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A Phase I Study of Local Treatment of Liver Metastases with Recombinant Tumour Necrosis Factor

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15 patients with therapy-resistant liver metastases were treated in a phase I study with recombinant tumour necrosis factor (rTNF). rTNF was injected into a liver metastasis by ultrasound guidance, using a 50 μg escalating dose schedule (3 patients/dosage) ranging from 100 to 350 μg per injection. Influenza-like symptoms such as fever, chills, nausea and vomiting were the main clinical side-effects. 2 patients experienced transient hypotension, probably due to concomitant use of morphine. Other toxicities, as reported after systemic use of rTNF, such as decrease in leucocytes and platelet counts, renal or liver toxicity were not observed. No difference was seen in subpopulations of lymphocytes ($CD3^+$, $CD4^+$ $CD8^+$, $CD16^+$ and $CD19^+$) prior to and after rTNF injection. In 8 patients stable disease occurred in rTNF-treated metastases. The maximal dose used by this route of administration is 350 μg per injection. Based on these observations we conclude that the toxicity of rTNF injected into liver metastases by sonographic control is transient and mild. The results suggest that intratumoral administration of rTNF might play a role in local tumour control.
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INTRODUCTION

TUMOUR NECROSIS FACTOR (TNF) was first identified in BCG-infected mice challenged with endotoxin. This serum factor caused haemorrhagic necrosis of subcutaneously implanted meth A sarcoma in mice [1]. Subsequently it was demonstrated that TNF-alpha is produced by activated cells of the monocyte/macrophage lineage, TNF-beta by lymphocytes [2]. After the isolation of TNF it was possible to identify the gene coding for this polypeptide [3, 4], and with the use of recombinant DNA technology TNF came available in large amounts of highly purified material. It was thought that by the production of TNF on a large scale the beginning was set for a selective form of immunotherapy in patients with advanced cancer.

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The exact antitumour activity of TNF is yet not clearly understood. Its direct cytotoxicity to sensitive tumour cells is mediated by specific cell surface receptors. Interaction of TNF with these receptors leads to membrane perturbations and DNA fragmentation [5]. Malignant cell lines which show no growth inhibition to TNF *in vitro*, can show tumour regression *in vivo*. This implies that other, indirect, mechanisms may be involved in the antitumour activity of TNF *in vivo*, such as activation of host immune defense mechanisms [6]. Recently it was demonstrated that TNF affects the haemostatic properties of vascular endothelium by stimulating procoagulant activity, thereby facilitating the formation of thrombi. TNF can also damage vascular endothelial cells directly. Thus, within a solid tumour TNF might lead to occlusion of its vessels and subsequent diminished perfusion which finally leads to necrosis of the tumour. Not only is the neovasculature of certain tumours sensitive to the activity of TNF, but normal tissue endothelium may also be damaged [7, 8].

Multiple phase I studies with TNF have been carried out. In most studies TNF have been administered intravenously. Overall, it appears that systemic TNF, in the doses and schedules examined, has little single agent activity in the treatment of advanced human malignancy [9–15]. Due to excessive toxicity

there is no further dose escalation possible. Dose-limiting toxicity mainly consisted of hypotension most probably caused by the effects of TNF on normal endothelium. Intratumoral application of TNF, however, has occasionally resulted in local tumour regression [16–18]. By this route higher concentrations can be achieved at the site of malignancy, without severe systemic toxicity. The lesions were mainly located superficially and therefore easy to inject.

In the present study we report results of a clinical phase I trial of recombinant human TNF (rTNF) administered by sonographic control into liver metastases of various types of adenocarcinomas.

MATERIALS AND METHODS

Patients

Patients with histologically proven liver metastases refractory to standard anticancer therapy were eligible for this study. Patients were informed about the design of the trial as a single-arm, non-randomised, open label study to evaluate the toxicity of intratumoral rTNF. Written informed consent was obtained from all patients. The study protocol had been approved by the institutional ethics committee.

Eligibility criteria included: age between 18 and 75 years, a Karnofsky performance status > 70, a life expectancy of more than 3 months, and an interval of more than 4 weeks since any prior antitumour therapy; a normal renal (creatinine < 110 μmol/l) and hepatic function (bilirubin < 20 umol/l and SGOT < 90 U/l); a normal peripheral blood count, including haematocrit > 25%, leucocytes > 3×10^9/l, and platelets > 100×10^9/l.

Excluded were patients with uncontrolled infection, cardiac disease, hypertension, bleeding disorders or neurological disorders. At entry of the study patients were evaluated by medical history, physical examination, Karnofsky performance status, coagulation profile, complete blood cell count, determination of liver and renal function, determination of serum electrolytes and urine analysis.

