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Pharmacokinetics, Single-Dose Tolerance, and Biological Activity of Recombinant γ-Interferon in Cancer Patients[1]

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ABSTRACT

We report a clinical study of the pharmacokinetics, toxicity, and biological activity of i.v.- and i.m.-administered recombinant γ-interferon (rIFN-γ) consisting of 143 amino acids. Ten patients with metastatic cancer were given rIFN-γ at doses of 0.01 to 2.5 mg/sq m by alternating i.m. and i.v. bolus injections with a minimum intervening period of 72 h. After i.v. administration, rIFN-γ was cleared monoexponentially with a short half-life of 25 to 35 min as determined by bioassay and enzyme immunoassay. After i.m. injection, a longer half-life of 227 to 462 min was measured by enzyme immunoassay. Serum titers were detected by bioassay only at high doses, suggesting partial loss of antiviral activity at the i.m. site. However, other biological effects were retained as evidenced by fever, chills, and fatigue after both routes of administration and granulocytopenia after i.m., but not i.v., doses. Two of ten patients showed objective evidence of tumor regression. These data suggest that further studies with i.m. as well as prolonged i.v. infusions of rIFN-γ are indicated.

INTRODUCTION

Interferons are a group of naturally occurring inducible proteins with potent antiviral, antiproliferative, and immunomodulating effects (1, 2). On the basis of their antigenic specificities, the interferons are divided into 3 classes. These classes include the large family of leukocyte interferons (IFN-α)[4] and at least one fibroblast interferon (IFN-β), all of which are structurally related, virally induced, acid-stable molecules (3). In contrast, IFN-γ is a structurally distinct, acid-labile glycoprotein produced by T-lymphocytes in response to antigen or mitogen (3). Antitumor activity in several cancers has been demonstrated in clinical investigations of partially pure IFN-α and IFN-β as well as recombinant IFN-α (4–10). *In vitro* studies indicate that different target cell lines show preferential sensitivity to the antiproliferative effects of IFN-γ *versus* IFN-α (11–14). Synergistic antiproliferative effects are obtained when IFN-γ is combined with other interferons (12). These synergistic effects may be related to the presence of distinct human cell surface receptors for IFN-γ *versus* IFN-α or IFN-β and may eventually be exploitable in clinical trials (15). As an immune modulator, IFN-γ has more pronounced activity than the other interferons including potent macrophage-activating effects with enhancement of both tumoricidal and microbicidal properties (16, 17). In addition to suggesting a potential role for IFN-γ in the treatment of human cancers, this agent's diverse range of biological activities may have significant clinical implications in the management of patients with viral diseases, intracellular infections, and acquired immune deficiency syndrome (17–19). Previous clinical investigations of IFN-γ were confined to preliminary Phase I studies of native IFN-γ. This material was not absorbed after i.m. administration and had a rapid serum disappearance curve after i.v. bolus administration (20, 21). Consistent serum antiviral activity was maintained with continuous i.v. infusions. The IFN-γ that was used in these studies was available in limited quantities and was only partially purified. Recently, however, the gene for human IFN-γ was cloned and expressed in *Escherichia coli* (22), permitting production of large amounts of highly purified material.

We report the results of a clinical investigation of the pharmacokinetics, single-dose tolerance, and biological activity of rIFN-γ in patients with disseminated cancer. This study will serve as a model for further development of this unique lymphokine as an antitumor and antiinfectious agent.

MATERIALS AND METHODS

The isolation, production, characterization, and purification of human rIFN-γ were done by Genentech, Inc. DNA containing the entire gene sequence for human IFN-γ was spliced into a plasmid which was inserted into *E. coli* (22). Replication of the IFN-γ gene occurs as the *E. coli* bacteria divide, allowing for the production of large quantities of rIFN-γ. The clinical material used in this study was genetically engineered to contain 143 amino acids identical in sequence to native human IFN-γ, except for the presence of a methionine residue at the *N*-terminal (23). The final purity of rIFN-γ was >98% as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Sterility was verified by the direct inoculation technique. The final product contains <0.5 ng of endotoxin per mg of protein as assessed by the *Limulus* amebocyte lysate assay. The specific activity of rIFN-γ was $\geq 1 \times 10^7$ units/mg of protein based on antiviral activity measured by inhibition of EMC virus replication in A549 cells (human lung carcinoma cell line), with reference to the IFN-γ standard Gg 23-901-530 of the NIH, Bethesda, MD.

