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A phase I study of recombinant interferon gamma administered by s.c. injection three times per week in patients with solid tumours

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Summary. Human recombinant DNA interferon gamma (IFN-G), with a specific activity of 2×10^6 IU/mg protein, was administered s.c. 3 days per week for 2 months to patients with solid tumors. The maximum tolerated dose (MTD) was 10×10^6 IU/m^2 (5.0 mg/m^2) per injection, and six patients were treated at the MTD. Two of these ceased treatment because of severe subjective toxity (headache, rigors and pyrexia) and three patients developed WHO grade 3 leucopenia. Subjective toxicity varied considerably between patients and some patients at low dose levels experienced severe constitutional symptoms whilst others treated at the MTD had few side effects. These differences were unrelated to pharmacokinetic parameters. Bioavailability of this IFN-G administered s.c. was very variable from one patient to another at the same dose level. We therefore counsel caution in using this IFN-G preparation s.c. in phase II studies.

Introduction

Interferon gamma (IFN-G) is distinct from the other two main types of interferon. It is produced by T lymphocytes in response to specific antigens or T cell mitogens and is acid labile [14]. IFN-G binds to a separate cell surface receptor compared to interferons alpha and beta [3], has greater anti-proliferative activity [2, 4, 12], better stimulation of macrophage function [9, 11] and effects on cell surface antigen expression and cellular differentiation [1, 6]. These differences make IFN-G a better candidate for anti-tumour therapy than either interferon alpha or beta. However the latter interferons were easier to produce and purify on a large scale than IFN-G and it is only recently that human recombinant IFN-G has become available for clinical evaluation.

A number of reports of phase I studies with recombinant IFN-G have appeared recently but these studies have largely involved i.v. or infusional administration [5, 7, 8, 13, 15, 16, 17]. These routes usually require hospitalisation causing considerable disruption to the life style of the patient, particulary when the treatment is prolonged. We have therefore investigated the tolerance and pharmacokinetics of IFN-G administered s.c. 3 days per week for 2 months as an outpatient therapy.

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Materials and methods

All the patients in this study had a histologically confirmed disseminated solid tumour, which was refractory to conventional therapies or of which no satisfactory treatment existed. Patients had to have a Karnofsky performance score of 50% or more, a life expectancy of at least 2 months and be willing and able to comply with the requirements of the study. Haematological, renal and hepatic function were confirmed to be normal by routine laboratory tests prior to study entry (total WBC $\geqslant 3.0 \times 10^9$/l, platelets $\geqslant 100 \times 10^9$/l, serum creatinine, creatinine clearance, calcium and hepatic enzymes all less than 25% above the upper limit of normal and a normal ECG). The patients had not received chemotherapy or radiotherapy for 4 weeks prior to study entry (6 weeks for nitrosoureas and mitomycin C) and did not have leucocyte or platelet transfusions for the same period. Patients with CNS metastases or serious concomitant medical illness were excluded. Patients with leukaemia, lymphoma or multiple myeloma were not considered for study entry.

Pre-treatment evaluation of the patients included full history and clinical examination, full blood count, serum chemistries, coagulation profile, 24-h urine collection for creatinine clearance, urinalysis, serum interferon and neutralisation assays and serum for storage at $-70\,^{\circ}$C. These investigations were performed weekly during the study. Following cessation of therapy they were performed at weekly intervals until all abnormal results due to IFN-G had returned to normal. A chest X-ray, and ECG were performed initially and repeated after 1 month of treatment and again at the end of the therapy. Toxicity was assessed using a 0–4 grading system [10] and recorded weekly. In order to allow comparison of the severity of toxicity in different groups of patients and to assess the average toxicity experienced by a particular patient during the course of the study a weighted severity score (WSS) was used:

$$\text{WSS} = \frac{\sum \text{number of reports of a particular grade of reaction} \times \text{that grade of toxicity}}{\text{total number of reports of every grade of toxicity}}$$

The IFN-G was supplied by the Schering Corporation via its Swiss subsidiary Werthenstein Chemie AG, and was recombinant DNA-produced human IFN-G (SCH 36850) with a specific activity of 2×10^6 IU/mg protein. The IFN-G was supplied as a lyophilized powder and the vials

contained either 0.5 or 1.0 mg of IFN-G. These vials were stored at 2 °–8 °C. The IFN-G was reconstituted by the addition of 1.0 ml of sterile water for injection and was always administered immediately after reconstitution.