Drug formulation

Human recombinant TNF (rTNF) was provided in lyophilised form by Knoll AG, FRG. The product had a specific activity of 6.63×10^6 U/mg and was reconstituted in sterile 0.9% NaCl in a way that each dose rTNF given had a volume of 1 ml. The preparation used was more than 99% pure and contained less than 10 pg of endotoxin per mg protein.

Ultrasound guided injection

Prior to rTNF administration a well-defined liver metastasis was selected with a sonography system (Aloka SSD 650, Japan). After the site of drug administration was anaesthetised with Xylocain 1%, rTNF was injected central into the selected liver metastasis with Shiba-needles (23 G) and sonographic control.

Treatment protocol and follow-up

rTNF was administered according to a 50 μg escalating dose schedule with 3 patients at each dose level. The study was initiated using a dose of 100 μg rTNF per injection. Patients were allowed to escalate to higher dose levels of rTNF provided that they were tolerating the lower dose of rTNF well. If indicated, patients could be treated with non-steroid anti-inflammatory drugs (NSAID). Prior to rTNF administration and 1–4 days thereafter, the following parameters were monitored: haemoglobin, white blood cell (WBC) and differential counts, platelets, SGOT, SGPT, lactic dehydrogenase (LDH), gamma-GT, total bilirubin, alkaline phosphatase, creatinine, total serum protein, uric acid, prothrombin time, and urine analysis. Vital signs (pulse, blood pressure and temperature) were controlled at 10, 20, 30 min, 1, 2, 4 and 8 h and daily until the fifth day after rTNF injection.

Response was measured after 4 weeks by sonographic control. Response determination and toxicity grading were performed according to WHO recommendations [19].

Table 1. Patients' characteristics

Total number of patients	15
Sex (M/F)	11/4
Age range in years (median)	37–73 (60)
No. of injections	23
Previous therapy	
Surgery	4
Surgery + CT	4
Surgery + RT	1
CT	4
RT	1
None	1
Type of malignancy	
Colorectal	9
Pancreatic	2
Gastric	2
Hepatic	1
Unknown	1
Diameter of metastases (median) (cm)	2–16 (5.3)

CT = chemotherapy, RT = radiotherapy.

Pharmacokinetics and immunology

Serum concentrations of rTNF were measured by IRMA (detection level of 5 pg rTNF/ml serum, Medgenix, Fleurus, Belgium) in 3 patients receiving 300 μg. Peripheral blood samples were taken prior to rTNF injection, and after 2, 5, 10, 20, 30, 60 and 120 min.

Subpopulations of lymphocytes were assessed by FACS analysis using monoclonal antibodies against several lymphocyte surface antigens (CD3, CD4, CD8, CD16, CD19) in 5 patients at a dose level of 150, 200 and 250 μg.

Statistics

Data before and after TNF administration were compared using the paired Student's *t*-test. A P value less than 0.05 was considered to indicate a significant difference.

RESULTS

15 patients have been entered to this study. Patient characteristics are listed in Table 1. A total of 23 injections of rTNF were administered and evaluable for toxicity. The same metastasis was injected twice in 4 patients and three times in 2 patients. The main clinical side-effects included fever, chills, nausea, vomiting, diarrhoea, headache, and pain at the injection site (Table 2).

Systemic toxicity

All patients treated by intratumoral injection of rTNF experienced some degree of constitutional symptoms. Independently of the dose rTNF, chills appeared within 10–20 min after injection and lasted for 10–40 min. Although antipyretic drugs (indomethacin, paracetamol) were administered prior to 21

Table 2. Side-effects of rTNF injected into liver metastases

Dose (μg/injection)	100	150	200	250	300	350		
(× 10^6 U/injection)	0.66	0.99	1.32	1.65	1.98	2.31	Total	
Injections	3	3	3	3	6	5	23	(%)
Chills	2	3	3	3	5	3	19	84
Fever*	3	2	3	2	5	3	18	78
Nausea	2	2	1	1	4	2	12	52
Vomiting	1	2	1	1	3	1	9	39
Pain†	1	0	2	0	1	2	6	26
Headache	1	1	2	0	0	0	4	18
Diarrhoea	0	0	0	0	1	2	3	13
Hypotension‡	0	0	1	0	1	0	2	9

The side-effects observed did not exceed grade II toxicity as defined by the WHO criteria.
* Fever > 38°C.
† Pain at injection site; 1 patient (300 μg) had pain at the site of local recurrence in the sigmoid.
‡ Hypotension < 100 mmHg systolic.

rTNF injections in 13 patients, chills followed by fever developed after 13 injections in 9 patients, mild rigors without fever occurred after 4 injections in 2 patients, and fever without chills occurred in 2 patients. 2 patients were not treated with NSAID and both developed chills followed by fever. Temperatures up to 39.9°C were found within 0.5–2 h after rTNF injection. Notably, some patients treated with rTNF at higher doses did not experience chills or a rise in body temperature.