Biological activity for the recombinant product was confirmed (data on file, Genentech, Inc.) by *in vitro* tests showing antiviral activity against EMC, Herpes Simplex I, rhinovirus, and vaccinia virus and *in vivo* antiviral activity in squirrel monkeys infected with EMC. In addition, *in vitro* antiproliferative activity against various human tumor cell lines including those derived from melanoma (G361) and osteosarcoma (G292) was demonstrated. rIFN-γ also demonstrated *in vitro* immunoregulatory activity including stimulation of immunoglobulin secretion and natural killer cell-mediated cytotoxicity as well as activation of macrophages.

The interferon was provided as a sterile lyophilized powder. Vials were reconstituted with sterile water immediately before injection to a concen-

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[4] The abbreviations used are: IFN-α, α-interferon (leukocyte interferon); IFN-β, β-interferon (fibroblast interferon); IFN-γ, γ-interferon; rIFN-γ, recombinant γ-interferon; EMC, encephalomyocarditis; ELISA, enzyme-linked immunosorbent assay.

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tration of 0.2 mg/ml for dose levels ≤0.1 mg/sq m and 1.0 mg/ml for higher dose levels.

Patient Selection and Study Design. Ten patients with disseminated cancer were entered on the study. All patients had histopathological confirmation of their diagnosis. Patients were ambulatory with Karnofsky performance status >50% (24) and had not received antitumor therapy for a minimum of 3 weeks prior to entrance in the study. Eligibility criteria included a life expectancy of at least 3 months and preserved hepatic (bilirubin, <1.5 mg/dl), renal (creatinine, <2 mg/dl), and hematological functions (granulocytes, ≥1,500/cu mm; platelets, ≥100,000/cu mm). Informed consent was obtained in accordance with institutional policy.

Two patients were entered sequentially at each dose level, starting with the lowest dose. The dose levels were 0.01 (0.2×10^6), 0.05 (1×10^6), 0.10 (2×10^6), 0.25 (5×10^6), 0.5 (10×10^6), 1.0 (20×10^6), and 2.5 mg/sq m (50×10^6 units/sq m). Each patient was treated on a twice weekly schedule with i.m. injections alternating with i.v. bolus injections, the latter being administered over 5 min. A minimum period of 72 h was required between injections. However, at the discretion of the investigators, longer intervals were used depending on biological effects. Each patient was escalated to the next dose level, providing there were no serious adverse effects after receiving treatment by each route of administration at the prior dose level. Each patient received a maximum of 4 dose levels.

Patients were monitored daily. All symptoms were recorded and classified as mild, moderate, or severe. "Mild" referred to symptoms that caused no change in performance levels and did not require medication for relief. "Moderate" symptoms were those that required medication for relief. "Severe" signified symptoms that were inadequately relieved by medication and caused a greater than 25% drop in performance status. Vital signs including heart rate, blood pressure, temperature, and respiratory rate were recorded before injection and at 1, 2, 4, 6, 8, 12, and 24 h after i.m. injections; and at 10 min, 30 min, and 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h after i.v. injections. A physical examination was done before the initial dose and twice weekly thereafter, while the patient was on the study. An electrocardiogram and chest roentgenogram were done before the study and after the final dose. A complete blood count was done every 24 h. Electrolytes and a chemistry profile including renal and liver function tests were measured before and 24 h after each dose. Triglycerides and cholesterol were measured weekly. A urinalysis, prothrombin time, partial thromboplastin time, and reticulocyte count were obtained before each injection and at the end of the study.

Evaluation of tumor size was done by physical examination and appropriate radiological studies and scans as well as tumor markers. The criteria for responses have been previously described (9).

Pharmacology Studies. Venous serum samples were collected prior to injection and at 1, 2, 4, 6, 8, 12, and 24 h following i.m. doses; and at 10 min, 30 min, and 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h after i.v. doses. Blood samples were centrifuged, and the serum was decanted and stored at 0°C until analysis. Serum rIFN-γ titers were measured by both a bioassay and ELISA at Genentech, Inc. The bioassay was performed using an antiviral cytopathic effect as described previously (25), with the human lung cancer cell line A549 as a target of EMC virus. The 50% end point was determined, and the interferon titer was expressed as the reciprocal of that dilution. All samples were corrected to the reference IFN-γ standard, Gg 23-901-530, of the NIH. A solid-phase ELISA was performed using a sandwich technique with 2 polyclonal antibodies to rIFN-γ, one of which was conjugated to a horseradish peroxidase label.

