Eur J Cancer Clin Oncol, Vol. 25, No. 2, pp. 379–388, 1989.
Printed in Great Britain

0277-5379/89$3.00 + 0.00

Clinical Trials Summaries

Phase I Study of Intratumoral Application of Recombinant Human Tumor Necrosis Factor

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TUMOR NECROSIS FACTOR (TNF) was first described in 1975 [1, 2]. TNF-α, which is identical with cachectin [3], is produced in cells of the monocyte/macrophage system, whereas mitogen-stimulated lymphocytes produce TNF-β [4, 5]. TNF can induce hemorrhage and necrosis in certain experimental tumors and human tumor grafts in mice [6]. This was the reason to conduct a phase I study in cancer patients after recombinant human TNF-α (rHuTNF [7–9]) became available. We chose to investigate the intratumoral application because we expected from this form of application an optimal ratio of tumor response versus side-effects as had been shown for other biological response modifiers such as BCG and interferon.

MATERIALS AND METHODS

Patients

Eligibility criteria: age 18–75 years; histologically proven malignant disease refractory to standard treament; life expectancy >2 months; Karnofsky index ≥70%; >4 weeks since chemotherapy, radiotherapy or surgery. Exclusion criteria: pregnancy, breast feeding, life-threatening conditions, major organ or psychiatric diseases, hypertension (systolic pressure >180 mmHg), hypotension (systolic pressure <100 mm Hg), allergic disposition, positive skin test with rHuTNF, platelets <100,000/mm^3, leucocytes <4000/mm^3, abnormal liver function or coagulation tests.

Accepted 2 September 1988.
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Application of rHuTNF

The study protocol had been approved by the ethics committee of the University of Cologne. rHuTNF (provided by Asahi Chemicals Inc., Tokyo) had a specific activity of 2.3×10^6 U/mg in the L-cell assay without cytotoxic drugs [10]. In our hands, rHuTNF provided by Asahi and Genentech had the same specific activity in this L-cell assay. Starting dose was 43 μg/m^2 (1×10^5 U/m^2). At each dose level, three patients were treated. Each patient received only one application of rHuTNF. The dose escalations were 2×, 2.5×, 5×, 7×, 9× and 12×10^5 U/m^2 (87, 109, 217, 304, 391, 522 μg/m^2). The maximum tolerated dose was defined as one dose level below the dose where life-threatening side-effects were observed. Strict intratumoral injection was assured by sonographic control.

Documentation of toxicity

The following parameters were controlled before therapy: vital signs, ECG, chest X-ray, abdominal sonogram or CT, lung function test, hemoglobin, WBC, platelets, SGOT, SGPT, yGT, LDH, bilirubin, alkaline phosphatase, cholinesterase, creatinine, total serum protein, uric acid, triglycerides, cholesterol, prothrombin time, partial thrombin time, immunoglobulins and urinalyis. Vital signs were checked again 10, 20, 30 min and 1, 2, 4, 8, 12, 24, 48, 72, 96 h and 1, 2, 3, 4 and 8 weeks after injection. ECG and laboratory parameters were repeated on days 1, 2, 3, 4, 7, 14, 21, 28 and 56 after injection. Chest X-rays, abdominal sonograms and lung function tests were repeated on day 28 after therapy.

Determination of rHuTNF plasma levels

Plasma levels of rHuTNF were determined immunologically by ELISA (detection limit 0.17 ng/ml or 0.4 U/ml) and functionally by the L-cell assay (detection limit 4.3 ng/ml or 10 U/ml as described in ref. [10] before and 20 min, 1, 2, 4, 8, 12, 24 and 48 h after injection of rHuTNF.

Antibodies to rHuTNF

The serum of patients was tested for antibodies against rHuTNF by a sandwich ELISA using monoclonal antibodies against rHuTNF before and 1, 2, 4 and 8 weeks after therapy.

RESULTS

Twenty-one patients (for characteristics see Table 1) were entered into this study.

Side-effects

The side-effects are listed in Table 2. They are described below in detail.

Fever and chills. Eighteen patients experienced rigors and fever up to 40.3°C during the first hour. Two patients without fever had taken pain killing drugs before the application of rHuTNF. In six patients receiving ≥ 217 $\mu g/m^2$ (5×10^5 U/m^2) a second temperature peak was observed 8–16 h later. All patients complained of 'flu-like symptoms'.