Patients received escalating doses of IFN-G by s.c. injection on Monday, Wednesday and Friday of each week for a total of 8 weeks. Patients were observed in hospital initially being discharged 24 h after the second injection of IFN-G (toxicity permitting). Further injections were administered at home either by the patient or the community nurse. The patients were seen at weekly intervals. In stable or responding patients treatment was continued until there was evidence of progressive disease. Three patients were entered at each dose level. If a patient did not receive 4 weeks treatment for reasons other than toxicity a further patient was entered at that dose level. When grade 3 or 4 toxicity was observed two additional patients were entered at that level. The maximum tolerated dose (MTD) was defined as that dose level which produced grade 3 or 4 toxicity in 3 out of 5 patients. If a patient developed grade 3 or 4 toxicity the IFN-G was either suspended and restarted at the next lower dose after recovery from toxicity or the dose reduced without suspension of treatment. This decision was at the discretion of the investigator and based on the type of toxic reaction and the condition of the patient. If grade 3 or 4 toxicity persisted at the reduced dose the IFN-G was stopped.

Pharmacokinetics were performed at each dose level. Blood (10 ml) was taken at 0, 0.5, 1, 2, 4, 8, 12 and 24 h after the first injection of IFN-G. The serum from these samples was separated and stored frozen at −70 °C. The samples were assayed for IFN-G content using an IRMA assay (Boots-CELLTECH Ltd., Berkshire, England). A polystyrene bead coated with anti-human IFN-G polyclonal sheep antibody was added to appropriate dilutions of sera. After a 3-h incubation period, the fluid was removed and the beads washed once. Then a monoclonal anti-IFN-G antibody labelled with ^{125}I was added and incubation was continued at ambient temperature for 1 h followed by 2°–8 °C overnight. The beads were then washed, the amount of bound radioactivity determined and compared to that of a standard curve.

Results

A total of 32 patients were entered into the study and their characteristics are shown in Table 1. The planned dose levels, actual doses administered and number of injections given are detailed in Table 2.

Subjective toxicity

There was no subjective toxicity at the lowest dose administered (0.05 mg/m^2, 0.1×10^6 IU/m^2). At doses between

Table 1. The characteristics of patients given IFN-G in a phase I study of three times weekly s.c. administration

Number of patients	32
Males	8
Median age (range)	53 (23–69) years
Median time from diagnosis	16 (1–84) months
Number with no prior therapy	10
Diagnosis: ovarian carcinoma	10
melanoma	8
sarcoma	5
renal cell carcinoma	4
colon carcinoma	2
unknown primary	2
non-small cell lung	1

Table 2. The details of dose levels of IFN-G administered by s.c. injection to patients with solid tumours in a phase I study

Dose level mg/m^2 (IU/m^2)	Number of patients	No prior therapy	Mean doses given (range)	Numbers stopping for toxicity
0.05 (0.1)	3	3	24 (24)	0
0.25 (0.5)	4	0	13 (4–18)	0
0.5 (1.0)	3	0	26 (24–28)	0
1.0 (2.0)	3	0	16 (11–24)	0
2.0 (4.0)	5	1	19 (8–24)	0
3.0 (6.0)	3	1	17 (4–25)	1
4.0 (8.0)	5	3	16 (7–24)	1
5.0 (10.0)	6	2	16 (4–24)	2

Table 3. Subjective adverse experiences recorded as the WSS and the number of patients at each dose level with grade 2 or more toxicity on at least one occassion during treatment with IFN-G

Dose IFN-G (mg/m^2)	0.05		0.25		0.5		1.0		2.0		3.0		4.0		5.0	
Number in group	3		4		3		3		5		3		5		6	
	Toxicity	WSS	Toxicity	WSS	Toxicity	WSS	Toxicity	WSS	Toxicity	WSS	Toxicity	WSS	Toxicity	WSS	Toxicity	WSS
Chills/rigors	0	0	1	0.5	1	1.0	1	0.9	0	0.7	2	0.8	4	0.9	1	0.5
Fatigue	0	0	4	1.0	1	0.2	1	0.6	4	0.8	2	0.9	3	0.7	3	0.9
Malaise	0	0	4	1.4	1	1.0	2	1.3	4	1.7	2	1.1	5	1.3	4	1.4
Headaches	0	0	0	0.2	1	0.6	0	0.3	0	0.1	1	0.3	2	0.5	3	0.8
Myalgia	0	0	0	0.2	0	0.2	1	0.6	0	0.1	1	0.5	0	0.1	0	0.1
Gastro-intestinal toxicity[a]	0	0	3	1.0[b]	0	0.1	1	0.3	2	1.4	1	0.3	4	0.9	2	0.5
Dry mouth	0	–	1	–	1	–	0	–	1	–	3	–	4	–	4	–

