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Phase I Study of Recombinant Tumor Necrosis Factor in Cancer Patients[1]

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ABSTRACT

Tumor necrosis factor is a cytokine derived from activated macrophages. This agent is cytostatic and cytolytic against transformed human cell lines *in vitro* and has *in vivo* activity against a variety of murine tumors. We report a clinical study of the pharmacokinetics, toxicity, and biological activity of i.v. and i.m. administered recombinant human tumor necrosis factor (rTNF). Twenty patients with metastatic cancer were given rTNF in doses ranging from 1 to 200 μg/m² by alternating i.m. and i.v. bolus injections with a minimal intervening period of 72 h. Each patient received a maximum of eight treatments given twice weekly over a 4-week period. With i.v. bolus administration, serum concentrations of rTNF were detected by enzyme-linked immunosorbent assay at doses of 25 μg/m² or greater. The clearance of rTNF in the serum was described by a monoexponential equation with a half-life calculated to be 14–18 min. After i.m. administration, serum concentrations of rTNF were consistently detected by enzyme-linked immunosorbent assay at doses of 150 μg/m² or greater. Peak concentrations were observed within 2 h and rTNF was occasionally detected, at the lower limit of sensitivity of the assay, at 24 h postinjection. rTNF was well tolerated clinically in this dose range, and there was evidence of antitumor effect.

INTRODUCTION

TNF[4] was discovered by Carswell *et al.* (1, 2) in 1975. Sera from endotoxin-treated mice, rabbits, or rats that had been sensitized with Bacillus Calmette-Guérin were found to contain an activity that caused hemorrhagic necrosis of rodent tumors. Subsequent experiments *in vitro* have shown that leukocytes produce at least two distinct cytotoxic factors by immune effector cells; activated macrophages produce TNF-α, whereas mitogen-stimulated lymphocytes produce TNF-β (previously called lymphotoxin) (3, 4). The genes encoding both TNF-α and TNF-β have recently been sequenced and cloned and have been shown to have an approximate 28% amino acid homology (5, 6). Both cytokines are known to have cytostatic and cytotoxic effects *in vitro* against a wide range of human tumor cells but have no such effects against normal human fibroblasts (1, 2, 8). Antitumor effects have been demonstrated in both syngeneic murine tumors and human tumor xenografts in nude mice (9). In addition, marked synergistic antiproliferative activity has been demonstrated with TNF-α (or TNF-β) and γ-interferon against both murine and human tumor cell lines *in vitro* (10, 11). Although the exact mechanism by which TNF exerts its antitumor activity is unknown, it has been hypothesized that TNF must first bind to a cell surface receptor, be internalized, and then perhaps trigger the release of lysosomal enzymes that lead to lysis of the target cell.

The availability of TNF produced through recombinant DNA technology has enabled the exploration of the therapeutic potential of TNF as an anticancer agent in human clinical trials. We report the results of a multiple escalating dose Phase I clinical trial of rTNF. This biological agent is well tolerated when given as a single i.v. or i.m. injection, and it has biological activity in cancer patients.

MATERIALS AND METHODS

Preparation of rTNF. The molecular cloning and protein characterization and purification of rTNF were performed by Genentech, Inc. (South San Francisco, CA) and have been described previously (3–6). The rTNF produced in *Escherichia coli* is nonglycosylated and has a molecular weight of approximately 17,000. rTNF is purified to more than 99% as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and has a specific activity of approximately 4×10^7 units/mg of protein as defined by the lysis of actinomycin D-treated mouse L929 cells (12). Sterility, general safety, and purity studies meet Office of Biologics, Food and Drug Administration standards.

Patient Selection and Study Design. Patients with histopathological confirmation of disseminated cancer who had not received antitumor therapy for at least four weeks were entered on the study. Eligibility criteria included a performance status of ≥50% (Karnofsky scale) (13), life expectancy of at least 3 months, preserved hepatic (bilirubin, <1.5 mg/dl), renal (creatinine, <2.0 mg/dl), and hematological (granulocytes, ≥1,500/mm³; platelet count, ≥100,000/mm³) function. Informed consent was obtained in accordance with institutional policy. Patients were observed in the hospital for 36 h after each dose.