Gastrointestinal tract. Grade 3 watery diarrhea was observed in four patients at doses ≥ 217 $\mu g/m^2$ (5×10^5 U/m^2). Nausea and vomiting was observed in 11/21 patients. Grade 2 vomiting was observed in two patients and grade 3 in one, all of whom received the highest dose level.

Circulation. Tachycardia (>110/min) during the period of chills occurred in 16 patients. No other arrhythmias (grade 2–4) were observed. Hypertension (systolic >160–220 mmHg, diastolic >100 mmHg) occurred during the first hour in nine patients and in all three receiving 522 $\mu g/m^2$ (12×10^5 U/m^2). Twelve to 24 h after the injection of rHuTNF a dose-dependent hypotension with systolic pressures <100 mmHg developed in nine patients, five of whom became symptomatic and had to be treated with fluids (grade 3). One patient (522 $\mu g/m^2$ or 12×10^5 U/m^2) did not respond to volume substitution, became oliguric and had to be treated with dopamine. All patients receiving ≥ 217 $\mu g/m^2$ (5×10^5 U/m^2) experienced symptomatic postural hypotension which was observed between 8 and 24 h after the injection of rHuTNF.

Respiratory system. All patients were tachypneic during the first hour. Two patients of four with primary tumors or metastases in the lung developed bronchospasms necessitating bronchodilators.

Liver. Elevations of SGOT or SGPT (>2 × base line value) occurred in three patients and elevation of bilirubin, alkaline phosphatase and GGT in six, four of whom had received ≥ 391 $\mu g/m^2$ ($\geq 9 \times 10^5$ U/m^2). Decreases of cholinesterase by >1500 U/l were observed in all patients receiving the maximal dose of 522 $\mu g/m^2$ ($12 \times 10^2 \times 10$ U/m^2).

Kidney. One patient (522 $\mu g/m^2$ or 12×10^5 U/m^2), who developed symptomatic hypotension and became oliguric, had a reversible increase in serum creatinine to 2.4 mg/dl. Slight proteinuria and microhematuria were observed in eight patients.

Fluid retention. All patients receiving $\geq 9 \times 10^5$ U/m^2 gained weight between 1 and 2 kg. Fluid retention responded to treatment with furosemide.

Blood. Platelets fell 20–60% below the base line value in 11 patients; however, the nadir was never <100,000/mm^3. Grade 1 leucocytopenia was observed in three patients receiving different doses of rHuTNF. A transient relative lymphocytopenia occurred in 14 patients. The red blood cell count dropped by 20% in two patients.

Coagulation. Thromboplastin time and partial thrombin time increased slightly in four patients but remained within normal ranges. No bleeding episodes were observed.

Central nervous system. All patients complained of fatigue and four of headache. Somnolence and confusion were observed in one patient at the highest dose. Six patients complained of unexplained thirst. There were no effects on the peripheral nervous system.

Metabolism. There was no effect on cholesterol or glycerides. The serum protein concentration fell in all patients receiving ≥ 304 $\mu g/m^2$ ($\geq 7 \times 10^5$ U/m^2) by up to 2.5 g/dl in those patients who had the highest dose.

Immune system. There was no effect on IgG, IgM, IgA or IgE levels. No allergic reactions against rHuTNF, positive skin tests or antibodies against rHuTNF developed.

Table 1. Characteristics of patients treated with intratumoral rHuTNF

Patient	Dose (10^5 U/m^2)	Dose (μg/m^2)	Diagnosis	Sex	Age	Prior therapy [ChTh/RT/S*]
1	1	43	Malignant melanoma	M	43	S, ChTh, αIFN
2	1	43	NHL, high grade	F	55	ChTh, RT
3	1	43	NHL, low grade	M	58	RT, ChTh
4	2	87	Liver cell CA	F	39	S
5	2	87	Malignant melanoma	F	63	S, ChTh
6	2	87	Poroma CA	F	61	S, ChTh
7	2.5	109	Stomach CA	M	49	ChTh
8	2.5	109	Head and neck CA (squamous)	M	54	S, ChTh
9	2.5	109	Lung CA (squamous c.)	M	58	RT
10	5	217	Lung CA (large c.)	F	54	S
11	5	217	Lung CA (large c.)	M	51	None
12	5	217	Malignant melanoma	M	48	S, ChTh
13	7	304	Liver cell CA	F	61	S, ChTh
14	7	304	Lung CA (squamous c.)	M	55	S, RT
15	7	304	Head and neck CA (squamous)	M	51	ChTh, RT
16	9	391	Leiomyosarcoma	M	57	S, RT
17	9	391	Breast CA	F	47	S, RT, ChTh
18	9	391	Colon CA	F	64	S, ChTh
19	12	522	Anal CA (squamous c.)	F	68	S, RT
20	12	522	Pancreas CA	F	56	S
21	12	522	Fibrosarcoma	F	56	S, ChTh