Hypotension requiring volume substitution was observed in 2 patients treated with rTNF at doses of 200 and 300 μg, respectively. These patients had been treated concomitantly with morphine. In later courses the addition of morphine was avoided and similar or higher doses of rTNF were not found to reduce blood pressure of the same patients.

Hepatic toxicity

There were no significant differences in liver tests prior to and after rTNF injection (Fig. 1). Changes in bilirubin, alkaline phosphatase, SGOT, SGPT, gamma-GT and LDH were not related to the size of the liver metastases treated or the dose of rTNF injected.

Haematological toxicity

No effect of rTNF treatment was observed on red blood cell (RBC), WBC, WBC differentials and platelet counts.

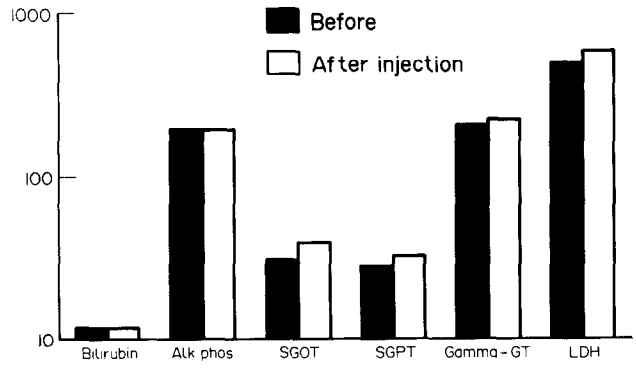

Fig. 1. Liver tests after various doses of rTNF (100–350 μg) injected into liver metastases. Bilirubin expressed in μmol/l; alkaline phosphatase, SGOT, SGPT, gamma-GT and LDG as U/l.

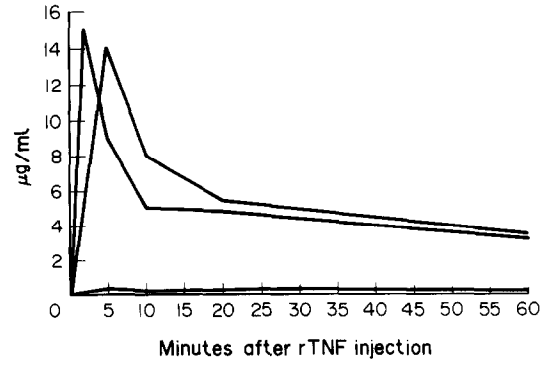

Fig. 2. Serum concentration rTNF after intratumoral injection of 300 μg rTNF in 3 patients.

Pharmacokinetics and immunology

There were variations in the maximum concentration and the time course of plasma levels rTNF in the 3 patients receiving the same dosage of 300 μg (Fig. 2). Lymphocyte-count and subpopulations of lymphocytes ($CD3^+$, $CD4^+$, $CD8^+$, $CD16^+$ and $CD19^+$) in peripheral blood samples prior to rTNF injection, and after 2 and 4 days, showed no significant difference (Fig. 3).

Other toxicity

5 patients experienced pain at the site of injection, despite the use of local anaesthetics. After rTNF administration into a liver metastasis 1 patient indicated pain at the site of a recurrent adenocarcinoma of the sigmoid. However, endoscopic control of the local recurrence revealed no effect on tumour size. Gastrointestinal effects such as nausea, vomiting and diarrhoea were mild and transient. Renal function as measured by serum creatinine remained unchanged.

Dose-limiting side-effects were not observed in any of the patients treated with intratumoral rTNF. As defined by the WHO criteria the most serious kind of complications observed in this trial can be indicated by grade II toxicity. All clinical side-effects were reversible within 12 h after drug administration. The severity and duration of symptoms were not related to the dose of rTNF injected.

Tumour response

Since the aim of a phase I trial is to define the toxicity of a certain regimen, no conclusions can be drawn on the effectiveness of rTNF as an anticancer drug by this route of administration.

In 8 patients the injected metastasis showed no progression. Characteristics of these patients are listed in Table 3. It should be noted that in all patients only the liver metastasis treated with rTNF showed stable disease, while other metastases, located nearby within the same liver lobe, showed progression.

