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A phase I toxicity study of human rDNA interferon in patients with solid tumours

J. Wagstaff, G. Chadwick, A. Howell, N. Thatcher, J. H. Scarffe, and D. Crowther

Cancer Research Campaign Department of Medical Oncology, University of Manchester, Christie Hospital and Holt Radium Institute, Wilmslow Road, Manchester M20 9BX, England

Summary. *This study was designed to evaluate the clinical tolerance to multiple IM injections of rDNA-produced human alpha-2 interferon (IFN) (Schering-Plough 30500) in patients with solid tumours. IFN was administered in escalating IM doses in separate groups of patients daily for 14 days and then twice weekly for a further 10 weeks. The dosage levels were 1, 3, 10, and 30 million U/injection. Subjective toxicity could be divided into two types, acute and chronic. The acute reactions took the form of an influenza-like syndrome consisting in chills, rigors, headache, tremor, nausea, vomiting, and myalgia. These symptoms were dose-related but tachyphylaxis developed with continued dosing. The chronic toxicity consisted of malaise, lethargy, fatigue, anorexia, and confusion. These symptoms were not so dose-dependent and tended to become more severe with prolonged treatment. Objective toxicity consisted of myelosuppression and liver dysfunction. Granulocyte counts below 1.0 × 10^9/l were seen in three patients at the 30-million-U level, with platelet counts less than 100 × 10^9/l in two of these. Elevation of the liver enzymes were seen in all five patients treated at 30 million U, but returned to normal after 1 week without IFN in all but one patient. A tolerable dose (IM) for phase II/III studies lies between 3 and 10 million U for daily scheduling and between 10 and 30 million U for twice-weekly injections.*

Introduction

Early preparations of leucocyte and fibroblast interferon (IFN) were scarce, of variable potency, and extremely impure. Clinical studies were designed without information from phase-I studies conducted according to conventional methods, and consequently the doses and schedules used were found rather by serendipity. Similarly, the toxicities encountered with these preparations were thought possibly to be due to inherent impurities rather than to the IFN. The recent introduction of new techniques in molecular biology and the development of recombinant DNA technology has allowed the cloning of the IFN molecule and its production in virtually unlimited amounts in extremely pure forms. It has become apparent that there are whole families of different interferons and that their biological properties may vary from subspecies to subspecies. Because of this heterogeneity it will be important to assess both the efficacy and toxicity of each of these IFN species in a uniform fashion. At present two rDNA alpha IFNs have become available for phase-I/II investigation. The results of studies with one of these (Hoffman La Roche IFL-rA) have recently been published [5], and the results of a phase I study with the human alpha-2 IFN used by us have also been reported [1]. In this paper we present the results of a phase-I toxicity study of human alpha-2 IFN (Schering-Plough Corp. 30500) given as either daily or twice-weekly IM injections.

Offprint requests to: John Wagstaff

Patients and methods

All patients in this study had a histologically confirmed disseminated neoplasm, which was refractory to conventional therapies or for which no satisfactory treatment existed. Patients were to have a Karnofsky performance status of 50% or more, to have a life expectancy of at least 4 months, and be able and willing to comply with the requirements of the study. Haematological, renal, and hepatic function were to be judged normal by routine laboratory tests prior to study entry (total WBC ≥ 3.0 × 10^9/l, platelets ≥ 100 × 10^9/l, serum creatinine, creatinine clearance, calcium, and hepatic enzymes all less than 25% above the upper limit of normal, bone marrow examination, and ECG were to be normal). The patients were not to have received chemotherapy or radiotherapy for 4 weeks prior to study entry (6 weeks for nitrosoureas and mitomycin C) and not to have had leucocyte or platelet transfusions for the same period. Patients with central nervous system metastases or serious concomitant medical illness were excluded. Patients with leukaemia, lymphoma or multiple myeloma were not considered eligible for entry.

Pretreatment evaluation of the patients included full history and clinical examination, full blood count, serum chemistry studies, 24-h urine collection for creatinine clearance, urinalysis, serum interferon, and neutralisation assays and serum for storage at −70° C. These investigations were performed twice weekly during the daily scheduling and weekly during the twice-weekly administration. Following cessation of therapy they were performed at weekly intervals until all abnormal results had returned to normal. A chest X-ray, ECG, bone marrow aspirate, and trephine biopsy were carried out initially and subsequently at 1 month and at the end of the study.