*ChTh: chemotherapy; RT: radiotherapy; S: surgery; αIFN: α-interferon.

Table 2. Side-effects of intratumoral application of rHuTNF

Dose (10^5 U/m^2) (μg/m^2)	1 (43)	2 (87)	2,5 (109)	5 (217)	7 (304)	9 (391)	12 (522)	Total
Fever >38.0°C	2/3	3/3	2/3	2/3	3/3	3/3	2/3	18/21
Chills	2/3	3/3	2/3	2/3	2/3	3/3	3/3	17/21
Tachycardia (>110 b/min)	2/3	3/3	1/3	3/3	3/3	3/3	3/3	16/21
Pain in tumor	1/3	3/3	1/3	3/3	3/3	1/3	2/3	14/21
Nausea/vomiting	0/3	1/3	0/3	3/3	2/3	2/3	3/3	11/21
Hypertension (>160 syst.)	0/3	2/3	2/3	1/3	0/3	1/3	3/3	9/21
Hypotension (<100 syst.)	0/3	0/3	1/3	1/3	2/3	2/3	3/3	9/21
Liver (GOT, GPT, GGT, aP, Bili 2× ↑)	0/3	1/3	0/3	0/3	1/3	2/3	2/3	6/21
Diarrhea	0/3	0/3	0/3	1/3	1/3	1/3	1/3	4/21
Leucopenia (<4000/mm^3)	0/3	1/3	1/2	0/3	0/3	0/3	1/3	3/21
Anemia (Hb ↓ >20%)	0/3	0/3	0/2	0/3	0/3	1/3	1/3	2/20
Kidney (creat. >2 mg/dl)	0/3	0/3	0/3	0/3	0/3	0/3	1/3	1/21
Herpes labialis	0/3	0/3	0/3	0/3	0/3	0/3	1/3	1/21
CNS	0/3	0/3	0/3	0/3	0/3	0/3	1/3	1/21*
Coagulation (PT, PTT)	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/21
Thrombocytopenia (<100,000/mm^3)	0/3	0/3	0/2	0/3	0/3	0/3	0/3	0/20

*Somnolence, confusion.

Other side-effects. One patient developed herpes labialis infection. Most patients complained of pain at the injection site, and four at distant tumor sites.

Chronic toxicities. All toxicities were reversible within 72 h. No chronic toxicities have been observed with a follow-up of 12–24 months.

Maximum tolerated dose/dose limiting toxicities

Life-threatening clinical toxicity was observed in all three patients receiving 522 μg/m^2 (12×10^5 U/m^2). Although no single grade 4 toxicity as defined by the WHO criteria was observed, these patients experienced a life-threatening prostration resulting from the sum of fever, hypotension, fluid

Table 3. Plasma levels of rHuTNF (detected by ELISA)