[a] Gastro-intestinal toxicity: grade 1 = anorexia, grade 2 = nausea, grade 3 = transient vomiting, grade 4 = intractable vomiting

[b] One patient had grade 4 reaction due to tumour-related small bowel obstruction. WSS excluding this patient was 0.7

0.25 and 2.0 mg/m^2 (0.5 and 4.0 × 10^6 IU/m^2) there was no clear relationship between the IFN-G dose administered and either the WSS or the proportion of patients reporting ≥ grade 2 toxicity (Table 3). For chills/rigors, headaches and dry mouth the proportion of patients reporting ≥ grade 2 toxicity was higher at 3.0, 4.0 and 5.0 mg/m^2. Subjective toxicity consisting of severe headaches, chills/rigors and pyrexia were the reason for stopping treatment in three patients, one at 3.0 mg/m^2 and two at 5.0 mg/m^2. In two of these patients severe toxicity continued despite reducing the IFN-G dose and there was no evidence of tachyphylaxis. One other patient receiving a dose of 4.0 mg/m^2, stopped IFN-G therapy after 15 injections because of profound tiredness and malaise which recovered completely within 2 weeks of cessation of treatment. There was no clear relationship between these acute reactions and either the peak serum IFN-G level or the area under the time concentration curve (AUC). Dryness of the mouth was a particular problem for the majority of patients at doses above 3.0 mg/m^2.

Objective toxicity

Changes in oral temperature during the first 24 h after the first dose of IFN-G are shown in Table 4. At 0.25 mg/m^2 two of the four patients had a fever between 38.0° and 38.9 °C. At the maximum tolerated dose of 5.0 mg/m^2 all six patients had a fever ≥38.0 °C and in one of these WHO grade toxicity (>40 °C) was observed. There were highly significant correlations between the rise in body temperature and the AUC in the pharmacokinetics studies (Pearson's correlation $r=0.44$ $t=2.2$ $df=20$; Spearman's correlation rho $=0.43$ $t=2.16$ $df=20$).

The number of patients at each dose level experiencing different degrees of myelosuppression as judged by nadir WBC are shown in Table 5. Grade 3/4 toxicity was observed first at an IFN-G dose of 2.0 mg/m^2. Half the patients at the MTD of 5.0 mg/m^2 had grade 3 toxicity. A scattergram of the WSS for neutropenia (grade by WHO cirteria) versus AUC is shown in Fig. 1. There was a highly significant correlation between the degree of neutropenia and the AUC (Pearson's correlation $r=0.51$ $t=2.68$ $df=21$), the total dose of IFN-G given (Pearson's correlation $r=0.43$ $t=2.62$ $df=30$) and the peak serum level of IFN-G (Pearson's correlation $r=0.44$ $t=2.22$ $df=21$). In no patient did the platelet count drop below 100 × 10^9/l.

Mild and reversible elevations of aspartate aminotransferase occurred in patients receiving 2.0 mg/m^2 or more. However WHO grades 2 (two patients) and 3 (one patient) were only observed at 5.0 mg/m^2. Isotope liver scans did not show metastases in these three patients. Aspartate aminotransferase was the most sensitive parameter of liver dysfunction with elevations in γ-glutamyltransferase and serum alkaline phosphatase being much less pronounced. No patient had bilirubin values above normal.

Of all the patients, 13 (41%) developed erythematous reactions at the sites of IFN-G injections. The frequency of this phenomenon tended to increase with increasing dose. The reaction was severe in one patient (2.0 mg/m^2) with associated oedema of the upper arm and moderately severe in two others (3.0 mg/m^2 and 4.0 mg/m^2). Two of these patients had skin biopsies which showed a mild dermal infiltrate of small lymphocytes.

There was no significant correlation between the dose of IFN-G given and changes in weight (Pearson's correlation $r=-0.31$ $t=-1.56$ $df=23$), serum albumin (Pearson's correlation $r=0.19$ $t=0.96$ $df=26$) or Karnofsky performance status.