The means of blood levels for each dose for all patients at that dose were calculated. Serum disappearance curves and nonlinear regression analyses were carried out using these values. Serum half-lives, volumes of distribution, and the area under the concentration curve ($C \times t$) were then calculated by standard techniques (20).

The presence of antibodies to rIFN-γ was determined on blood samples obtained before study and 1 and 28 days after completion of the study. A modification of the bioassay was used in which neutralization of the antiviral effect of interferon-containing samples was measured.

RESULTS

Ten patients with metastatic cancer were treated. There were 6 women and 4 men with a median age of 55 years (range, 35 to 80 years). Three patients had renal cell carcinoma; 2 had sarcomas; and one each had adenocarcinoma of colon, nodular poorly differentiated lymphocytic lymphoma, carcinoid, multiple myeloma, and adenocarcinoma of the lung. Seven of the patients had previously received chemotherapy, and one patient had previously received partially purified buffy coat IFN-α.

The pharmacokinetic parameters of rIFN-γ are shown in Tables 1 and 2. In patients receiving rIFN-γ by the i.m. route, titers detected by ELISA were adequate for pharmacology studies in 3 to 8 treatments at each dose level from 0.1 to 2.5 mg/sq m (Table 1). However, titers were detectable by bioassay only at the higher dose levels, and the titers detected were low. After i.v. bolus administration of rIFN-γ, titers were below the limits of sensitivity of the ELISA assay (0.4 ng/ml) at doses below 0.1 mg/sq m and of the bioassay (40 units/ml) at doses below 0.25 mg/sq m. At doses above these levels, adequate pharmacoki-

Table 1

Serum pharmacokinetics of rIFN-γ by ELISA after i.m. administration

Dose (mg/sq m)	$t_{½}$ ABS[a] (min)	$t_{½}$ CL (min)	$C \times t$ (ng/ml × min)	% of dose absorbed
0.1 (6)[b]	91.6[c]	345	1,029	50
0.25 (8)	96	227	2,558	77.2
0.5 (7)	91	462	8,962	52.3
1.0 (5)	144	266	10,000	32.7
2.5 (3)	142	248	24,640	52.3

[a] $t_{½}$ ABS, absorbance half-life; $t_{½}$ CL, clearance half-life; $C \times t$, exposure time (concentration × time).
[b] Numbers in parentheses, number of patients.
[c] Values expressed as means.

Table 2

Serum pharmacokinetics of rIFN-γ by ELISA and by bioassay after i.v. administration

Dose (mg/sq m)	No. of patients		Pharmacokinetic parameters							
			$t_{½}$ (min)		V_d[a] (liters)		$C \times t$ (ng/ml × min)		Clp (ml/kg × min)	
	ELISA	Bioassay	ELISA	Bioassay	ELISA	Bioassay	ELISA	Bioassay	ELISA	Bioassay
0.1	6		32[b]		25		275		7.3	
0.25	8	7	34.5	33.0	24.3	17.2	766	1,025	6.5	4.8
0.5	7	5	29.7	32.7	15.7	12.8	2,051	2,766	4.0	3.6
1.0	5	5	25.2	25.9	10.9	10.2	4,982	5,222	4.0	3.6
2.5	3	2	33.6	29.9	11.9	12.8	15,267	12,550	3.3	4.0

[a] V_d, volume of distribution; $C \times t$, exposure time (concentration × time); Clp, plasma clearance.
[b] Values expressed as means.