Table 1 describes the treatment plan. Two patients were entered sequentially at DOSE LEVELS 1 through 10 and received twice weekly treatment for 4 weeks. The first two patients received 5 μg/m² i.m. and 1 μg/m² i.v. (LEVEL 1), the next two patients received 10 μg/m² i.m. and 2 μg/m² i.v. (LEVEL 2), etc. A minimum interval of 72 h was maintained between doses. Each patient received a maximum of four sequential dose escalations weekly over a 4-week period. This study design was used to observe single dose tolerance and pharmacokinetics over a wide dose range. It was recognized that the maximum tolerated dose for Phase II trials could not be determined due to the possible occurrence of cumulative toxicity from intrapatient dose escalations. Fixed multiple dose Phase I trials to achieve this objective are now in progress.

Patients were monitored daily. All constitutional symptoms were recorded and classified as minimal (Grade I), moderate (Grade II), severe (Grade III), and life threatening (Grade IV). Vital signs were recorded before injections and at 20 min and at 1, 2, 4, 6, 8, 12, 18, and 24 h after each injection. A history and physical examination were done before the initial dose and twice weekly (preinjection) thereafter. Patients were weighed daily. An electrocardiogram and chest x-ray were done before the study and after the final dose. A complete blood count, coagulation profile, and determination of serum electrolytes were done prior to each dose and at 4 and 24 h after each dose. A serum chemistry profile (including renal and liver function tests and triglyceride and cholesterol levels), urinalyses, and reticulocyte counts were obtained before and 24 h after each dose. Tumor size was evaluated by physical examination and appropriate radiological studies and scans, as well as by tumor markers. The criteria for antitumor response have been described previously (14).

Pharmacokinetic Studies. Venous blood samples were collected before injection and at 20 min, 40 min, and 1, 1.5, 2, 4, 6, 8, 12, 18, and 24 h following i.m. doses and at 5, 10, 20, and 40 min and 1, 1.5, 2, 4, 6, 8, 12, 18, and 24 h after i.v. doses. Blood samples were centrifuged and the serum was decanted and stored at ≤−20°C until analysis.

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[4] The abbreviations used are: TNF, tumor necrosis factor; rTNF, recombinant tumor necrosis factor; ELISA, enzyme-linked immunosorbent assay.

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Table 1 *rTNF dose levels*

Dose levels	Dose ($\mu g/m^2$) i.m.	i.v.
1	5	1
2	10	2
3	15	5
4	25	10
5	35	15
6	50	25
7	65	35
8	80	50
9	100	65
10	125	80
11	150	100
12	175	125
13	200	150

Serum rTNF titers were measured by ELISA at Genentech, Inc. A solid-phase ELISA was performed using a sandwich technique with two polyclonal antibodies to rTNF, one of which was conjugated to a horseradish peroxidase label. The ELISA assay can detect rTNF reliably at 100 to 2800 pg/m. In addition, a bioassay (L-M cell cytotoxicity with actinomycin D added) (15) was performed on specimens that were positive in the ELISA.

The mean serum concentrations at each time point for all patients at a given dose were calculated. Individual serum rTNF disposition curves were constructed and nonlinear regression analyses were performed for each patient. Serum concentration half-lives, volumes of distribution, and the area under the concentration curve were then calculated for each patient individually in standard fashion (16).

The presence of antibodies to rTNF was determined on blood samples obtained before the study and 3 to 4 and 28 to 30 days after completion of the study.

Statistics. The Wilcoxon signed rank test was used for the determination of statistical significance between pre- and post-laboratory values.

RESULTS

Twenty patients with disseminated cancer were treated, 7 men and 13 women ranging in age from 26 to 75 years, with a median age of 54 years. Five patients had colon cancer, 2 had renal cell carcinoma, 4 had breast cancer, 2 had pancreatic cancer, 3 had multiple myeloma, and 1 each had nodular poorly differentiated lymphoma, melanoma, adenocarcinoma of the gastroesophageal junction, and Hodgkin's disease. The patients with multiple myeloma and pancreatic cancer had received no prior therapy, whereas all other patients had been treated with radiation therapy, chemotherapy, and/or immunotherapy. Two of the 20 patients were removed from the study after they received 1 dose of rTNF because they did not meet the eligibility criteria.