Toxicity was assessed using a 0–4 grading system [2]. To allow comparison between groups of patients experiencing different toxicities a weighted severity score (WSS) was used:

$$\mathrm{WSS} = \sum \frac{\text{Number of pts reporting reaction} \times \text{severity}}{\text{Number of pts in group}}.$$

Table 1. Patients' pretreatment characteristics and cumulative dose tolerated

Patient number	Primary diagnosis (duration in years)		Previous therapies[a]	Dose group ($\times 10^6$ IU)	Duration of treatment (days)	Cumulative dose ($\times 10^6$ IU)
001	Ca lung	(0.3)	XRT	1	81	34
002	Ca ovary	(1.6)	CT	1	46	24
003	Ca lung	(0.1)	None	1	39	22
004	Sarcoma	(0.7)	S	3	81	102
005	Sarcoma	(0.4)	S + CT	3	32	60
006	Ca breast	(2.8)	S CT + XRT	3	32	60
007	Ca colon	(0.1)	S	10	81	340
008	Ca lung	(0.1)	None	10	28	190
009	Ca breast	(15.6)	S XRT + CT	10	28	190
010	Ca stomach	(0.4)	None	30	21	930
011	Ca ovary	(0.5)	S + CR	30	15	450
011A	Sarcoma	(1.2)	XRT + CT	30	15	180
012	Sarcoma	(1.8)	S + CT	30	95	1,020
013	Ca colon	(4.0)	S	30	17	420

[a] XRT, radiotherapy; CT, chemotherapy; S, surgery

Moderate intolerance was defined as grade 1 or 2 toxicity and severe intolerance was defined as grade 3 or 4 toxicity.

The interferon (IFN) placebo, and diluent were supplied by the Schering-Plough Corporation via its Swiss subsidiary, Werthenstein Chemie AG. The IFN was rDNA produced human alpha-2 IFN (Sch 30500) purified to 10^8U/mg protein and prepared as previously described [3, 7]. It was dissolved in phosphate-buffered saline (pH 7.2) and freeze-dried in sterile 6-ml glass vials. The IFN was stored at 4° C. The IFN was reconstituted with diluent and the volume of injection was always 2 ml.

On day 1 a 2-ml injection of diluent was given by deep IM injection as a placebo. This was done because many of the patients were expected to have had cytotoxic chemotherapy previously and to experience psychologically induced toxicity from any injection. The injection of placebo allowed the magnitude of this response to be evaluated.

The planned dosage escalations are shown in Table 1. Patients were to receive 14 days of IFN by deep IM injection from day 2 and subsequently twice-weekly injections for a further 10 weeks. Initiation of patients to the next highest treatment group depended on the toxicity experienced by patients at the previous dose level. At a given dose of IFN, if no more than one patient developed moderate intolerance then progression the the next dose level could be initiated. If two of three patients developed moderate intolerance two further patients would be included at that dose, and if three of five patients showed moderate intolerance then escalation to the next dose level would not be instigated. At any given dose level of IFN, if any one of three patients developed severe intolerance three further patients would be added at that level. If three out of six patients developed severe intolerance then progression to the next dosage would not occur.

The maximum tolerated dose (MTD) was defined as the dose that produces moderate intolerance in three of five patients or severe intolerance in two of six patients.

If a patient developed moderate intolerance the IFN could be continued or stopped at the discretion of the clinician. If therapy was stopped recovery to baseline values would be allowed to occur. Treatment could then be reinstituted and if moderate intolerance again developed treatment would be stopped. If severe intolerance developed IFN would be discontinued until recovery had occurred. Treatment would then be recommenced at the next lower dose. If severe intolerance again developed then the patient would be withdrawn from the study.