No.	Dose (10^5 U/m^2)	Dose (μg/m^2)	Plasma levels (ng/ml) after injection: 0	20	1 h	2 h	4 h	8 h	12 h	24 h	48 h
1	1	43	0	0	0	0	0	0	0	0	0
2	1	43	0	0	0	0	0	0	0	0	0
3	1	43	0	0	0	0	0	0	0	0	0
4	2	87	0	0	0	0	0	0	0	0	0
5	2	87	0	1.7	0.9	0.3	0	0.2	0	0	0
6	2	87	0	1.4	0.7	0.2	0	0	0	0	n.d.
7	2.5	109	0	2.2	0.8	0	0	0	0	0	n.d.
8	2.5	109	0	0.2	0.2	0	0	0	0	0	n.d.
9	2.5	109	0	0	0	0	0	0	0	0	n.d.
10	5	217	0	26.0	14.3	2.9	0	0	0	0	n.d.
11	5	217	0	3.2	0.8	0.2	0	0	0	0	n.d.
12	5	217	0	0	0.2	0	0	0	0	0	n.d.
13	7	304	4.2	n.d.	6.4	n.d.	4.7	4.5	5.6	5.0	n.d.
14	7	304	0	0	0	0	0	0	0	0	n.d.
15	7	304	0	0.6	0.6	0.7	0.2	0.2	0.2	0	n.d.
16	9	391	0	0	0	0	0	0	0	0	n.d.
17	9	391	0	126.1	76.5	81.7	16.5	0	0	0	0
18	9	391	0.5	2.5	2.1	0.7	0.6	0.5	0.4	0.4	n.d.
19	12	522	0	1.4	2.5	3.2	1.5	0.5	0	0	0
20	12	522	0	49.6	24.5	9.7	0	0	0	0	n.d.
21	12	522	0	0.6	0.5	0.5	0.3	0.2	0	0	0

n.d. = not determined.

retention, watery diarrhea and central nervous side-effects. Maximum tolerated dose (MTD) for the intratumoral application of rHuTNF was therefore defined as 391 μg/m^2 (9 × 10^5 U/m^2).

Pharmacokinetics

Plasma levels were detected by L-cell assay in 4/18 and by ELISA (Table 3) in 13/18 patients who received dosages of ≥87 μg/m^2 (≥2 × 10^5 U/m^2) rHuTNF. Plasma levels peaked within 20 min after injection and were detectable for up to 12 h. The highest plasma level observed was 126 ng/ml (290 U/ml) in a patient who received 391 μg/m^2 (9 × 10^5 U/m^2). Even though there was a tendency for higher plasma levels after increased dosages of rHuTNF, there were great variations between patients who received the same dosage (Fig. 1). In 2/4 patients with plasma levels above the detection limit of the L-cell assay, the values in the bioassay were nearly twice as high as those determined immunologically.

Two patients had measurable plasma levels of TNF before the application of rHuTNF. The plasma level persisted throughout the period of observation, suggesting an endogenous production of TNF. The levels of endogenous TNF ranged from 3.5 to 7.0 ng/ml (8–16 U/ml) in the first and from 0.35 to 0.43 ng/ml (0.8–1 U/ml) in the second patient.

Response

A biological effect of the intratumoral application of rHuTNF was observed in 11/21 patients (Table 4). The effects of rHuTNF could be well observed and documented in superficial tumors (Fig. 2). They followed a typical course: a massive local inflammation developed 8–24 h after the injection, progressing in some cases to hemorrhagic necrosis which appeared after 2–5 days. This was followed by invasion of granulocytes causing sterile abscesses after one week, as was demonstrated by cytological and microbiological examination of the aspirate in two patients. After 2–3 weeks, there was demarkation of the tumor.

We observed one complete clinical local regression, four partial local regressions (decrease of the size of the injected tumor by >50%), four minor local regressions (decrease by 25–50%) and two massive local inflammations without effect on the size of the local tumor (Table 4). The complete local regression occurred in a lymph node infiltrated by a low grade non-Hodgkin's lymphoma and lasted for 2 months. In a patient with non-small cell lung cancer there was a complete resolution of a distant bone metastasis (in a rib) after injection of rHuTNF into a liver metastasis. The injected metastasis responded with a necrosis (presumably hemorrhagic as judged by sonography) and showed a partial local regression. The effect