The most common clinical side effects associated with rTNF therapy are described in Table 2. The side effects were similar following i.m. and i.v. administration. Although fever and chills occurred in almost every patient at all dose levels, they were not dose limiting and generally resolved within 12–24 h. Headaches were common but were easily treated with acetaminophen. Although mild soreness at the i.m. injection site was common, no ulceration occurred. The onset of muscle soreness was at 6–12 h and the duration was 24–72 h. With increased dose, muscle soreness became more severe but was never dose limiting. Further, there were no consistent adverse hemodynamic changes. Significant hypotension was not observed. No patient experienced a significant (≥10%) weight loss. Respiratory insufficiency and hepatic dysfunction occurred following two doses of rTNF (12 $\mu g/m^2$ total dose) in a patient with breast cancer and extensive hepatic and pulmonary lymphangitic metastases. This patient died 7 days following the last rTNF injection. A second patient with colon cancer and pulmonary metastases was removed from the study after experiencing transient respiratory insufficiency. A full recovery to the pretreatment clinical condition followed. The onset of acute respiratory insufficiency in both patients occurred within 6–12 h after receiving rTNF.

Table 2 *Adverse clinical effects of rTNF*

	1–15 $\mu g/m^2$		25–65 $\mu g/m^2$		80–200 $\mu g/m^2$	
	i.m.	i.v.	i.m.	i.v.	i.m.	i.v.
No. of patients	7	10	13	13	10	5
No. of evaluable treatments	14	25	29	28	28	13
Adverse effects						
Fever	14[a] (100)[b]	24 (96)	29 (100)	27 (96)	28 (100)	13 (100)
Chills	9 (64)	20 (80)	22 (76)	24 (80)	23 (82)	11 (85)
Headaches	3 (21)	8 (32)	13 (45)	7 (25)	15 (54)	10 (77)
Fatigue	3 (21)	5 (20)	9 (31)	9 (32)	11 (40)	8 (62)
Anorexia	3 (21)	8 (32)	8 (28)	3 (11)	11 (40)	6 (46)
Nausea	2 (14)	3 (12)	3 (10)	2 (7)	8 (29)	7 (54)
Vomiting	2 (14)	1 (4)	4 (14)	2 (7)	2 (7)	3 (23)
Diarrhea	1 (7)	0 (0)	2 (7)	1 (4)	2 (7)	1 (8)
Dizziness	3 (21)	1 (4)	4 (14)	2 (7)	6 (21)	1 (8)
Myalgia	0 (0)	1 (4)	3 (10)	1 (4)	4 (14)	1 (8)

[a] Values represent the number of treatments associated with adverse effects.
[b] Numbers in parentheses, percentage of patients experiencing adverse effects.

The effect of rTNF on platelet, granulocyte, and lymphocyte counts is presented in Table 3. Twenty-four h after i.m. administration of rTNF, there was a statistically significant, although clinically unimportant, decrease in platelet count. This effect was neither cumulative nor dose dependent. The platelet counts recovered by 48–72 h. In contrast, 24 h after i.v. administration of rTNF, no significant decrease in platelet counts occurred. Four h after the administration of either i.m. or i.v. rTNF, there was a significant decrease in the absolute lymphocyte count and an increase in the granulocyte count (Table 3). By 24 h the absolute granulocytes had returned to normal while the absolute lymphocytes had not. With respect to hemoglobin, among the 16 patients who completed 1 month of therapy there was a significant decrease from pretreatment hemoglobin levels that was not dependent on the cumulative TNF dose (Table 4). The drop was significantly below the level that we observed after 1 month of therapy in our γ-interferon trial which had similar phlebotomy requirements (17). rTNF also affected certain biochemical parameters. After patients completed the 8 doses of rTNF or 1 month of therapy, there was a statistically significant increase in serum triglyceride and a significant decrease in cholesterol (Table 5) and serum albumin levels (data not shown). There was no laboratory evidence of renal or hepatic toxicity.

