients receiving hIFN-β. The present study confirms that hIFN-β can raise the number of ER and PGR in endometrial adenocarcinomas that express these receptors. Like Sica et al., we too found that PGR increased more than ER. However the hIFN-β dosage schedules were different in the two studies. In Sica's study hIFN-β was given at the dose of 2×10^6 or 6×10^6 IU/day 3×/week, whereas we gave hIFN-β at the dose of 3×10^6 IU/every other day for 3 weeks.

In both Sica's study and ours the hIFN-β induced increase in ER and PGR in patients with endometrial adenocarcinoma was statistically significant but in approximately half of the patients there was either a decrease or no change in receptor levels. The lack of consistency may be partly due to intrapatient heterogeneity in the receptor levels, which was evident in simultaneous biopsies from the same patient. It is not clear whether these differences between biopsies are due to a different degree of contamination with normal cells or to variable receptor levels in different cancer cell populations. Another aspect that has not been

TABLE 3
ER and PGR before and after hIFN-β Treatment in Individual Patients[a]

Pat no.	Clinical stage	Histologic grade	ER before hIFN-β	ER after hIFN-β	PGR before hIFN-β	PGR after hIFN-β
1	IB	2	0	18	0	13
2	IIIB	3	3	2	0	27
3	IIB	2	0	8	4	43
4	IB	2	4	2	7	13
5	IB	3	9	8	10	0
6	IB	2	44	79	12	41
7	IB	2	4	9	14	35
8	IB	3	118	232	15	13
9	IIIA	3	19	0	17	0
10	IA	1	20	26	19	30
11	IIIB	3	2	0	20	12
12	IIIA	2	24	111	23	190
13	IIIB	3	23	18	23	3
14	IIB	2	27	40	24	39
15	IB	3	37	0	61	19
16	IB	2	100	380	66	796
17	IB	1	70	105	69	434
18	IC	2	166	423	73	146
19	IV	2	31	380	80	396
20	IB	3	122	39	83	955
21	IB	1	51	77	83	180
22	IC	1	120	202	88	599
23	IC	2	56	20	120	45
24	IB	2	65	236	143	187
25	IB	1	18	30	153	224
26	IB	1	279	160	158	201
27	IB	2	53	197	161	211
28	IIB	1	34	49	162	231
29	IB	2	29	22	204	288
30	IB	3	148	202	294	395
31	IA	1	66	154	316	3099
32	IB	2	69	67	559	576
33	IIB	1	156	441	811	1851
34	IB	2	177	158	867	791
35	IB	2	382	169	917	42
36	IB	1	180	70	1332	341
Mean			75.166	114.833	194.111	346.278
SD			84.039	128.065	311.373	600.038
Median			47.5	68.5	76.5	183.5
Minimum			0	0	0	0
Maximum			382	441	1332	3099

ER: estrogen receptors; PGR: progesterone receptors; hIFN-β: human interferon-β; SD: standard deviation.
[a] ER and PGR are expressed as fmoles/mg cytosol proteins.

investigated is whether the surgical operation itself influences the number of receptors. We cannot exclude this, as we did not establish whether the number of receptors changed in patients not receiving interferon. However, considering that our results are consistent with those obtained in vitro by De Cicco et al.,[13] the difference is more likely to be due to the effect of interferon.

These results might have clinical application. Progestins are currently used for therapy for patients with endometrial cancer and presumably their growth inhibitory activity is mediated by their receptor binding.

The finding that hINF-β increases tumor PGR levels provides a rationale for combining hINF-β with progestins. The results of this and previous studies suggest that a sequential treatment of hINF-β followed by progestins might be more effective than progestins alone. However, hIFN-β appeared to increase the

TABLE 4
Effect of hIFN-β Therapy on ER and PGR Levels in Endometrial Adenocarcinoma

		Increase in receptors Mean ± SD fmol/mg protein	P^a After vs. before
ER			
Evaluable cases[b]	30	47 ± 125	0.2
Change >35%: total cases	25		
cases with increase	18	57 ± 134	0.04
Change >50%: total cases	20		
cases with increase	14	72 ± 144	0.11
PGR			
Evaluable cases[b]	30	183 ± 628	0.001
Change >35%: total cases	24		
cases with increase	19	221 ± 699	0.006
Change >50%: total cases	21		
cases with increase	16	242 ± 744	0.02

ER: estrogen receptor; PGR: progesterone receptor; hIFN-β: human interferon-β; SD: standard deviation.
[a] Statistical analysis was by Wilcoxon's Signed Rank Test.
[b] Evaluable cases were those which were receptor-positive before or after treatment.

TABLE 5
Levels of ER and PGR in Two Endometrial Carcinoma Biopsies Taken Simultaneously from the Same Patient

	ER		PGR	
	fmol/mg protein			
Case	a	b	a	b
8	59	178	19	11
5	9	9	3	16
7	4	4	16	13
7[a]	13	6	59	11
32[a]	46	89	367	785
14[b]	8	73	11	67
37[b]	10	0	9	3
38[b]	16	21	31	29
39[b]	10	56	6	74

[a] Biopsies taken after hIFN-β therapy.
[b] Cases not included in the study because they were only biopsied before treatment.

number of receptors in cases which were already positive and it is not known whether any quantitative increase in PGR receptors is related to an increase in sensitivity to progestins. From our results it would appear that hIFN-β does not induce PGR in endometrial adenocarcinomas which do not express detectable receptor levels before therapy.

In conclusion, these findings indicate that hINF-β can increase the number of PGR and–to a lesser extent–ER in endometrial adenocarcinoma. Whether this effect has any potential therapeutic application is a question that only a properly designed randomized clinical trial can answer.

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