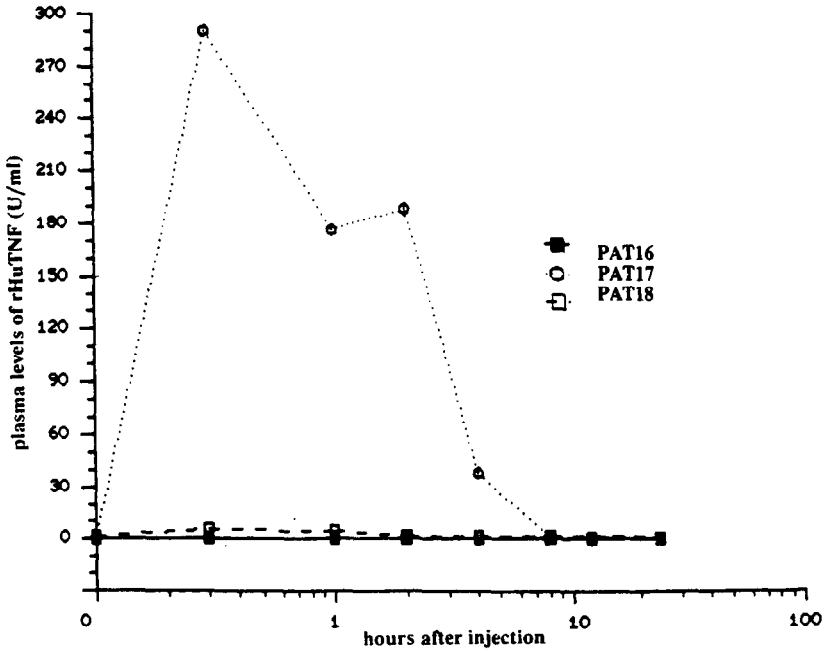

Fig. 1. Plasma levels of rHuTNF of patients receiving 391 μg/m² (9 × 10⁵ U/m²) *rHuTNF as determined by ELISA. It is evident that there are great variations in the time course and peak plasma levels of patients receiving equal doses of rHuTNF.*

on the bone metastasis lasted for nearly 1 year and was documented by X-ray and bone scan.

DISCUSSION

Fever and chills were the most frequent side-effects in this study of intratumoral application of rHuTNF. The mechanisms of most of the side-effects of rHuTNF are unknown. Further studies are needed to determine whether they are due to direct effects of rHuTNF or may be caused by the induction of biosynthesis of other cytokines, such as interleukin-1 [11].

The spectrum and intensity of the side-effects at higher doses of rHuTNF are strongly reminiscent of the side-effects experienced with interleukin-2 [12]. Indeed, it appears that, as with the interleukin-2 treatment, a capillary leak syndrome may be responsible for fluid retention, development of watery diarrhea, confusion (by cerebral edema) and hypotension. The second factor contributing to the hypotension is vasodilatation, which also causes the profound postural hypotension observed in patients receiving higher doses of rHuTNF. The effects on hematopoiesis were not of clinical significance even though TNF is known to inhibit erythropoiesis [13] and granulocyte–macrophage progenitor cells *in vitro* [14], possibly because these effects may be compensated *in vivo* by the release of granulocyte-colony stimulated factor (G-CSF) by endothelial cells [15]. We observed a dose-dependent suppression of liver function (drop in cholinesterase activity) without a significant rise in transaminases. Similarly, after one single injection of rHuTNF we did not observe development of cachexia and changes in triglycerides and cholesterol levels which might be expected from the fact that rHuTNF causes suppression of lipoprotein lipase [16]. In fact, changes in cholesterol and triglycerides have been observed in a phase-1 study of repeated systemic application of rHuTNF [17]. From our study of a single injection of rHuTNF we cannot exclude the possibility that there might be cumulative effects of rHuTNF on hematopoiesis and metabolism. Apart from a rapid, yet mild and transient decrease of platelet counts, no striking clotting abnormalities were discovered, nor did we observe effects on the immune system, especially no allergic reactions, positive skin tests or antibodies against rHuTNF.

Dose-limiting in our trial was the life-threatening prostation of the patient resulting from the sum of the described side-effects. It might be possible to increase the dosage of rHuTNF beyond the MTD (391 μg/m² or 9 × 10⁵ U/m²) defined under the conditions of this trial if rHuTNF is given in an intensive care unit or to patients with an excellent performance status, as these patients tolerated the side-effects of rHuTNF better. Similarly, the tolerance to rHuTNF might be increased by prophylactic treatment with prostaglandin synthetase inhibitors, as it is known from experimental data that pretreatment with such drugs (e.g. indomethacine, ketoprofen) may attenuate some of the side-effects of rHuTNF. Our protocol did not permit dose escalation within one patient because prior exposure to biological response modifiers may alter

