Cancer Chemother Pharmacol (1984) 12: 39–42

Cancer Chemotherapy and Pharmacology

Phase-I trial of Ultrapure™ human leukocyte interferon in human malignancy

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Summary. *A phase-I trial of Ultrapure™ human leukocyte (α) interferon was performed, in which 15 patients were treated according to a dose-ranging protocol. Five patients were treated at each of three dosage levels: 3×10^6 IU/dose, 9×10^6 IU/dose, and 15×10^6 IU/dose. Doses were given on days 1–5 and 8–12 of a 28-day study period. Serial NK-cell assays were performed in all patients, and failed to show consistent effects referable to treatment. Serum interferon levels were assayed on one patient at the 9×10^6 IU and one patient at the 15×10^6 IU dose level. In both cases, a significant interferon titer (≥ 160) was detected in the serum, and this persisted for as long as 12 h. Fever, malaise, and myalgias were associated with therapy. The dose-limiting toxicity was a dose-related leukopenia, with median white blood cell nadirs of 6,500/mm^3 (3×10^6 IU/dose), 3,200/mm^3 (9×10^6 IU/dose), and 1,800/mm^3 (15×10^6 IU/dose) being produced. One patient died in ventricular fibrillation while suffering chest pain after receiving 5 days of treatment at the 15×10^6 IU/dose level. Three patients showed minor responses, insufficient to be called partial responses, in association with interferon therapy. We conclude that dose-limiting leukopenia occurs with this schedule of administration of Ultrapure™ human leukocyte interferon at 15×10^6 IU/dose.*

Introduction

The interferons are a group of naturally occurring glycoproteins produced in response to a variety of stimuli, including viruses, and other inducing agents [1, 13]. Interferons have also shown evidence of antitumor activity, both in vitro and in vivo [1, 13]. Early human trials confirmed that they have clinically significant antitumor effects [4, 8], and led to great interest in their therapeutic effects. In this paper we report on a phase I trial of human leukocyte (α) interferon that we conducted in patients with a variety of malignancies refractory to conventional treatments.

Materials and methods

Ultrapure™ interferon (IFNα) was supplied by Interferon Sciences (Brunswick, NJ, USA). This compound was produced from normal human leukocytes. Following partial purification by standard techniques, the IFN was further purified by antibody affinity chromotography. The final product used in this clinical trial had a specific activity of $\geq 1 \times 10^6$ IU/mg protein.

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A total of 15 patients were studied; nine were males and six were females. All had metastatic malignancy refractory to any conventional treatment of proven value. The age range was from 35 to 72 years with a median of 55 years. The diagnoses were: gastric cancer (2), colorectal cancer (3), renal cell carcinoma (2), breast cancer (2), malignant melanoma (1), multiple myeloma (1), fibrosarcoma (1), non-Hodgkin's lymphoma (1), squamous cell carcinoma of the soft palate (1), and adenocarcinoma of presumed ovarian origin (1). All patients were negative for serum anti-IFN antibodies prior to the start of treatment.

A dose-ranging schedule was used. The first five patients were treated with 3×10^6 IU/dose, the second five patients were treated with 9×10^6 IU/dose, and the last five were treated with 15×10^6 IU/dose. All patients were given one dose daily on days 1 through 5 and 8 through 12 of the study. After a 2-week rest period, patients showing objective evidence of tumor regression were treated with either a second reinduction course or with twice-weekly maintenance treatments. Two patients, both receiving 15×10^6 IU/dose, received 10 doses in 11 days. One of these patients was treated on days 1 through 7 and 9 through 11, while the other was treated on days 1 through 6 and 8 through 11 of the induction course.

Patients were asked to record any side-effects and to record their temperatures at 4 h and 6 h after each dose. Physical examinations, blood counts, and serum chemistries were performed as needed and on days 0, 5, 12, and 28. Tumor measurements were taken at the start of therapy, at the end of the induction period, and 2 weeks after the last dose of the induction. Chest X-rays, blood counts, serum chemistries, and physical examinations were repeated 2 weeks after the last dose of IFN and as medically indicated thereafter.

Serial measurements of NK-cell activity were performed in all patients, and two patients were tested for serum clearance of IFN. All patients were tested for the development of anti-IFN antibodies.