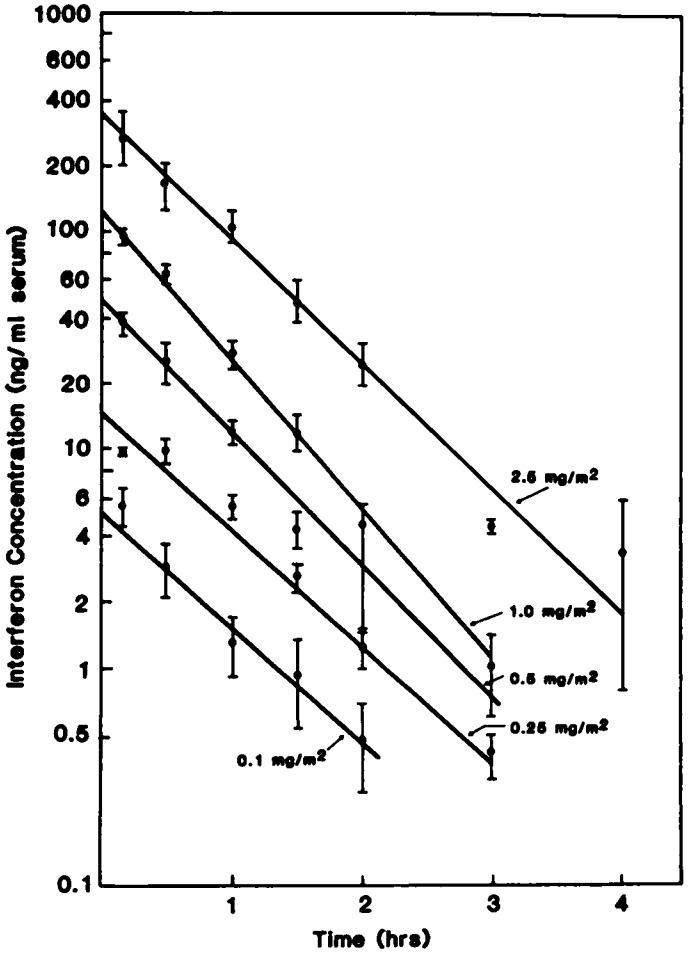

Chart 1. Serum disappearance of rIFN-γ after i.v. bolus administration as measured by ELISA. *Points*, mean of blood levels for each dose for all patients at that dose; *bars*, SE. *Solid lines* indicate the least-square regression lines calculated for each set of values.

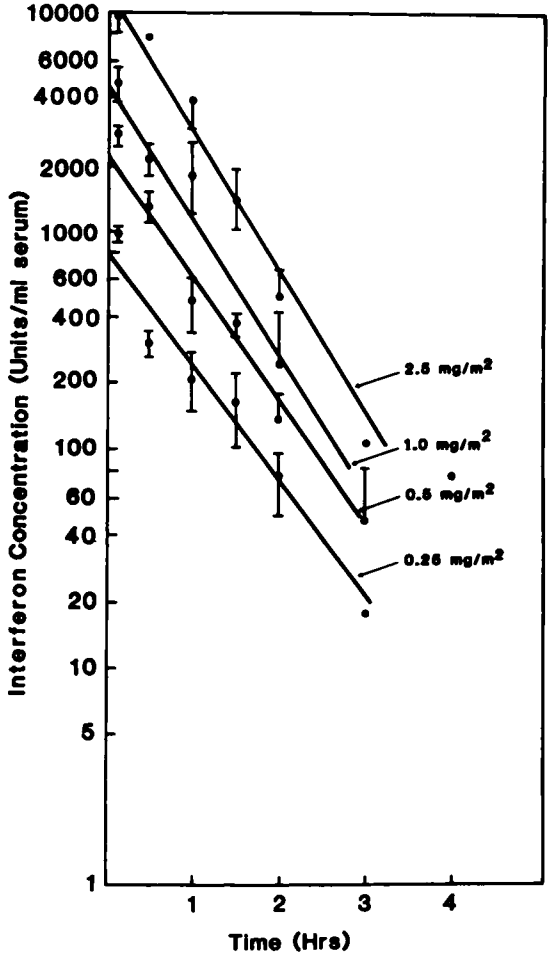

Chart 2. Serum disappearance of antiviral activity after i.v. bolus administration. *Points*, mean of blood levels for each dose for all patients at that dose; *bars*, SE. *Solid lines* indicate the least-square regression lines calculated for each set of values.

netic measurements were obtained in 3 to 8 treatments at each dose level (Table 2).

According to data from both assays, the half-life of rIFN-γ given by the i.v. route was 25 to 35 min and was not dependent on dose (Table 2). In contrast, the half-life of rIFN-γ given by the i.m. route varied from 227 to 462 min independently of the dose (Table 1). After i.v. injection, the clearance of biologically active material and the clearance of ELISA-positive material both fit ($r^2 > 0.95$) an open one-compartment model (Charts 1 and 2). After i.m. injection, the pharmacological data fit an open 2-compartment mathematical model (Chart 3). Between 33 and 77% of the dose administered i.m. was absorbed (Table 1). The exposure time calculated by concentration × time ($C \times t$) was higher for i.m. than for i.v. injection and was directly proportional to the dose, increasing from 1,029 to 24,640 ng/ml × min over the dose range of 0.1 to 2.5 mg/sq m after i.m. injection and from 275 to 15,267 ng/ml × min after i.v. injection, as determined by ELISA assay, at the corresponding dose levels (Tables 1 and 2).