Results

Fourteen patients were entered in the study and their characteristics are shown in Table 1. The planned dosage escalations were not completed because the maximum tolerated dose was reached at 30 million U. The dose levels given and the total cumulative doses per patient are shown in Table 1. Two patients experienced transient mild influenza-like symptoms after the placebo injection and one of these complained of a little nausea. The reactions were thought to be due to the explanation of the possible side-effects given to the patients prior to study entry. Both these patients experienced more dramatic symptoms following their first IFN injection.

Subjective toxicity

The adverse experiences consistently reported by the patients are shown in Tables 2 and 3. Table 2 shows dosage relationships and Table 3, correlation with schedule. These experiences can be divided into two groups, acute and chronic.

Acute reactions (upper part of Table 3) developed within a few hours of administration of IFN. An acute influenza-like syndrome consisting of fever, chills/rigors, myalgia, headache, nausea, and vomiting came on dramatically, usually at about 2 h after injection and was dose-dependent. With 30 million U these experiences were quite dramatic and very disturbing for the patients. By the third to fourth day of IFN the patients had begun to develop tolerance and by the end of the first week of injections no longer experienced these symptoms. Four patients reported a return of milder influenza-like symptoms when they commenced on the twice-weekly injections, but these again resolved when treatment was continued.

Chronic reactions (lower part of Table 3) tended to occur gradually and were not significantly ameliorated by changing to the twice-weekly schedule. They became apparent even at

Table 2. Adverse experiences related to dose (recorded as weighted severity score)

	1×10^6 ($n=3$)	3×10^6 ($n=3$)	10×10^6 ($n=3$)	30×10^6 ($n=5$)	Total in all groups ($n=14$)
Chills/rigors	0.3	1.7	3.3	3.4	2.3
Fatigue	3.0	3.3	3.0	3.4	3.2
Malaise	3.7	3.7	3.3	3.0	3.4
Headaches	0.7	2.3	1.0	0.6	1.1
Tremor	0	1.3	3.3	3.6	2.3
Myalgia	0.3	1.0	1.7	1.4	1.1
Confusion	0	1.3	1.0	0	0.5
Dry mouth	0	1.7	2.3	2.0	1.6
Anorexia	2.7	2.7	2.7	3.0	2.8
Nausea	1.7	2.7	0.3	1.8	1.4
Vomiting	1.7	1.7	0.3	1.8	1.4

Table 3. Adverse experiences[a] related to schedule

	Daily injections (n = 14)			Twice-weekly injections (n = 13)		
	No.	%	WSS	No.	%	WSS
Acute						
Chills/rigors	11	79	2.1	4	31	0.5
Headache	8	57	0.9	2	15	0.3
Tremor	10	71	2.3	3	23	0.5
Loose stools	3	21	0.2	1	8	0.1
Nausea	10	71	1.6	6	46	1.0
Vomiting	7	50	0.9	4	31	0.8
Myalgia	8	57	0.9	4	31	0.8
Anxiety	3	14	0.4	1	8	0.2
Chronic						
Fatigue	13	93	2.7	13	100	3.0
Malaise	13	93	2.4	13	100	3.3
Anorexia	12	86	2.1	13	100	2.6
Confusion	2	14	0.3	2	15	0.5

[a] Figures give the number of patients experiencing the reaction, the percentage within the group, and the weighted severity score (WSS) in each case

low doses (1 million units) and although they became more severe at higher doses the magnitude of this increase was not proportional to the increasing doses. This syndrome of central nervous system depression consisted of malaise, somnolence, lethargy, and anorexia. Some patients found that these symptoms interfered with their life-style to a significant degree. They became much more dependent on their next of kin and felt insecure about performing routine daily chores such as boiling a kettle, for fear of falling asleep and leaving it on the stove. Two elderly patients developed a mildly confused state whilst receiving IFN, which resolved once the drug was stopped. Dryness of the mouth and altered taste were a problem in some patients, and these contributed to the anorexia experienced by these patients.

Objective toxicity

1. Haematological. Changes in the blood count were seen at all dose levels (Fig. 1), but these were only clinically significant at the 30-million-U level. With 1 million U daily the white blood count (WBC) fell to a nadir of 4.3 × 10^9/l on day 11 of treatment. However, it recovered to starting values once the twice-weekly scheduling was started. The platelet count did not decrease significantly with the daily schedule but rose above baseline values on the twice-weekly dosing.