NK cell assay. Natural killer (NK) cell assays were done according to routinely used procedures. Briefly, after separation of peripheral blood leukocytes (PBL) on sodium diatrizoate-Ficoll (LSM, Litton Bionetics) gradients, the mononuclear fraction was collected and washed three times in complete RPMI-1640 culture medium (GIBCO, Grand Island, NY, USA) containing 5% non-mitogenic fetal bovine serum (FBS). The washed cells were then suspended at a concentration of 10^7 viable cells per milliliter in complete RPMI-1640

and appropriately diluted for addition to NK-cell-sensitive K562 target cells [14] at effector-to-target (E : T) ratios of 1 : 1, 5 : 1, 10 : 1, 25 : 1, 50 : 1, and 100 : 1. Prior to assay, approximately 10^7 K562 cells in 1 ml complete RPMI-1640 were labeled for 90 min with 300 μCi Na^{51} CrO_4 (New England Nuclear, Boston, USA), washed three times, then suspended in medium at a concentration of 10^5 viable cells/ml. The target cells were added to the wells of U-bottom 96-well cluster plates in volumes of 100 μl (10^4 cells). Effector cells (100 μl) were then added to triplicate wells for each dilution. After incubation for 4 h, the plates were centrifuged at 400 *g* for 5 min; 100 μl of supernatant fluid was removed from each well and the mean amount of ^{51}Cr released for each dilution was determined in a Beckman Gamma 4000 counter. Maximum ^{51}Cr release was measured by lysing triplicate wells of 10^4 target cells with 1% sodium laural sulfate. Spontaneous release was measured in target cells to which medium alone was added. For each E : T ratio, percent specific lysis was calculated using the following formula:

$$\% \text{ Specific lysis} = \frac{(\text{Experimental cpm}) - (\text{Spontaneous cpm})}{(\text{Maximum cpm}) - (\text{Spontaneous cpm})} \times 100.$$

NK-cell activity is expressed as lytic units per 10^7 mononuclear cells tested as determined from linear regression analysis of dose-response curves in which E : T ratios were plotted against percent specific lysis [2]. One lytic unit is defined as the number of effector cells required to produce 30% specific lysis of 10^4 K562 target cells.

Interferon assay. IFN titers in the sera of patients who had received IFN injections were determined by their capacity to inhibit the cytopathic effect (CPE) of encephalomyocarditis (EMC) virus on human lung carcinoma cells (strain A549 provided by Dr S. E. Grossberg, Milwaukee). A549 cells were grown in Dulbecco's modified Eagle's medium with 10% fetal bovine serum (complete DMEM). IFN was assayed, with slight modification, by the dye-elution procedure of Jameson et al. [12]. One unit of a WHO (NIH #G-023-901-527) IFNα reference standard titrated as approximately one unit in our assay.

Anti-IFN assay. The neutralization of functional IFN activity was used as a measure of anti-IFN activity in serum [17]. Serial two-fold dilutions of a test serum were made in complete DMEM in U-bottom 96-well cluster plates. Five units of Ultrapure™ IFNα in 100 μl were then added in duplicate to each well containing an equal volume of a given serum dilution. A reference sheep antiserum to HuIFNα (NIH #G-026-502-568) was used as a positive control. After incubation of the plate for 1 h at 37° C the contents of each well were assayed as above to determine whether neutralization of IFN functional activity had occurred.

Results

The most severe toxicity encountered was a dose-related leukopenia. The median white cell nadirs for each treatment group were 6,500/mm³ at 3 × 10^6 IU/dose (range 3,100–13,100/mm³), 3,200/mm³ at 9 × 10^6 IU/dose (range 2,700–4,400/mm³), and 1,800/mm³ at 15 × 10^6 IU/dose (range 1,700–3,200/mm³). In each group the white cell nadir occurred on a mode of day 5. The leukocyte count rose slightly by day 8 and fell again in association with the second 5-day cycle of treatment. With cessation of treatment the white blood cell count returned to normal (Table 1). A similar pattern of a reversible decrement in the platelet count was seen, but was not severe (Table 2). The hematocrit changed minimally (Table 3).

Most patients reported chills lasting from 15 min to 3 h occurring 1–8 h after each injection of IFN. The severity of the chills seemed dose-related. Associated with these chills was a fever occurring 4–6 h after each injection. The mean maximal temperature was 37.9° C at 3 × 10^6 IU/dose and 39.2° C at both the 9 × 10^6 and 15 × 10^6 IU/dose levels. Fevers and chills were most severe on the earlier days of each 5-day cycle, particularly on days 1 and 8. Acetaminophen was somewhat helpful in ameliorating these side-effects.

Most patients reported anorexia and malaise, which persisted for 1–2 weeks after the last dose of IFN. Generalized myalgias were noted by most patients but were not severe.