A tachycardia ≥120/min occurred in four patients after their first IFN-G injections. Two were given 0.25 mg/m^2, a third 2.0 mg/m^2 and the fourth 5.0 mg/m^2. Signifi-

Table 4. Maximum oral temperature during the first 24 h after IFN-G. The doses are given on the left in mg/m^2

Dose	Temperature				
	<37.0°C	37.0° –37.9°C	38.0° –38.9°C	≥39.0°C	Number in group
0.05	3	0	0	0	3
0.25	0	2	2	0	4
0.5	0	3	0	0	3
1.0	0	1	1	1	3
2.0	0	3	1	1	5
3.0	0	1	2	0	3
4.0	0	3	1	1	5
5.0	0	0	3	3	6

Table 5. Nadir total WBC graded by WHO toxicity scale. The range of each WHO grade is given at the head of each column (x10^9/l) and the IFN-G doses (mg/m^2) are given in the left hand column

	WHO toxicity grade				
	0 ≥4.0	1 3.9–3.0	2 2.9–2.0	3 1.9–1.0	4 <1.0
0.05	1	2	0	0	0
0.25	2	1	1	0	0
0.5	0	0	3	0	0
1.0	0	2	1	0	0
2.0	1	1	1	1	1
3.0	0	0	3	0	0
4.0	1	2	1	1	0
5.0	2	1	0	3	0

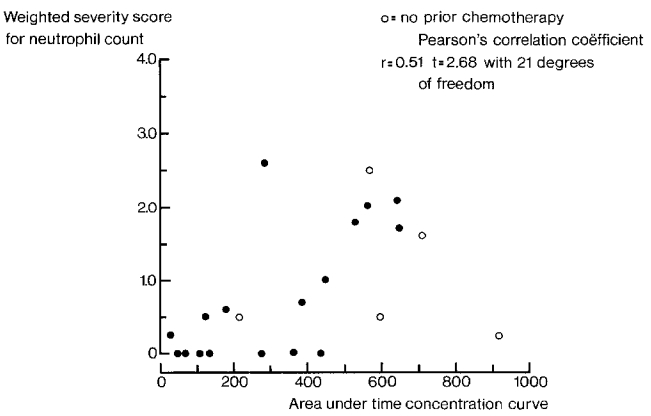

Fig. 1. A scattergram of the toxicity to neutrophils (recorded as the weighted severity score, WSS) versus the area under the time concentration curves (AUC) during the first 24 h after s.c. administration of recombinant DNA gamma interferon (IFN-G)

cant falls in systolic blood pressure (≥20 mm Hg) occurred in nine patients but none of these had symptoms of hypotension. There was no correlation between IFN-G dose or AUC and change in heart rate or systolic blood pressure from baseline values using both Pearson's and Spearman's correlation tests.

Pharmacokinetics

Results of the pharmacokinetic investigations are given in Fig. 2 and the AUC for each dosage group in Fig. 3. There was considerable interpatient variation in the AUC for patients receiving the same IFN-G dose. Substantial levels of IFN-G were still present in the serum 24 h after administration. The mean peak serum level in four patients given 5.0 mg/m^2 (10 × 10^6 IU/m^2) was 35 IU/ml (11–56 IU/ml). In two patients the pharmacokinetics were different from the other patients. The serum levels rose progressively throughout the 24 h of the study. IFN-G could not be measured in the urine of these patients.