DISCUSSION

The aim of this study was to establish the toxicity and safety of rTNF injected into liver metastases by sonographic control. The main clinical side-effects were chills and fever despite pretreatment with indomethacin. No conclusions can be drawn about the effectiveness of indomethacin to attenuate toxicity. Although administration of indomethacin did not prevent side-effects, it may have reduced the severity and duration of rTNF toxicity. This assumption is invigorated by the results of a

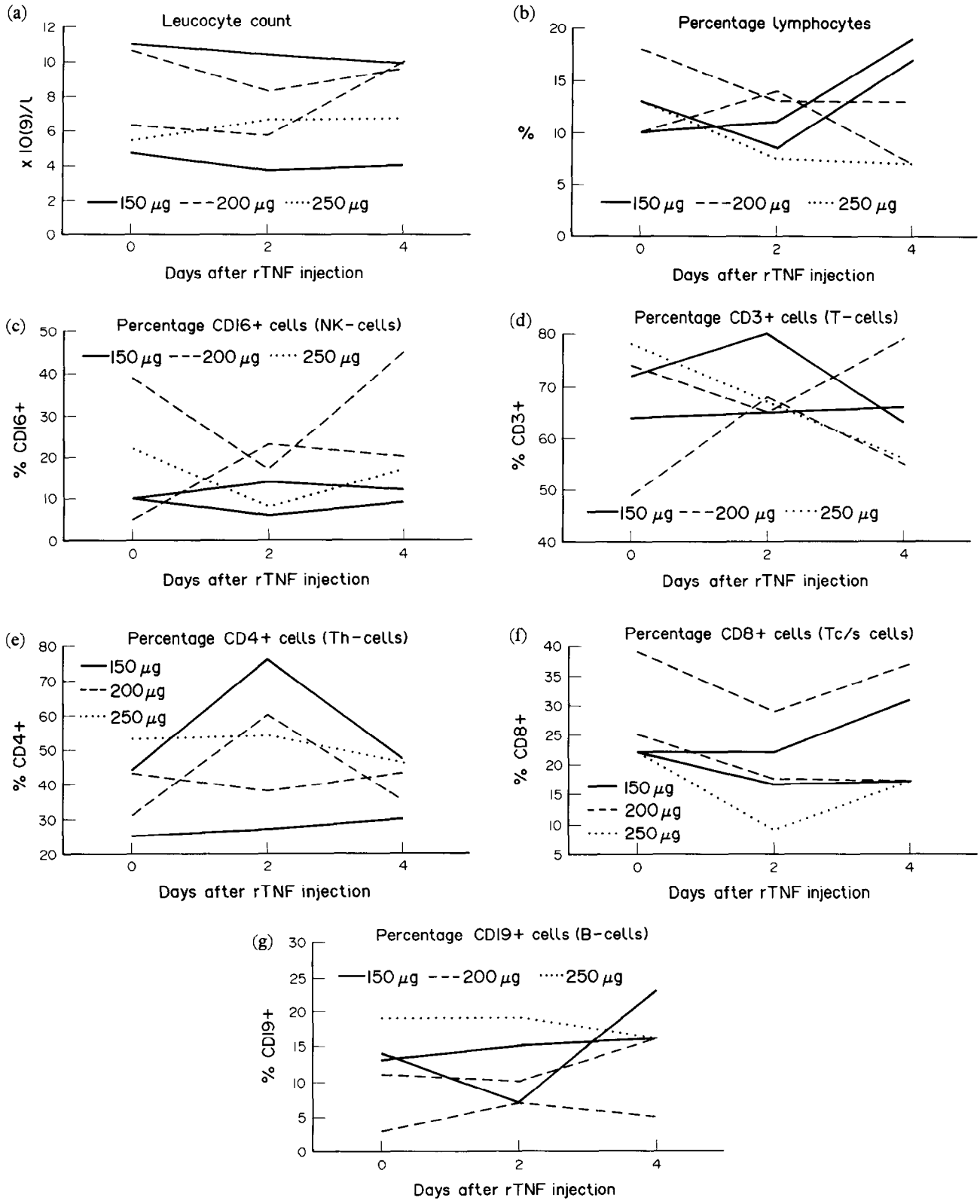

Fig. 3. Leucocyte count, percentage lymphocytes and lymphocyte subpopulations in peripheral blood samples prior to (= 0) and after 2 and 4 days of rTNF injection in 5 patients.

