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# Intraperitoneal Human Recombinant Interferon alpha-2b in Minimal Residual Ovarian Cancer

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**Abstract**—*Twenty evaluable patients with minimal residual ovarian cancer at second look laparotomy were treated with human recombinant interferon alpha-2b (IFN) intraperitoneally. The dose administered was* 50 × $10^6$ *units once weekly for* 8 *weeks. Seventeen patients were evaluated by a relaparotomy: five had a pathological complete remission, four a partial response, six patients disease stabilization and two patients had progression. Three patients, two stable and one with clinical progression, had no laparotomy. Nine of the* 11 *patients with residual tumor smaller than* 5 mm *had a response, while no response was found in six patients with residuals over* 5 mm. *The median duration of CR is* 11+ *months* (6–13+ *months) after evaluation.*

*For toxicity,* 156 *treatment cycles could be studied. Fever was seen in* 80% *of all cycles within* 24 h *following administration of IFN, in* 58 *cycles* (37%) *over* 38°C *and in* 65 *cycles* (43%) *over* 39°C. *Abdominal pain was slight in* 32% *and moderate in* 3% *of all cycles. The peripheral blood leukocyte counts dropped after* 52% *of all cycles, in* 27% *below* 4.0, *in* 22% *below* 3.0, *and in one patient below* 2.0 × $10^9$/l. *IFN dosage was not reduced for leukopenia, but in one patient reduction was necessary for thrombopenia, resulting from insufficient marrow reserve after a previous autologous bone marrow transfusion.*

*Pharmacokinetic studies showed i.p. IFN levels* 50–100 *times the blood levels. Blood levels were still elevated* 2 *days after i.p. infusion, but normalized within* 1 *week on repeated administration. At the second instillation, lower peak serum levels were reached.*

*In conclusion, high doses of i.p. IFN appear to be active in patients with minimal residual disease, with ongoing response in CR patients.*

*Apart from general malaise on the day of treatment, toxicity was acceptable. IFN may be active in patients with minimal residual ovarian cancer through local as well as systemic effects.*

## INTRODUCTION

OVER PAST YEARS, there has been a growing interest in the application of interferons in the treatment of solid tumors. For some of these compounds a dose–response relation may exist, which has led to the local treatment of certain disease localizations such as ovarian cancer in the peritoneal cavity [1]. We have studied the effect, pharmacokinetics and toxicity of i.p. administered human recombinant interferon alpha-2b (rhIFN alpha-2b) (IFN) in patients with minimal residual ovarian carcinoma after treatment with standard combination chemotherapy, in order to confirm these results.

## PATIENTS AND METHODS

Twenty patients entered this study, ages ranged from 38 to 72 years, median 54 years. All patients were surgically evaluated before treatment with IFN and had FIGO stage III: i.p. tumor without documented extraperitoneal disease at the start of treatment. Tumor residuals after second look ranged from microscopic disease in six patients, residuals under 0.5 cm in five patients and 0.5–2 cm in six patients.

Prior to i.p. treatment, each patient had undergone two or three laparotomies for staging or resection of tumor, and all patients had been treated with at least six cycles of combination chemotherapy, comprising carboplatin or *cis*-platinum and cyclophosphamide. No patient had prior irradiation to the pelvis or abdomen. During the staging laparotomy an intraperitoneal access port (Port-A-Cath®) was implanted subcutaneously with the tip directed toward the pelvis. Subsequently, after wound healing, an abdominal scan was done with $Tc^{99m}$ colloid in 2 l of normal saline to demonstrate adequate spread throughout the abdominal cavity. Treatment with i.p. IFN was started within 3

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weeks after the last operation and an evaluation laparotomy was performed within 4 weeks after the last instillation.

The protocol was approved by the local Medical Ethical Committee.

*Intraperitoneal therapy*

Fifty million units of human rIFN alpha-2b (Intron-A, Schering Company) was instilled weekly i.p. in 250 ml normal saline preceeded and followed by 2 l of normal saline at 37°C i.p., without addition of heparin, remaining in the abdominal cavity without evacuation. The patients were repositioned every 30 min for 2 h after instillation. Samples of lavage fluid were taken after 1 and 2 h for IFN levels, cytology specimens and bacterial cultures, and were repeated 24 h later. The port and catheter were then flushed with heparinized saline. A total of eight cycles was given. There was no dose escalation of IFN, but IFN could be reduced for grade III–IV WHO toxicity.

Patients were premedicated with 20 mg morphine HCl slow release oral tablets and 10 mg diazepam rectally. For pain or fever during treatment acetaminophen 4 × 500 mg daily was given, and the patients were discharged after 24 h.

*Response criteria*

Standard WHO criteria for response were used, for all lesions found during reassessment laparotomy. Multiple sites were biopsied and peritoneal washings done to document CR.