Table 6 is a summary of the pharmacokinetic parameters calculated from the ELISA-detectable concentrations of rTNF in the serum of patients receiving i.v. bolus doses of rTNF ranging from 25 to 100 $\mu g/m^2$. The half-life and dose-corrected area under the curve for rTNF given by i.v. bolus administration appear to increase with increasing dose (Fig. 1; Table 6). The apparent volume of distribution (V_d) was 66 liters at the 25-$\mu g/m^2$ dose and decreased to 12 liters at the 100-$\mu g/m^2$ dose. After i.v. bolus injection, the clearance of ELISA-detectable material closely fit (r^2>0.95) an open one-compartment mathematical model (Fig. 1). Moreover, determinations of rTNF activity in the serum following i.v. bolus administration using the L-M cell cytotoxicity bioassay correlated with ELISA measurements (data not shown). In contrast to the i.v. bolus route, i.m. administered rTNF was not consistently detected in patients'

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Table 3 *Changes in hematological indices 4 and 24 h after rTNF administration*

Hematological index	Route of administration	No. of treatments	Pretreatment mean ± SE (×10³ cells/mm³)	4 h posttreatment mean ± SE (×10³ cells/mm³)	24 h posttreatment mean ± SE (×10³ cells/mm³)	P
Platelets	i.m.	59	343 ± 21		292 ± 19	<0.001
	i.v.	60	322 ± 20		332 ± 22	NS[a]
Granulocytes	i.m.	59	4.8 ± 0.29	7.03 ± 0.43		<0.001
			4.8 ± 0.29		4.8 ± 0.23	NS
	i.v.	60	4.36 ± 0.28	7.48 ± 0.39		<0.001
			4.36 ± 0.28		4.57 ± 0.27	NS
Lymphocytes	i.m.	59	1.05 ± 0.1	0.56 ± 0.05		0.001
			1.05 ± 0.1		0.79 ± 0.08	0.001
	i.v.	60	1.0 ± 0.07	0.56 ± 0.05		<0.001
			1.0 ± 0.07		1.12 ± 0.07	0.05

[a] NS, not significant.

Table 4 *Change in hemoglobin after 1 month of rTNF therapy*

Cumulative rTNF dose (μg/m²)	No. of patients	Pretreatment hemoglobin (g/dl) median (range)	Posttreatment hemoglobin (g/dl) median (range)	P
73–180	5	11.1 (10.7–15.2)	9.7 (6.8–9.8)	<0.05
255–470	6	12.4 (10.3–14.0)	8.9 (7.0–11.5)	<0.01
595–1105	5	11.6 (10.3–14.3)	9.3 (8.5–12.1)	<0.001

Table 5 *Change in lipids after 1 month of rTNF therapy*

Lipids	No. of patients	Pretreatment median (range) (mg/dl)	Posttreatment median (range) (mg/dl)	P
Triglyceride	16	119 (59–296)	125 (68–384)	0.025
Cholesterol	16	164 (100–284)	142.5 (88–231)	<0.001

Table 6 *Serum pharmacokinetics of rTNF by ELISA after i.v. bolus administration*

Dose (μg/m²)	No. of studies	Half-life[a] (min ± SE)	Apparent V_d[b] (liters ± SE)	AUC (ng/ml × min)
25	3	15.9 ± 3.6	66 ± 30	10.5 ± 2.7
35	5	13.9 ± 1	31.3 ± 5	19.7 ± 5.3
50	4	16 ± 2	13.4 ± 1.1	89.6 ± 13.9
65	5	18 ± 0.4	17.7 ± 4	114.6 ± 26.5
100	3	17 ± 2	12 ± 4	223.8 ± 69

[a] Values expressed as means.
[b] V_d, volume of distribution; AUC, area under the concentration curve.

sera by ELISA until doses reached 150 μg/m² or greater. Peak serum levels were usually obtained with 2 h and ELISA-positive material occasionally persisted for 24 h after the injection (Fig. 2).

None of the patients had circulating antibodies to TNF prior to the study, nor did any patient develop antibodies by the completion of the study.

Among the 16 evaluable patients that completed 4 weeks of therapy, there was evidence for antitumor effect in 2 patients. There was complete regression of a 5- x 5-cm neck lesion but no demonstrable effect on extensive retroperitoneal disease in a patient with renal cell carcinoma; there was resolution of malignant ascites in another patient with metastatic colon cancer.