Three patients in this trial have died. Two clearly died of progressive disease on days 31 and 50 of the trial period. One patient, receiving 15 × 10^6 IU/dose, developed acute, severe chest pain and died suddenly in ventricular fibrillation on day 6. This patient had a SWOG performance status of 1 and no prior history of cardiac disease. Permission to perform an

Table 1. WBC × 10^3/mm³ by dose level and day of treatment

	3 × 10^6 IU/dose		9 × 10^6 IU/dose		15 × 10^6 IU/dose	
	Mean	Median	Mean	Median	Mean	Median
Day 0	10.4	9.9	7.5	7.5	6.4	6.4
Day 5[a]	8.8	6.5	3.4	3.2	2.7	2.2
Day 8[b]	7.8	6.5	3.6	3.6	3.0	2.9
Day 12	7.7	4.0	4.1	4.2	2.4	2.1
Day 28	9.4	7.8	6.6	5.9	7.4	5.3

[a] Last day of the 1st treatment course
[b] First day of the 2nd treatment course

Table 2. Platelet count × 10^3/mm³ by dose level and day of treatment

	3 × 10^6 IU/dose		9 × 10^6 IU/dose		15 × 10^6 IU/dose	
	Mean	Median	Mean	Median	Mean	Median
Day 0	326	351	345	344	266	291
Day 5	296	295	241	241	179	194
Day 8	171	187	222	222	173	120
Day 12	256	186	283	302	177	132
Day 28	288	320	318	256	271	273

Table 3. Hematocrit by dose level and day of treatment

	3 × 10^6 IU/dose		9 × 10^6 IU/dose		15 × 10^6 IU/dose	
	Mean	Median	Mean	Median	Mean	Median
Day 0	32.1	34.2	34.6	33.1	38.2	37.1
Day 5	38.3	38.2	34.5	36.6	37.0	37.0
Day 8	31.4	29.2	30.6	30.6	35.2	34.0
Day 12	35.9	35.0	32.5	30.7	38.2	37.0
Day 28	34.7	32.1	30.1	27.8	36.2	30.5

autopsy was denied. Two other patients had serial electrocardiograms while receiving treatment, and no changes were notable.

Three minor responses of insufficient magnitude to be deemed partial responses were produced in the 15 patients. These responses were seen in one patient each with renal cell carcinoma, breast cancer, and multiple myeloma. Despite reinduction treatment in the two patients with solid tumors and twice-weekly maintenance treatments in the patient with multiple myeloma, these minor responses lasted only 8, 12, and 15 weeks, respectively.

To ascertain whether detectable levels of circulating IFN are achieved in patients receiving Ultrapure™ IFNα by IM injection, on days 3, 5, 8, and 10 of therapy serum IFN titers were checked in two patients whose blood was drawn approximately 1, 3, 5, 7, 9, and 12 h after IFN injection. By 3 h after injection with either 9×10^6 or 15×10^6 IFN units, significant levels of IFN (titer > 160) were detectable in the serum. In both patients the levels peaked (as high as 300 U/ml) by about 7 h and were declining (< 160 U/ml) by 12 h. Thus, substantial circulating levels of IFN were achieved in these patients for 12 h or more after injection.

Natural killer (NK) cell activity in the peripheral blood of each patient was evaluated prior to, at the conclusion of, and approximately 2 weeks after completion of their therapy (Table 4). Initial activities ranged broadly from approximately 1 lytic unit/10^7 cells in patient G. M. to 76 lytic units/10^7 cells in patient C. P. Overall, NK-cell activity was increased in eight of 11 evaluable patients at conclusion of therapy (day 12) and was decreased in three of 11 patients. In T. R. and J. A., the increases were marginal at best. By approximately 2 weeks after completion of therapy, in six of 12 evaluable patients NK-cell activity had declined from the levels seen on day 12. Interestingly, in patient M. H., whose NK activity initially, on day 12, and on day 17 post therapy was 2, 58, and 1 lytic unit/10^7 cells, respectively, that activity was boosted to 49 lytic units/10^7 cells when it was measured after initiation of maintenance therapy.

Table 4. NK cell activity (lytic units/10^7 cells) in peripheral blood mononuclear leukocytes of cancer patients before injection of IFNα, at completion of therapy (day 12), and 13–18 days post therapy

Patient	IFN dose	Time of assay		
		Pre-therapy[a]	Day 12[b]	Post therapy[c]
R. T.	3×10^6 U	43	46	21 (14)
N. T.	3×10^6 U	ND	26	ND
J. A.	3×10^6 U	37	53	ND
E. M.	3×10^6 U	1.5	18	5 (14)
P. M.	3×10^6 U	43	30	46 (14)
C. P.	9×10^6 U	76	ND	150 (15)
A. B.	9×10^6 U	9.8	126	16 (17)
M. H.	9×10^6 U	2	58	1 (17)
L. N.	9×10^6 U	72	176	65 (14)
E. S.	9×10^6 U	9	ND	73 (18)
E. K.	15×10^6 U	32[d]	ND	ND
B. S.	15×10^6 U	43	10.4	38 (12)
P. U.	15×10^6 U	6	0.4	15 (13)
A. C.	15×10^6 U	33	431	189 (16)
G. M.	15×10^6 U	0.9	10.4	58 (14)