At 3 million and 10 million U per injection the WBC and platelet counts both fell with the daily treatments. But whereas the platelets recovered with twice-weekly scheduling the WBC remained depressed. The nadirs did not drop below 1.9 × 10^9/l for WBC or 150 × 10^9/l for platelets in any patient at these dose levels.

Changes in the platelets and WBC were more dramatic with doses of 30 million U (Fig. 2). In patient 11A the WBC fell rapidly, to reach a nadir of 0.9 × 10^9/l by day 5 of daily treatment. The platelet count fell to a lesser degree, reaching a nadir of 95 × 10^9/l, also on day 5. Treatment was suspended for 9 days after only 4 days' therapy, with rapid recovery of both the WBC and platelets. However, when treatment was reinstituted the WBC fell from 5.1 to 1.1 × 10^9/l and the platelet count fell from 218 to 99 × 10^9/l after only 2 further days of IFN. Because of this IFN was stopped. Similar events occurred in patient 12, with nadirs of 2.0 (5 days) and 32 × 10^9/l (9 days). This degree of thrombocytopenia was associated with epistaxis. After recovery of the blood count (9 days) IFN was restarted and the planned 14 days of daily injections could be completed. It was then possible to give twice-weekly injections without clinically significant problems with myelosuppression. The other three patients treated at 30 million U of IFN all developed myelosuppression (nadirs: patient 10, WBC = 1.7 and platelets = 123, both on day 9; patient 11, WBC = 2.9 and platelets 297, both on day 8; patient 13, WBC = 1.5 and platelets = 109, both on day 18).

2. Hepatotoxicity. At 1, 3, and 10 million U per injection there were no changes in liver function. One patient ((no. 7) had liver metastases before entry to the study and had elevated liver enzymes which were not made worse by treatment with IFN at a dose of 10 million U per injection. Four of five patients treated at 30 million U developed rises in the SGOT, SGPT, LDH, and gamma GT whilst receiving daily injections (Table 4). These abnormalities were less marked with twice-weekly scheduling, and the SGOT and SGPT had returned to normal by the final visit 1 week after stopping IFN. The gamma GT remained elevated in one of three patients at the final visit. This patient was subsequently shown to have liver metastases.

3. Other toxicity. No changes were seen in renal function and there was no symptomatic evidence of pulmonary dysfunction during this study. One patient with advanced ovarian carcinoma (patient 11) died after 15 days of IFN and was found to have had a pulmonary embolism at post mortem. Premortem anticoagulation studies were not performed in this patient. No cardiac arrythmias were detected and ECGs remained normal during treatment.

4. IFN Neutralisation factors. No neutralising activity to human alpha-2 IFN developed in the serum of any patient at any time during this study.