The clinical side effects associated with rIFN-γ are shown in Tables 3 and 4. The majority of i.m. injections at a dose level ≥0.1 mg/sq m resulted in fever, chills, fatigue, and myalgias, with escalation in dose level corresponding to increasing severity of symptoms (Table 3). Fever began 2 to 4 h after i.m. injection and peaked at 6 to 12 h. Gastrointestinal symptoms were mild and usually consisted of nausea with an occasional episode of vomiting. The site of i.m. injection was monitored and showed only rare minimal local erythema.

Administration of rIFN-γ by the i.v. route was accompanied by similar side effects (Table 4). However, the patients noted that symptoms appeared sooner after i.v. injections but were short-lived, resolving completely within 24 h, whereas after i.m. doses, side effects often persisted for 48 h. The only changes in vital signs that were noted were a mild tachycardia and increase in blood pressure that often accompanied elevation in temperature. Occasional side effects reported included emotional lability consisting of crying episodes on the day of injection in one patient. In addition, a brief episode of blurred vision occurred in one patient after a single treatment but did not recur when higher doses were administered to the same patient. Clinical neurological examination was normal in both of these patients. Weight loss ranging from 0 to 8 kg occurred over the 4-week treatment period. Lesions indicative of recurrent herpes simplex developed in one patient after 3 doses.

Dose-limiting toxicity consisting of severe fatigue accompanied by high fever was seen in 5 of 10 patients. The maximum doses achieved in each of these patients were 0.25, 1.0, 2.5, 2.5, and 2.5 mg/sq m after both i.m. and i.v. injections.

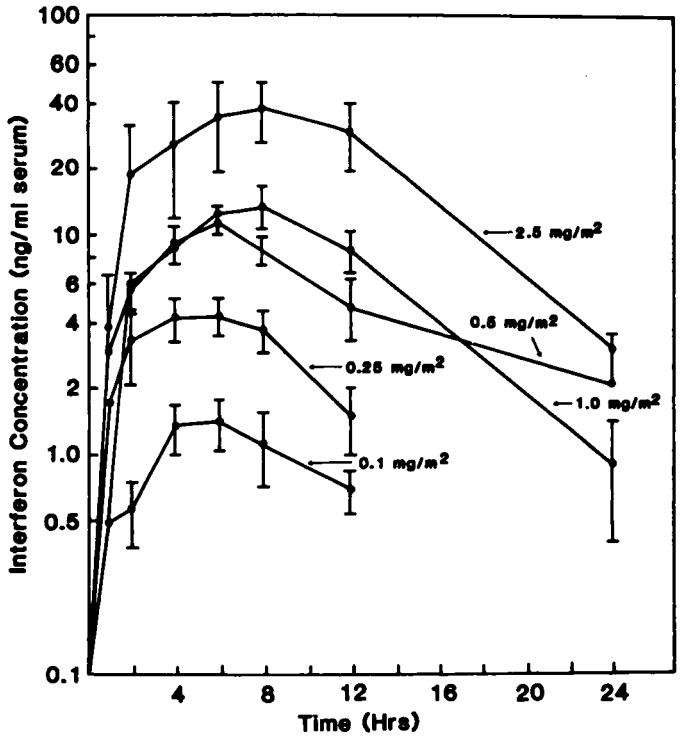

Chart 3. Serum disappearance of rIFN-γ after i.m. administration as measured by ELISA. *Points*, mean of blood levels for each dose for all patients at that dose; *bars*, SE.