*Toxicity*

The specific side-effects of IFN such as fever, flu-like symptoms, anorexia and malaise were graded for severity (Table 1). For other kinds of toxicity, standard WHO criteria were applied. For any grade III toxicity IFN was held until symptoms had subsided, and reduced 50% during further treatment.

*CA*-125

The serum levels of this marker were determined every week during treatment by ELISA (Abbott Inc.). Marker levels in the peritoneal washings were measured after every instillation.

*Pharmacokinetics*

Serum samples for IFN were determined at 0, 2, 24 and 48 h in eight patients. Samples of i.p. fluid were taken 1, 2 and 24 h after instillation. The specimens were assayed by the Primate Centre TNO by bioassay, measuring the prevention of CPE by Sendai virus on fibroblast cultures according to Armstrong [2].

## RESULTS

Nine patients responded to treatment, five reaching a complete and four a partial remission. Four complete responders started IFN treatment without macroscopic residuals, but had positive biopsies on multiple sites, the other had 5 mm nodules diffusely on the visceral peritoneum. At evaluation laparotomy, biopsies were taken from multiple sites and peritoneal washings were done, and proved all to be negative. Median duration of CR is 11+ months (6, 7, 11+, 12+, 13+ months) after treatment. The four partial responders all had tumor nodules smaller than 5 mm on the peritoneum and had microscopic residuals after IFN treatment, detected by random biopsies or peritoneal washings.

Of six patients with stable disease, one started with microscopic residuals, the others had tumor up to 2 cm. In two patients with tumor residuals of 2 cm diameter in the cul de sac, no surgical evaluation was performed for clinically stable tumor.

In three patients the tumor was progressive. One of them started with residuals over 2 cm and did not require laparotomy to document progression. Of the two others, one initially had nodules of 1 cm diameter and one microscopic disease, and both had evident progression at second look. The results of surgical evaluation are listed in Table 2.

In conclusion, nine out of 11 patients with residuals smaller than 5 mm responded to treatment with IFN.

*Toxicity*

The objective and subjective toxicity was scored weekly after each i.p. instillation of IFN, for a total

*Table* 1. *Grading of IFN toxicity*

| Grade | 1 | 2 | 3 |
|---|---|---|---|
| Fever | ≥38°C | >39°C | >40°C for 24 h |
| Chills | <10 min | 10–30 min | >30 min |
| Malaise | <50% in bed | >50% in bed | Unable to rise |
| Abdominal pain | <24 h intermittent no analgesics | >24 h relieved by analgesics | >24 h not relieved by analgesics |
| Anorexia | <24 h | 24–72 h | >72 h |
| Weight loss | ≤2 kg | 2–5 kg | >5 kg |
| Pruritus | <24 h | 24–48 h | >48 h |

*Table 2. Patients with surgically documented response by tumor residuals* (%)

| Response | CR | PR | SD | PD | No. of patients |
|---|---|---|---|---|---|
| Max diameter | | | | | |
| Microscopic | 4 (23) | — | 1 (6) | 1 (6) | 6 |
| <5 mm | 1 (6) | 4 (23) | — | — | 5 |
| 5–20 mm | — | — | 5 (30) | 1 (6) | 6 |
| Total | 5 (29) | 4 (23) | 6 (36) | 2 (12) | 17 |

*Table 3. WHO grade of toxicity in 156 cycles* (%)

| WHO grade | 0 | I | II | III | all > 0 |
|---|---|---|---|---|---|
| Anemia | 47 | 45 | 8 | — | 53 |
| Leukopenia | 48 | 27 | 22 | 3 | 52 |
| Thombopenia | 93 | 7 | — | — | 7 |
| Fever | 20 | 37 | 42 | 1 | 80 |
| Anorexia | 9 | 91 | — | — | 91 |
| Weight loss | 94 | 5 | 1 | — | 6 |
| Nausea/vomiting | 39 | 13 | 48 | — | 61 |
| General malaise | 20 | 77 | 3 | — | 80 |
| Rigors/aches | 69 | 23 | 8 | — | 31 |
| Headache | 32 | 65 | 3 | — | 68 |
| Dizziness | 94 | 6 | — | — | 7 |
| Abdominal pain | 65 | 32 | 3 | — | 35 |
| Obstipation | 90 | 10 | — | — | 10 |
| Diarrhea | 69 | 29 | 2 | — | 31 |
| Alopecia | 98 | 2 | — | — | 2 |
| Itching | 95 | 3 | 2 | — | 5 |
| Herpes simplex | 90 | 10 | — | — | 10 |

of 156 cycles. Subjective reactions specific for IFN were graded slight, moderate or severe, and their incidence is given in Table 3.

The severity and incidence of these symptoms were very variable between individual patients. Most could perform their normal daily activities, while a few had persistent malaise, anorexia and fatigue between cycles.