[a] Peripheral blood was drawn just prior to the patient's first injection of IFN

[b] Peripheral blood was drawn on the last day of two 5-day courses of IFN with a 2-day rest between the two courses

[c] Peripheral blood was drawn approximately 2 weeks after completion of therapy. The number in ( ) indicates days after completion of therapy that blood was drawn

[d] Patient E. K. died before therapy was completed

One of the criteria for entering a patient on this study was the initial absence from his/her serum of antibodies capable of neutralizing IFNα. As it has been reported that occasional patients who receive IFN injections may develop antibodies to IFN, serum was collected from 11 of 14 of the surviving patients in our study approximately 2 weeks after completion of their therapy. In one patient, serum was collected at approximately 7 weeks (patient L. N.). When these sera were tested for their ability to neutralize 5U of Ultrapure™ IFNα none did so, although a reference sheep anti-human IFNα (NIH #G-026-502-568) completely neutralized the IFN activity.

Discussion

Ultrapure™ human leukocyte interferon can be given safely according to the dosage schedule used. It is possible that continuous daily treatment for more than 5 consecutive days could result in more severe, dose-limiting myelosuppression. It is our experience that myelosuppression is rapidly reversible upon cessation of treatment, so that close monitoring of blood counts should prevent the complications of life-threatening neutropenia. Two of five patients treated with 15×10^6 IU/dose developed white counts of less than 2,000, and it is likely that continuous treatment at this dosage level would produce dose-limiting neutropenia in a large proportion of patients. Thrombocytopenia and anemia were noted in some patients, but were not dose-limiting.

Fevers and chills were bothersome to patients, but were not dangerous. Some control of these side-effects was provided by treatment with standard antipyretics. The anorexia and malaise noted by most patients was occasionally profound, and in two patients led to a significant decrease in performance status.

No deaths could be clearly related to Ultrapure™ IFNα. The patient dying suddenly on day 6 might represent a cardiotoxic death, but this cannot be stated with certainty. This patient's maximum temperature (39° C) was less than the mean for those at his dosage level, and no undue toxicity was manifest in his case. None of our other patients showed evidence of cardiac toxicity, though an excessive incidence of myocardial infarction in patients receiving interferon has been noted in France [3].

The antitumor effect of Ultrapure™ IFNα in this heavily pretreated group of patients was minimal. Of the 15 patients, 11 had received prior radiation therapy and 12 had received prior chemotherapy. As a whole, the group had received prior treatment with a mean of 2.3 drug regimens and 4.3 drugs. The activity of IFNα in this group of patients was insufficient to allow any conclusions regarding response as a function of dose.

Lymphocytes generally referred to as natural killer (NK) cells continue to be actively investigated because of evidence that they are important in immunosurveillance against cancer cells [9] because the expression of NK-cell activity may be diminished in at least some cancer patients [5, 16] and because interferons are capable of augmenting NK-cell activity [10, 11, 15]. When this activity was monitored in the peripheral blood leukocytes of the cancer patients in our study, no consistent

pattern of NK-cell activity or change in that activity were apparent in all patients. Changes were evident in individual patients, however, and this observation is consistent with that made in similar studies [6, 7]. The latter studies, as well as studies presently under way in our laboratory, indicate that the decreases in NK-cell activity where they occur in cancer patients probably are not attributable to the presence of suppressive adherent mononuclear cells.

In summary, Ultrapure™ human leukocyte interferon can be given safely in the dose range employed, and substantial circulating levels of IFN activity are demonstrable at these doses. Phase-II studies of this IFN could be undertaken at the 15×10^6 IU/dose level if close monitoring of blood counts were provided. Although antibodies capable of neutralizing Ultrapure™ IFNα were not detected in our patients, we cannot exclude the possibility that such antibodies might develop in other patients, in patients given IFN for a longer time, or at a time later than that evaluated.

Acknowledgements. Data on NK cell activity were computed using Prophet, a national computer resource supported in part by the Biotechnology Resources Program, Division of Research Resources, NIH.

Funding for the study described in this paper was provided by Interferon Sciences, Inc., New Brunswick, NJ 08901.

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Received May 23, 1983/Accepted August 23, 1983