Table 4. Effects of intratumoral application of rHuTNF

No.	Dose (10^5 U/m^2)	Doses (μg/m^2)	Diagnosis	Injection site	Effect on tumor
1	1	43	Malignant melanoma	Skin met.	None
2	1	43	NHL, high grade	Axill. LN.	Total local regression
3	1	43	NHL, low grade	Inguin. LN.	Minor local regression
4	2	87	Liver cell CA	Supraclav. LN.	None
5	2	87	Malignant melanoma	Skin met.	None
6	2	87	Poroma CA	Muscle met.	None
7	2.5	109	Stomach CA	LN met.	Minor local regression
8	2.5	109	Head and neck CA (sqamous)	Skin met.	Minor local regression
9	2.5	109	Lung CA (squamous c.)	Muscle met.	Partial local regression
10	5	217	Lung CA (large c.)	Liver met.	Partial local regression*
11	5	217	Lung CA (large c.)	Skin met.	Massive local inflammation
12	5	217	Malignant melanoma	Skin met.	None
13	7	304	Liver cell CA	Liver tumor	None
14	7	304	Lung CA (squamous c.)	Muscle met.	Local inflammation
15	7	304	Head and neck CA (squamous)	Oral tumor	Partial local regression
16	9	391	Leiomyosarcoma	Liver met.	Minor local regression
17	9	391	Breast CA	Liver met.	None
18	9	391	Colon CA	Liver met.	None
19	12	522	Anal CA (squamous C.)	Liver met.	None
20	12	522	Pancreas CA	Liver met.	None
21	12	522	Fibrosarcoma	Breast tumor	Minor local regression (massive necrosis)

*In addition to the complete resolution of a distant bone metastasis.