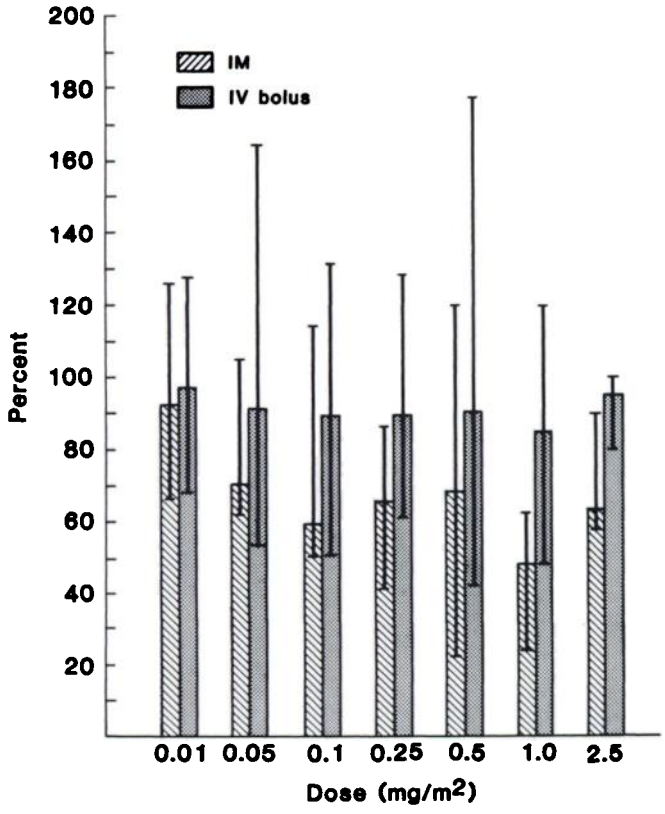

Chart 4. Granulocyte counts after i.m. and i.v. bolus administration of rIFN-γ. *Columns*, median of the granulocyte count observed 24 h after injection of interferon, expressed as a percentage of the base-line counts; *bars*, range.

The effect of i.m. and i.v. bolus administration of rIFN-γ on absolute granulocyte counts is shown in Chart 4. Minimal effects were observed after i.v. bolus rIFN-γ regardless of the dose level. In contrast, a substantial decrease in absolute granulocyte counts was seen starting at rIFN-γ doses of 0.05 mg/sq m given by the i.m. route. However, increasing the dose level did not significantly increase the degree of granulocytopenia, with a median decrement in granulocytes of 30% at a dose of 0.05 mg/sq m and 37% at 2.5 mg/sq m. The lowest granulocyte counts were generally reached at 24 h, and recovery to base line occurred within 48 to 72 h after i.m. injection. Lymphocyte and platelet counts were unaffected by treatment. Hematocrit values showed a median drop of 7% over the 4-week study period, which was most likely due to phlebotomy requirements. Liver and renal function tests remained unchanged throughout the study except for a moderate increase in serum glutamic oxaloacetic transaminase to 229 units/ml in one patient which occurred in association with radiologically documented infarction of hepatic metastases. This value returned to normal 2 weeks after discontinuation of rIFN-γ. Antibodies against rIFN-γ were not detected in any patient.

Two of 10 patients in this study showed objective evidence of tumor regression. One patient with nodular poorly differentiated lymphocytic lymphoma achieved a minor response with disappearance of peripheral adenopathy, a <50% decrease in retroperitoneal adenopathy, and a decrease in lymphocytic infiltrate of the bone marrow from 32 to 18%. The other patient who had metastatic sarcoma previously treated with multiple chemotherapeutic agents, including an Adriamycin-containing regimen and 4 investigational agents, achieved a ≥25% but <50% regression of lung nodules. After treatment was interrupted for approximately 3 weeks, the patient's disease progressed in spite of reinitiation of therapy.

DISCUSSION

IFN-γ is a T-cell-derived lymphokine with the ability to function as a differentiation factor, an antiviral agent, an antiproliferative agent, an immune regulator with potent macrophage-activating effects, and as an antimicrobial agent against intracellular protozoa and bacteria (14, 16–18, 26–28). This unusually broad spectrum of biological activities suggests a potential therapeutic role for this material in patients with cancer, viral diseases, and opportunistic infections. To date, reported clinical studies of IFN-γ have been limited to preliminary pharmacokinetic evaluation of native IFN-γ which was available in limited amounts and was only approximately 40% pure (20, 21). However, recently, synthesis of human IFN-γ in *E. coli* using recombinant DNA technology has been achieved (22). In contrast to the α-interferons which have at least 12 different genetic loci (29), there appears to be only one gene coding for human IFN-γ (30). The material used in this study was genetically engineered to have 143 amino acids, identical in sequence to native IFN-γ except for a methionine residue at the *N*-terminal (23). Preclinical testing has demonstrated that synthetic IFN-γ is biologically active. The major aims of this study were to ascertain the pharmacokinetics, single-dose tolerance, and biological effects of rIFN-γ in patients with disseminated cancer.

The pharmacological parameters of rIFN-γ when measured by ELISA and by bioassay after i.v. bolus administration were similar