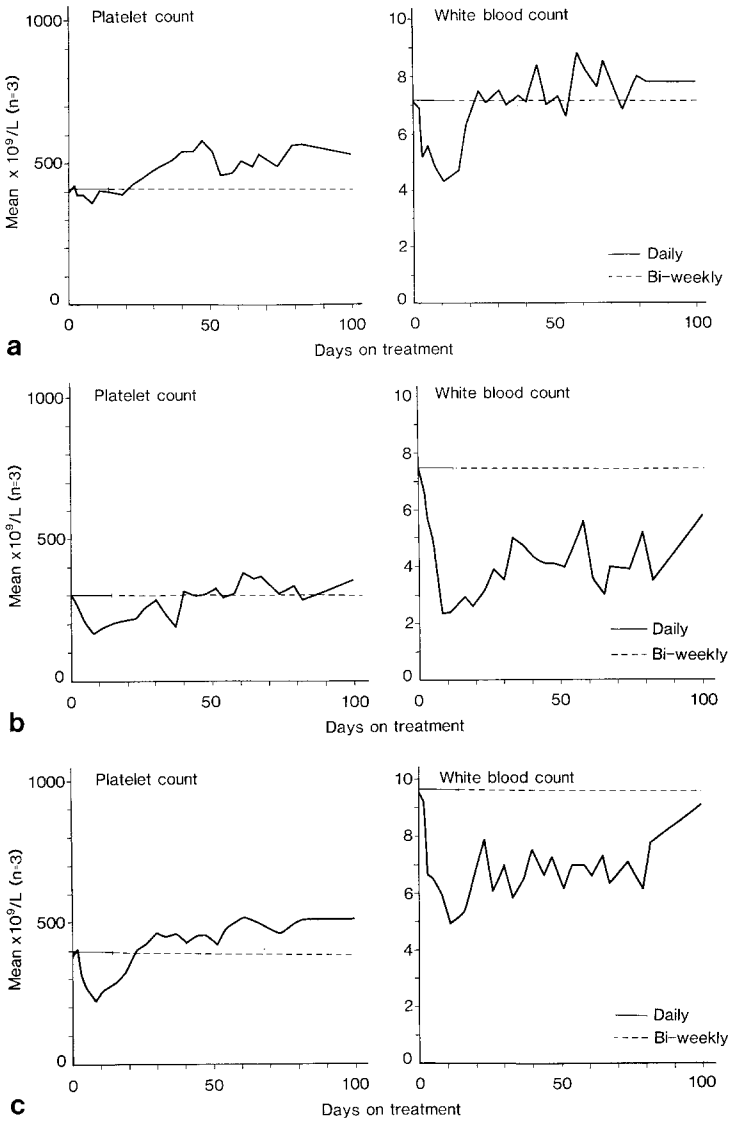

Fig. 1a–c. White blood cell and platelet counts in patients treated with human alpha-2 interferon daily for 14 days and then twice weekly for a further 10 weeks: **a** 1 million U, **b** 3 million U; **c** 10 million U

Discussion

The spectrum of toxicities seen with this highly pure genetically engineered human IFN were very much the same as those reported with the less pure heterogeneous mixtures of IFNs that have been used in clinical trials previously [4]. It is therefore likely that the influenza-like syndrome associated with the administration of IFN is related to the IFN and not to impurities. This was a somewhat unexpected finding, because preclinical testing in a number of animal models had suggested that this IFN would be nonpyrogenic even at doses much higher than have been given in this study (Schering Corp. USA, unpublished results). It follows that there are species differences in biological responses to various IFNs and that animal toxicity screening may be of limited value in the preclinical evaluation of IFNs.

The maximum tolerated dose for daily administration of this IFN was 30 million U per day. The dose-limiting toxicity was myelosuppression, consisting of both granulocytopenia and thrombocytopenia. Only one of five patients treated at this dose level was able to complete the planned 14 days of IFN without interruption. This patient (no. 10) had not had prior chemotherapy; however, one other patient (no. 13) without previous chemotherapy did develop dose-limiting myelosuppression. On the basis of the results obtained in this study we suggest that a suitable dose for daily scheduling in phase II/III studies would lie between 10 and 30 million U per injection, but because of the chronic neurological toxicity would probably be closer to 10 million than to 30 million U per injection.

With twice-weekly scheduling myelosuppression was not a clinical problem. Two patients (nos. 10 and 12) were able to continue to receive 30 million U twice weekly for 8 and 10 weeks. Patient 10 then had to stop therapy because of severe lethargy, somnolence, and malaise. Patient 12 found it very difficult to continue to the end of the planned 10 weeks of twice-weekly administration, due to symptoms similar to those described by patient 10. The maximum tolerated dose for twice-weekly administration was therefore also 30 million U. We feel that a reasonable dose for phase II/III studies with twice-weekly scheduling would again lie between 10 and 30 million U per injection, and might be as high as 20 million U.