Vital signs were monitored over the first 24 h after treatment. Fever occurred often during this period, and its degree is listed in Table 2. For fever over 38.5°C or chills 500 mg acetaminophen was given and repeated if necessary. Three patients experienced slight hypotension, bradycardia, bowel cramps and diarrhea, probably as a vasovagal reaction to the i.p. instillation itself or the opiate premedication, and never lasting longer than the first day of treatment. No allergic symptoms like continuous fever, exanthema, hypotension or wheezing were reported. Some patients, however, complained about periods of severe itching, without visible exanthema or skin eruptions.

Hematologic parameters were followed weekly. Baseline blood hemoglobin levels showed grade I anemia in three patients, but grade I anemia occurred after 45% of cycles, and grade II after 8%. No patient needed or received blood transfusions during treatment. Only one patient had grade III thrombopenia during IFN treatment due to insufficient marrow reserve after a previous autologous bone marrow transfusion. For this, IFN treatment was delayed three times and the IFN dosage had to be reduced 50% in four courses. There were no other patients with thrombocytopenia. Leukopenia occurred in half of the patients, grade I in 27% of all cycles, grade II in 22%. Grade III leukopenia was only found in the patient mentioned above.

Two patients experienced deterioration of preexistent peripheral neuropathy during IFN treatment. One patient, who had no surgical evaluation due to tumor progression after six cycles, experienced slight motor dysfunction and ataxia of arms and legs and progression of numbness and tingling. The other patient developed a motoric paresis of both legs after two cycles of IFN, which recovered 2 weeks after stopping treatment, but recurred on reinstitution of 50% IFN dosage. IFN treatment then had to be terminated. Both patients already suffered from minor (grade I) peripheral neuropathy resulting from previous treatment with cisplatin.

There were no biochemical abnormalities during treatment, all patients had and retained normal liver enzymes and renal function tests.

There were no problems with the implanted access ports in these 20 patients, except some abdominal discomfort during the first week after implantation. Bacterial cultures were never positive. We encountered no bowel perforation, bleeding, sepsis or other catheter-related problems. The projected treatment with IFN was not possible in all patients, however. In one patient surgery for the introduction of a catheter was complicated by a bowel lesion due to dense adhesions, which precluded the placement of the catheter and i.p. treatment. In a second patient the i.p. Tc colloid scan showed insufficient fluid distribution, which excluded the patient from this study.

*IFN pharmacokinetics*

*Serum.* The serum IFN levels rose from pretreatment baseline levels <20 U/ml to maximum levels of 30–70 U/ml at 24 h after instillation (Fig. 1). After 48 h there was hardly any decrease, but after a week the serum levels were not measurable anymore. The 24 h-serum levels after the second instillation were slightly lower than after the first in all six patients in whom the measurement was repeated.

*Peritoneal fluid.* The peritoneal IFN levels rose from baseline levels <20 U/ml to levels 2000–3200 U/ml directly after instillation, decreasing very little during the first 24 h (Fig. 1). No samples after this time were taken, but IFN was undetectable after 7 days, at the start of the next cycle, despite the fact that no fluid was removed after instillation of IFN.

The mean peritoneal IFN level exceeded that in the serum with a factor of 50–100 at 24 h.

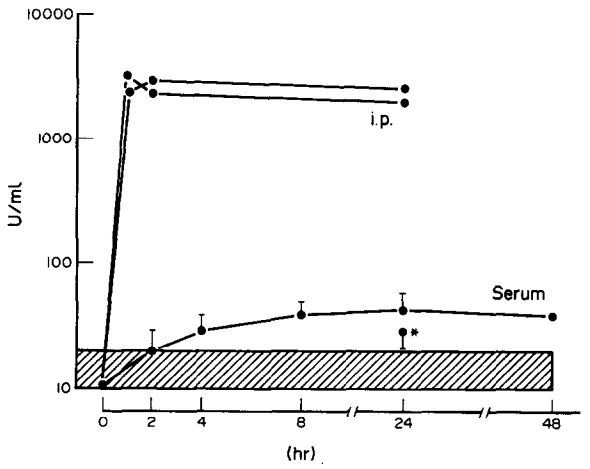


*Fig. 1. Levels of IFN in serum and in peritoneal fluid (mean and S.D. in six patients). *Indicates the serum IFN level 24 h after the second instillation, which was lower in all patients* ($P < 0.05$).

*Serum marker levels*

The marker levels in serum correlated well with tumor response, rising in two patients with tumor progression, and stable in two with stable disease. Disappearance of the marker, however, did not differentiate between a complete or partial response. The marker was not elevated in the washings after instillation or 24 h later. There was no systemic effect of i.p. treatment on serum marker levels.