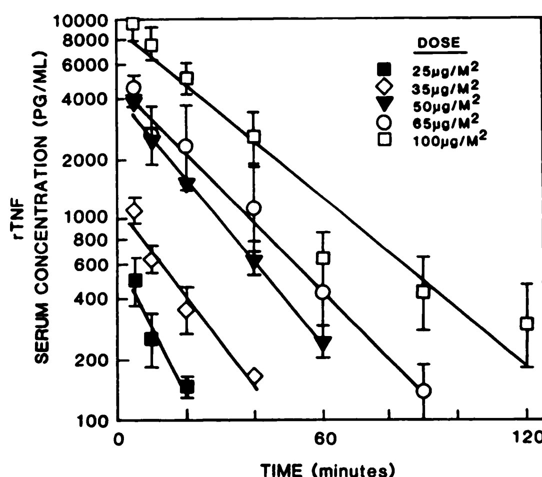

Fig. 1. Serum disappearance of rTNF after i.v. administration as measured by ELISA. *Symbols* represent the mean of blood levels for each dose for all patients at that dose. *Standard error bars* are shown unless insufficient data points were available.

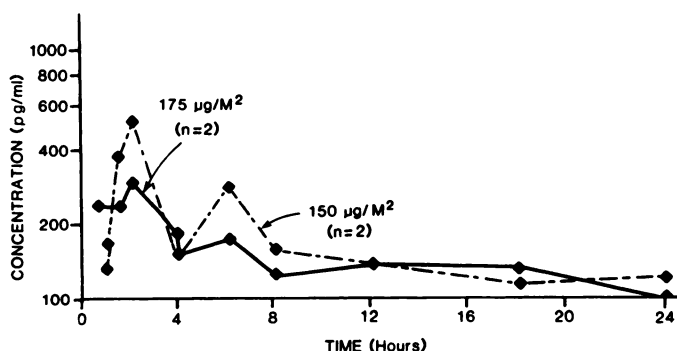

Fig. 2. Serum disappearance of rTNF after i.m. administration as measured by ELISA. ◆, mean of the serum levels for each dose for all patients at that dose.

DISCUSSION

Mononuclear cells secrete a variety of cytokines in response to foreign antigens: interleukins (18); interferons (19); and TNFs (3–6). TNF has a wide range of biological properties (for review see Ref. 20). Since the discovery that this factor was able to elicit hemorrhagic necrosis of tumors *in vivo* (1, 2), considerable effort has gone into the large scale production of TNF by recombinant DNA methodology. This report describes the pharmacokinetics, biological effects, single dose tolerance, and toxicity of rTNF in cancer patients.

The clearance of rTNF as measured by ELISA following i.v. bolus administration was monoexponential, suggesting an open one-compartment mathematical model (Fig. 1). This appears to be similar to the pharmacokinetics of other recombinant lymphokines (17, 21). In contrast to the i.v. route of administration, i.m.-administered rTNF occasionally resulted in sustained concentrations of rTNF in the serum. Because of the

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small number of measurements for each patient studied, pharmacokinetic calculations were not possible after i.m. administration.

Fever and chills were nearly universal following rTNF therapy irrespective of the route of administration. The mechanism of the pyrogenic effect of rTNF is not known but may involve a direct effect on the hypothalamic thermoregulatory centers or an induction of the biosynthesis of another monokine, interleukin 1 (22). An inflammatory reaction occurred at the i.m. injection site but was not dose limiting. Significant hypotension and weight loss were not observed. Respiratory insufficiency occurred in two patients but in both cases these events were attributed to the patient's underlying tumor.

TNF administered in the dose schedule used in our study did not result in granulocytopenia. Since TNF is known to inhibit granulocyte-macrophage progenitor cells *in vitro* (23),[5] perhaps higher doses and/or a more prolonged exposure to TNF will be necessary to achieve this effect *in vivo*. The fact that platelet counts were suppressed only after TNF was administered by the i.m. but not the i.v. route suggests that prolonged exposure to circulating levels of TNF may have accounted for the suppression. The effects we noted on peripheral WBC after 4 h of rTNF administration in all likelihood were a result of an acute phase reaction which is in part known to be mediated by TNF (20). TNF is also known to inhibit erythropoiesis (24). The significant drop in hemoglobin noted in our study population may indicate that this inhibition occurred *in vivo*. TNF is also thought to inhibit lipoprotein lipase activity (25, 26). The decrease in cholesterol and elevated triglyceride levels that we noted may have been a consequence of this inhibition.

In conclusion, we have shown that rTNF is well-tolerated when administered in single doses up to 200 $\mu g/m^2$, can be detected in the serum by ELISA following i.v. and i.m. injection, and shows evidence of antitumor effects in this dose range.

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