We conclude from this study that interferon is not a nontoxic form of chemotherapy when it is given in doses greater than 5 million U daily. Due to the peculiar biological

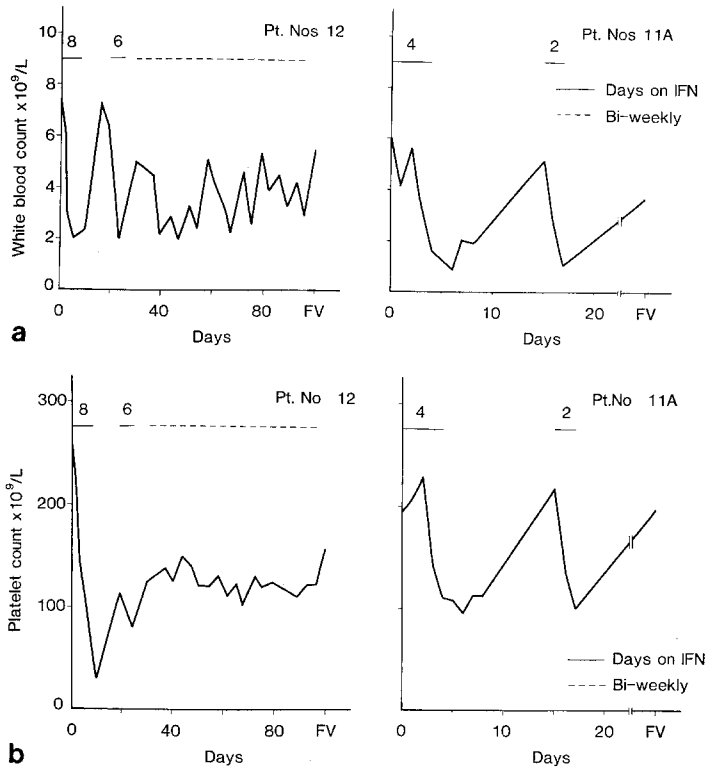

Fig. 2a, b. White blood cell **(a)** and platelet **(b)** counts of two patients treated with 30 million U of human alpha-2 interferon by IM injection

Table 4. Mean values of the liver enzymes in five patients given α-2-IFN by IM injection at 30×10^6 IU per injection daily for 14 days and twice weekly for 10 days

	PRE treatment ($n = 5$)	Day 5 ($n = 5$)	Day 8 ($n = 5$)	Day 15 ($n = 5$)	Day 40 ($n = 2$)[b]	Final visit ($n = 3$)[c]
SGOT (n[a] = 4–45 IU/l)	23.0 (18–33)	58 (23–135)	155 (56–261)	142 (20–168)	63 (54–72)	18 (11–30)
SGPT (n = 5–40 IU/l)	15 (7–23)	23 (7–44)	90 (21–246)	86 (13–99)	38 (34–41)	13 (10–17)
Gamma-GT (n = 0–65 IU/l)	46 (13–96)	63 (20–75)	194 (44–614)	293 (71–291)	149 (107–190)	105 (20–234)

[a] Normal
[b] Only two patients were still receiving IFN at day 40
[c] One patient died; other sample lost

properties of IFN it is not yet clear whether it should be administered at the maximum tolerated dose. We know that some of the enhancement of immune function seen with IFN is not found at higher doses, and indeed at these doses IFN may become immunosuppressive [8]. Careful phase II/III studies in conjunction with immunological testing will be required to establish the optimum dose and scheduling for this agent.

The toxicity which causes most distress to patients receiving IFN is that of central nervous system depression. This syndrome consists in malaise, lethargy, somnolence, depression, and in some patients even confusion. Abnormal EEGs have been documented in patients receiving even low doses of IFN [6]. It is uncertain what the mechanism of this phenomenon is, since IFN is known to pass poorly into the cerebrospinal fluid. A better understanding of the causes of this syndrome might permit pharmacological intervention, thus allowing the quality of life of patients receiving IFN to be improved.

The acute influenza-like syndrome can be severe and distressing at times. We used paracetamol in an attempt to ameliorate this, but we did not find it particularly effective. We were reluctant to use prostaglandin synthetase inhibitors since we were uncertain of the nature of the interaction between these agents and the biological properties of IFN. Tachyphylaxis developed within a few days and this syndrome ceased to be a significant clinical problem.

In conclusion, we feel that IFN justifies further clinical study to define its role in the management of patients with

malignancy. In this study we have produced data which will allow acceptable doses for phase II/III testing to be selected and also permit the toxic spectra of other recombinant DNA species of IFN (i.e., gamma and beta IFN), which will shortly become available, to be compared with this agent.

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