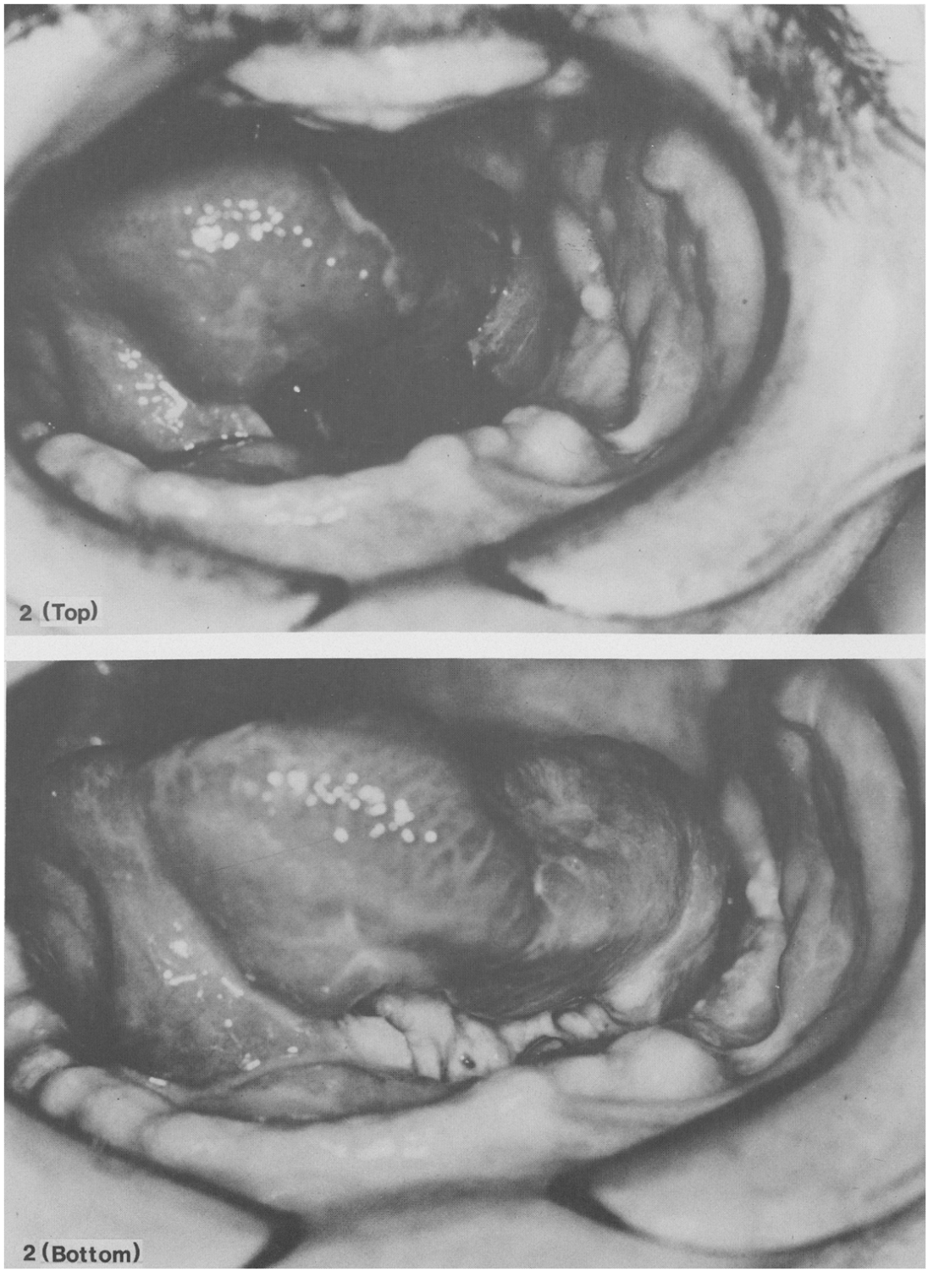

Fig. 2. Effect of rHuTNF on a squamous carcinoma of the tongue. Four days after the injection there is a massive hemorrhagic necrosis of the tumor (top). After 2 weeks the necrosis is demarkated and the area is free of tumor (bottom).

the responsiveness to subsequent doses [18]. Indeed, we observed an increased tolerance to rHuTNF in several patients who were treated off protocol and received repeated administrations of rHuTNF for compassionate treatment.

There were great variations both in the maximum plasma levels observed and in the time course of the plasma levels of patients receiving the same dosage. This may be due to several factors: different capacity of the injected tumor to bind rHuTNF, different vascularization of the tumor or individually different metabolization of rHuTNF. Another interesting point is the fact that in some patients the values of plasma concentrations of rHuTNF as determined in the functional L-cell assay were significantly higher than those determined immunologically by the ELISA. Reasons for this phenomenon may be that the ELISA does not detect metabolic products of rHuTNF which are functional in the L-cell assay or that rHuTNF induce secondary factors which induce cytotoxic effects in the L-cell assay. Investigations to answer this question are under way.

Two patients had an endogenous production of TNF, one with relatively high plasma levels (3.5–7.0 ng/ml). Preliminary data indicate that TNF was produced by the monocytes of this patient. The underlying mechanisms responsible for the production of TNF by the patient's monocytes are unclear. The clinical course of this patient with hepatocarcinoma was characterized by otherwise unexplained chronic fever, which is untypical for this disease. The patient died from progressing tumor 6 months after the therapy with rHuTNF.

We observed biological effects of the intratumoral application of rHuTNF in about half of the patients. As there was no correlation between these local effects with plasma levels of rHuTNF, absolute and differential WBC before and after rHuTNF, lymphocyte subpopulations or levels of immunoglobulin subclasses, we believe that the cytotoxic effects were caused by a direct effect of rHuTNF on the tumor or its vascular bed. The same lack of correlation between biological parameters and effect on the tumor holds true for distant tumor sites (one complete regression of a rib metastasis, development of sterile abscesses and pain in several patients). Therefore, none of the above mentioned parameters seems to be useful for the evaluation of the optimal biological dose of rHuTNF.

In several studies of systemic application of rHuTNF, including one of a 24 h continuous intravenous infusion performed at our institution [19], rHuTNF has shown little activity, but considerably higher toxicity. As maximal plasma levels achieved in these studies were much lower, it seems that the effects of rHuTNF on the tumor depend mostly on the concentration of rHuTNF at the tumor site, whereas side-effects are related to the duration of rHuTNF in the plasma and not to the maximal plasma concentration. Based on these observations one might conceive that intratumoral application of rHuTNF will play a role in local tumor control, possibly in conjunction with local radiotherapy or systemic chemotherapy, while systemic application of rHuTNF in cancer treatment holds little promise unless parameters are identified which help in designing the optimal biologic dose.

Acknowledgements—We thank Dr. L. J. Old for his advice in designing this study.

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