Table 3
Side effects associated with rIFN-γ: i.m. route

	% of treatments at the following doses (mg/sq m)						
	0.01 (2)[a]	0.05 (4)	0.10 (6)	0.25 (10)	0.50 (8)	1.0 (6)	2.5 (4)
Fever							
37.2°C–38.3°C	50	25	0	0	12.5	17	25
38.3°C–39.4°C	0	25	67	60	50	33	25
≥39.4°C	0	0	17	40	37.5	50	50
Chills							
Mild	50	50	50	30	37.5	33	25
Moderate	0	25	17	50	25	50	25
Severe	0	0	17	10	25	17	50
Fatigue							
Mild	50	50	33	20	37.5	33	25
Moderate	0	0	33	10	37.5	50	25
Severe	0	0	0	20	12.5	17	50
Myalgia							
Mild	100	25	0	30	12.5	33	50
Moderate	0	0	33	20	37.5	17	0
Severe	0	0	0	0	0	0	0
Headache							
Mild	100	50	33	0	12.5	17	0
Moderate	0	0	33	20	25	17	50
Severe	0	0	0	0	0	0	0
Nausea and vomiting							
Mild	50	25	67	40	37.5	33	25
Moderate	0	0	17	40	0	50	25
Severe	0	0	0	0	0	0	0
Diarrhea							
Mild	0	0	0	0	0	0	0
Moderate	0	0	0	0	12.5	17	0
Severe	0	0	0	0	0	0	0
Dry mouth	0	0	17	30	12.5	17	25

[a] Numbers in parentheses, number of treatments.

(Table 2). The clearance of biologically active material as measured by bioassay and of ELISA-positive material was monoexponential, suggesting an open one-compartment mathematical model (Charts 1 and 2). This clearance pattern is similar to that observed previously for partially pure IFN-γ (20) but differs from the biphasic clearance reported for human IFN-α (31). Using both assays, after i.v. bolus administration, the plasma half-life was between 25 and 35 min and was not dependent on dose (Table 2). This short half-life is similar to that previously reported for native IFN-γ (20). The apparent volume of distribution (V_d) was inversely related to the dose administered. The largest difference in the volume of distribution occurred between the 0.25- and 0.5-mg/sq m dose (Table 2). This result may suggest a saturation of receptor sites or metabolic or excretory mechanisms.

The pharmacokinetic parameters of rIFN-γ after i.m. administration were complex, and there were major differences observed in the bioavailability of rIFN-γ administered by the i.m. as compared to the i.v. route. Serum titers of rIFN-γ were detected by ELISA assay at dose levels ≥0.1 mg/sq m. The clearance $t_{½}$ was much longer than that observed after i.v. injection with values ranging from 227 to 462 min. Recombinant IFN-γ was apparently well absorbed after i.m. administration (Chart 3) with the percentage of dose absorbed ranging from 33 to 77% (Table 1). Interestingly, only low levels of rIFN-γ were detected in the serum by the antiviral bioassay. Given the dissimilar plasma $t_{½}$ for clearance after i.v. *versus* i.m. administration and the high percentage of dose absorbed after i.m. administration, these data suggest that i.m. administration of rIFN-γ may have resulted in absorbance into the plasma of ELISA-positive material which had lost some of its antiviral activity. Nonetheless, this material retained certain biological activities as demonstrated clinically by its ability to induce fever (Table 3) and granulocytopenia (Chart 4). In contrast, our previous studies of native IFN-γ indicated that, after i.m. injection, this material was undetectable in serum by both bioassay (20) and radioimmunoassay[5] up to a dose of 9 × 10^6 units. In addition, native IFN-γ resulted in minimal clinical side effects and granulocytopenia (20) as compared to rIFN-γ. Since the exact correlation between unit measurements of native and recombinant IFN-γ has not been determined, the reasons for the differences in pharmacokinetics between these molecules remain unknown. The partial loss of antiviral activity of rIFN-γ may be explained on the basis of evidence suggesting that the functional antiviral unit of IFN-γ is a tetramer (32). It is possible that disaggregation of rIFN-γ or partial degradation by tissue enzymes resulted in reduction in antiviral as compared to myelosuppressive activity after i.m. injection. In contrast, IFN-α, whose functional unit is a monomer, appears to retain more of its antiviral activity after i.m. administration (32). Since the antiproliferative properties of interferon may be mediated by response pathways different from those associated with the antiviral properties (33), the effect of i.m. injection on the ability of rIFN-γ to inhibit tumor cell growth remains speculative. Furthermore, a recent study showing the successful eradication of

[5] J. U. Gutterman, unpublished data.