*Cytology*

The presence or absence of malignant cells in the aspirated i.p. fluid before second laparotomy was not very helpful, as negative examinations did not prove CR and positive ones indicated no partial response. In four patients no samples could be obtained at all via the peritoneal catheter, which is a drawback of the implanted small lumen catheter.

## DISCUSSION

*Efficacy*

This is the second study reporting efficacy for treatment with i.p. human rIFN alpha-2b in patients with minimal residual ovarian cancer [1]. The occurrence of a response in 11 of the 17 surgically evaluated patients (65%) shows that IFN does have activity in ovarian cancer. All responses occurred in patients having tumor residuals under 5 mm diameter, nine out of 11 patients (82%), which confirms the findings of Berek *et al.* [1]. In the latter study there was a dose escalation over the first 4 weeks, and patients were then treated for 8 weeks with the same weekly dosage of 50 million units human rIFN alpha-2b as in this study. Berék *et al.* found four CR in 11 surgically evaluated patients. In their study, the IFN was removed 24 h after instillation, while in this study IFN remained in the abdominal cavity. Other studies of the systemic administration of IFN for the treatment of ovarian cancer in Scandinavia and the U.S.A. only found partial responses in two out of 18 (11%) patients [3] and no response in 13 patients [4], using lower dosages. It may be that the local i.p. administration or higher dosage indeed does have a more outspoken effect on residual cancer by exposing the malignant cells to higher IFN concentrations than after systemic use. It may also be that the selection of patients with minimal residual disease has produced the more favorable results in the two i.p. studies. Four of the five patients in the present study started without macroscopic residuals. The absence of tumor in multiple site biopsies and washings and the median CR duration of 11+ months supports the validity of these findings. As a protein, IFN should not be able to penetrate tumor tissue to great depth, although it is known that it has to be bound to specific receptors on the cells in order to be active [5]. Apart from the persistently high i.p. levels,

elevated blood levels can be measured as early as 2 h after instillation, lasting for several days after treatment. This may ensure a systemic effect of IFN as well, as could be expected from its systemic side-effects. The serum levels reached seem to be equivalent to those after s.c. of i.m. administration in other studies [6, 7]. The abdominal cavity thus seems to function as a reservoir for the release of IFN. After the second instillation all patients had somewhat lower drug levels at 24 h. This could indicate a faster take-up by (tumor) cells or enhanced protein breakdown or inactivation by neutralizing antibodies. Production of IFN-specific neutralizing antibodies has been described, but in most cases this occurred after several weeks of treatment, and more often after the 2α-a than 2α-b subtype [8, 9]. The development of a kind of tolerance to the typical flu-like side-effects of IFN in some patients could also be the reflection of neutralizing antibody production. We have not specifically looked into this problem, which could be a drawback for chronic IFN treatment.

The precise mechanism of action of IFN is not known exactly. It blocks viral DNA replication and might inhibit cellular protein synthesis and replication in the same way [10. 11]. IFN has an antiproliferative effect against ovarian cancer cells grown in a human tumor stem cell assay [12]. Part of its effect may be mediated through the production of lymphokines like interleukin, by activation of macrophages [12] and NK cells [13, 14], and the enhancement of cell-mediated cytotoxicity [1, 15]. It may also act synergistically with cytotoxic drugs *in vitro* [16]. Therefore, i.p. IFN may be potentially useful when combined with cytostatic agents in patients with ovarian cancer, especially in patients with minimal residual disease or in an adjuvant setting after a negative second look laparotomy.

*Toxicity*

The registration of untoward signs and symptoms after i.p. IFN was done meticulously in this study by a M.D. and an oncology nurse. All patients were admitted for one night, which was convenient in view of the fever and malaise occurring over the first 24 h after IFN. In most cases the fever and chills responded well to acetaminophen, and treatment with NSAID was refrained from, as its effect on IFN's mechanism of action is unknown and unpredictable. Most patients did have fever and malaise after IFN, but most of them seemed to develop some tolerance to IFN. There also was a striking variation in the grade of symptoms between individuals. The only kind of toxicity which has not been extensively reported earlier was the deterioration of peripheral neuropathy, originating from previous cisplatin treatment. The same has been described after vinca-induced neuropathy [17, 20]. We did not try to correlate the symptoms to individual IFN levels or to the occurrence of IFN specific, neutralizing antibody. The absence of complications of an implanted i.p. catheter is noteworthy compared with the problems encountered with the open-ended Tenckhof catheter. Draining fluid, however, sometimes can be a problem with this system.

## CONCLUSION

Intraperitoneal treatment with human rIFN alpha-2b may be effective in patients with minimal residual disease, with ongoing CR in three out of five patients. Toxicity seems to be acceptable, while IFN may be detectable in the serum for days. The exact mechanism of action still remains to be determined.

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