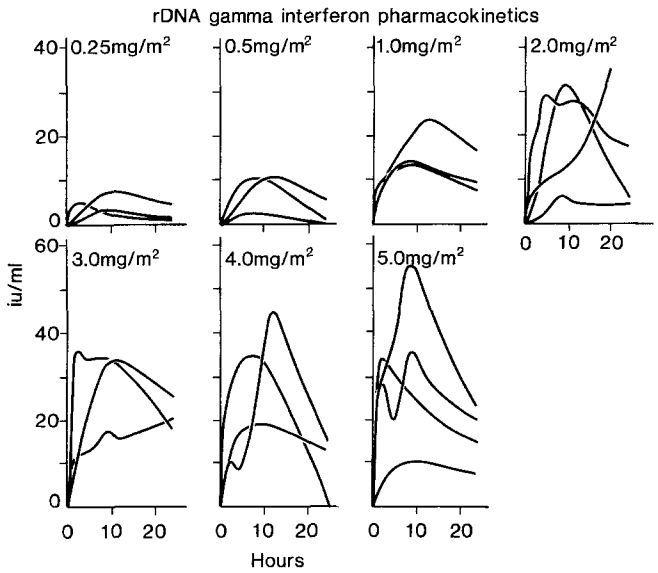

Fig. 2. The pharmacokinetic curves for patients given s.c. IFN-G

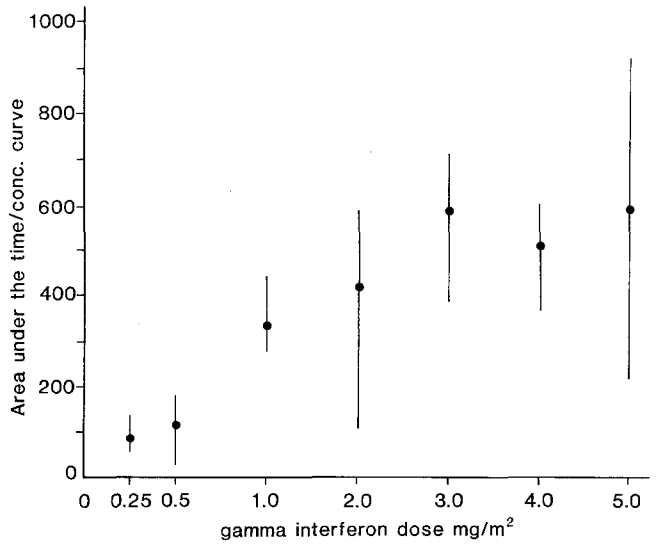

Fig. 3. The AUC during the first 24 h after s.c. administration of IFN-G plotted against the dose of IFN-G given. The bars show the variation in AUC between patients at a given dose

Deaths

Two patients died during the course of the study. One patient, with a non-small cell lung cancer developed rapidly progressive disease from which she died. The second had advanced ovarian carcinoma and had a Le Veen shunt in situ to control intractable ascites. She received four injections of IFN-G and was then admitted to hospital with dyspnoea. A chest X-ray was normal but a ventilation-perfusion radioisotope lung scan was consistent with multiple pulmonary emboli. She developed thrombocytopenia (<20 × 10^9/l) but had no laboratory evidence of disseminated intravascular coagulation or renal failure. It was felt that the pulmonary emboli were due to tumour cells entering the pulmonary vasculature via the Le Veen shunt and that platelets were being sequestered in the lungs. Despite ligation of the Le Veen shunt and anti-coagulation with heparin she developed progressive respiratory failure and died. The relatives refused an autopsy.

Anti-tumour effects

No objective responses were observed in this study. One patient experienced reduction in the size of her pleural effusion but subsequently developed increased fluid.

Discussion

In this study we administered human recombinant DNA IFN-G by three times weekly s.c. injection to patients with solid tumours. The MTD was 5.0 mg/m^2 (10 × 10^6 IU/m^2). At this dose level three of six patients developed WHO grade 3 neutropenia, one a WHO grade 3 rise in aspartate aminotransferase and one WHO grade 3 pyrexia. Two patients had to have IFN-G stopped at the MTD because of severe headache, chills/rigors and malaise. On the basis of the toxicity observed a tolerable dose for phase II studies would be 1.0 mg/m^2 (2 × 10^6 IU/m^2).

There are no reports of phase I studies with IFN-G given s.c., although data are available concerning i.m. administration. The MTD for single i.m. injections was 2.5 mg/m^2. However this IFN-G preparation had a specific activity of 20 × 10^6 IU/mg of protein giving an MTD of 50 × 10^6 IU/m^2 [7, 8]. When this IFN-G was given i.m. daily for 6 weeks the MTD was 0.25–0.5 mg/m^2 (5.0–10.0 × 10^6 IU/m^2) [8]. Thus, as might be expected, in terms of anti-viral units the MTD is of the same order for three times per week s.c. injection (10 × 10^6 IU/m^2) as daily i.m. injections (5.0–10.0 × 10^6 IU/m^2). In these latter two studies the dose limiting toxicities were very similar to those reported here.

A major problem with s.c. administration of this IFN-G lies in the bioavailability. There was considerable inter-patient variation in the AUC during the first 24 h which became more prominent at the higher doses. This wide variation in AUC was not observed following i.m. administration of IFN-G [7].

Although in general terms there was a dose-response relationship between toxicity and administered dose of IFN-G a degree of idiosyncracy was observed in these patients. Some patients at low dose had quite marked consti-

tutional symptoms whilst others at the MTD tolerated the drug with little toxicity. This difference was unrelated to either the peak serum IFN-G levels or the AUC.

In conclusion we feel that there would be considerable problems in using the s.c. route of administration for phase II/III studies with this IFN-G preparation. The variability in bioavailability would mean that pharmacokinetic profiles would be needed for each patient and the dose of drug adjusted so that the peak levels and AUC were similar for each of the patients in the study. Even then the idiosyncratic nature of the subjective toxicities may mean that an unacceptable number of patients would stop therapy at relatively low doses of IFN-G, thus prejudicing the results of phase II studies. It may be that s.c. administration of this IFN-G is not a viable route for further clinical studies with this drug.

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