Table 4
Side effects associated with rIFN-γ: i.v. route

	% of treatments at the following doses (mg/sq m)						
	0.01 (2)[a]	0.05 (4)	0.10 (6)	0.25 (10)	0.50 (8)	1.0 (6)	2.5 (4)
Fever							
37.2°C–38.3°C	0[a]	0	17	10	12.5	17	0
37.3°C–39.4°C	50	50	50	40	50	50	50
>39.4°C	0	50	34	60	37.5	34	50
Chills							
Mild	50	75	67	50	50	11	25
Moderate	0	25	34	30	12.5	50	25
Severe	0	0	0	20	12.5	17	50
Fatigue							
Mild	0	50	50	60	50	33	25
Moderate	0	25	0	10	25	17	25
Severe	0	0	17	20	0	17	50
Myalgia							
Mild	50	25	0	20	25	50	50
Moderate	50	25	33	30	0	17	0
Severe	0	0	0	10	12.5	0	0
Headache							
Mild	0	50	17	40	12.5	17	0
Moderate	50	25	34	10	25	0	25
Severe	0	0	0	0	0	0	0
Nausea and vomiting							
Mild	0	25	50	60	37.5	34	50
Moderate	0	25	34	30	12.5	17	0
Severe	0	0	0	0	0	0	0
Diarrhea							
Mild	0	0	0	10	0	0	0
Moderate	0	0	0	0	0	0	0
Severe	0	0	0	0	0	0	0
Dry mouth	0	25	0	40	37.5	17	50

[a] Numbers in parentheses, number of treatments.

condyloma acuminata by native IFN-β (34), in spite of no or low serum antiviral levels, suggests that the clinical antiviral efficacy of the interferons may not correlate with serum antiviral activity.

Side effects associated with rIFN-γ administration were qualitatively similar to those previously reported for native IFN-γ (20) and rIFN-α treatment (5) and consisted of fever and flu-like symptoms (Tables 3 and 4). However, the mechanisms of fever induction by these 2 types of interferons may be different. IFN-α has been shown to be intrinsically pyrogenic with its action possibly mediated by prostaglandin E_2 production in the hypothalamus (35). While the mechanisms of fever induction by IFN-γ have not been studied directly, recent evidence suggests that administration of this lymphokine stimulates release of other lymphokines, such as Interleukin-1 and Interleukin-2 (17), which could be responsible for some of the clinical biological activity of rIFN-γ; *i.e.*, release of Interleukin-1, an endogenous pyrogen (36), may account for the fever. Doses i.m. ≥0.05 mg/sq m produced a significant drop in granulocyte counts not seen after i.v. bolus injection at any dose level (Chart 4). This effect may be related to the longer exposure time ($C \times t$) after i.m. *versus* i.v. bolus injection (Tables 1 and 2). Previous studies of partially pure IFN-γ also show little effect on circulating granulocytes after i.v. bolus administration (20). In contrast, consistent granulocytopenia was observed after exposure time was prolonged by giving the interferon by a 6-h i.v. infusion. The necessity of prolonged *in vivo* exposure to IFN-γ before biological activity is evident is supported by *in vitro* work demonstrating that IFN-γ activates cells more slowly than IFN-α or IFN-β (37).

Dose-limiting toxicities of high fever and generalized weakness were observed during this study after both i.v. and i.m. injections in the 3 patients who reached a dose level of 2.5 mg/sq m. Clinical side effects including granulocytopenia were rapidly reversible, with granulocyte counts returning to base line within 48 to 72 h after administration of rIFN-γ. The mechanism by which this agent decreases the number of circulating granulocytes is not clear. Although the rapidity of recovery suggests that rIFN-γ may produce margination or redistribution of these cells, *in vitro* work showing that IFN-γ suppresses myeloid colony formation (38) supports the possibility of bone marrow myelosuppression as the mechanism of granulocytopenia.

Recently, IFN-γ has been reported to stimulate differentiation of both normal and tumor B-cells *in vitro* (26, 27). Interestingly, during the short period of treatment in this investigation, administration of rIFN-γ, a T-cell-derived lymphokine, resulted in objective evidence of tumor regression in a patient with a B-cell lymphoma.

In conclusion, we have demonstrated that rIFN-γ was well tolerated in single i.v. bolus or i.m. doses below 2.5 mg/sq m, with most patients experiencing transient flu-like symptoms. The short half-life and absence of granulocytopenia after i.v. bolus injections indicate that continuous i.v. infusions will be needed in order to prolong exposure time and thus produce consistent biological effects. In contrast, i.m. injection of rIFN-γ resulted in biological activity manifested by fever and granulocytopenia, but

pharmacokinetic studies indicate that this agent was altered before reaching the bloodstream, resulting in partial loss of antiviral activity. These data suggest that further investigations with i.m. injections as well as prolonged i.v. infusions of rIFN-γ are warranted. This study will form the framework for future Phase I, II, and III evaluations of the role of rIFN-γ in the therapy of human viral, microbial, and malignant diseases.

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