similar study where no NSAID were used to reduce toxicity. At a lower maximal dose, in comparison with our study, toxicity was more severe and resulted in life-threatening prostration [16]. Bartsch *et al.* reported that the severity and duration of chills, fever, local oedema and pain at the injection site was dose-related [17]. Our study did not confirm this. 5 patients experienced pain at the site of injection, although local anaesthetics were used prior to injection. 1 patient indicated pain at the site of a recurrent adenocarcinoma of the sigmoid. This phenomenon has also been reported by other investigators [16]. From results of the present study it is not clear whether these symptoms were caused by rTNF itself, or maybe caused by

Table 3. Characteristics of responders

Patients Age (y)	Sex	Dose of rTNF 1st inj.	2nd inj.	3rd inj.	Diameter of metastasis (cm)	Response*	Duration of response (weeks)	Type of malignancy	Previous therapy
62	M	100	—	—	6.3	SD	4	Pancreatic	S + CT
44	M	100	150	—	9.7	SD	4	Unknown	None
52	M	200	—	—	6.3	SD	4	Colon	S + CT
53	F	250	300	—	2.5	SD	4	Colon	S
53	M	300	—	—	4.0	SD	4	Colon	CT
59	M	300	300	—	4.1	SD	8	Gastric	CT
71	M	200	250	350	7.0	SD	8	Colon	CT
63	M	300	300	350	7.6	SD	8	Colon	S

* Only response of injected metastasis, SD = stable disease, S = surgery, CT = chemotherapy.

other cytokines induced by rTNF, e.g. interleukin-1 [20]. In our study hypotension was observed in 2 patients treated concomitantly with rTNF and morphine. As both agents can induce a decrease of blood pressure and may act synergistically, concomitant use should be avoided. Hepatic toxicity is documented in several phase I studies of systemic administration of rTNF [12, 21, 22]. We did not see any significant changes in liver tests after rTNF injection in a liver metastasis. Intratumoral application of a lower-dose rTNF, in comparison with the doses used in this study, showed elevations of SGOT or SGPT (> 2 × baseline value) in 3 out of 21 patients, and elevation of bilirubin, alkaline phosphatase and gamma-GT in 6 patients [16]. However, other investigators reported no deranged liver tests after intratumoral application of rTNF, even at a higher dose than used in this trial [17]. These controversial results may be related to the accuracy of the intratumoral rTNF administration, and to the size and vascularisation of the tumour, leading to differences in the rate of absorption and metabolisation of the injected drug. This also applies for the differences in plasma levels of rTNF measured after intratumoral injection of the same dosage. In other trials rTNF was administered intravenously for 24 h and lower plasma levels were found for a prolonged time [15, 23]. It seems that the effect of rTNF on the tumour depends mostly on the concentration of rTNF at the tumour site, whereas side-effects are related to the duration of rTNF in the plasma and not to the maximal plasma concentration. It is suggested that prolonged intravascular exposure of rTNF increases the number of rTNF receptors on endothelial cells and that this mechanism explains the more severe side-effects seen by continuous intravenous administration of rTNF. It should be noted that others have found more serious side-effects, in comparison with our study, after an intravenous injection of a lower-dose rTNF than used in our study [10, 21]. This finding stresses the importance of proper intratumoral injection to prevent intravascular leakage with, consequently, more severe side-effects.

Only sporadic tumour responses have been reported after systemic administration of rTNF in a phase I study [21, 24–26], while no responses have been reported in phase II studies of intravenous rTNF application [27–30]. Regression of tumour growth and even complete responses were found after intratumoral injection. Pfreundschuh *et al.* reported a phase I trial of intratumoral application of rTNF in 21 patients. Complete local response was seen in 1 patient, partial local response in 4 patients, minor local response in 4 patients, and stable disease in 2 patients [16].

Bartsch *et al.* found three partial responses, two minor responses, and three stable diseases in a total of 14 injected tumours with rTNF in a phase I study. All responses were restricted to the site of TNF application [17]. In our study eight injected metastases showed stable disease. As this antitumour effect could not be related to changes in overall host defence parameters, systemic activation of the immune system by rTNF seems unlikely to be of importance. In this study phenotypical parameters of peripheral lymphocytes were measured in 5 patients at three different dose levels. No significant changes in relative numbers of subpopulations were found. It is not certain whether these phenotypical parameters reflect a functional property of the identified cells. However, it is unlikely that the immunological parameters as measured in peripheral blood samples can reflect local activation or proliferation of tumour-infiltrating cells by rTNF, especially when rTNF is given intratumorally. This concept of local activation of tumour-infiltrating lymphocytes (TILs) by cytokines has been demonstrated in primary and metastatic liver tumours in an in-vitro model [31]. Another mechanism by which rTNF may conduct its antitumour effect is the effect on endothelial cells which results in thrombi formation and finally haemorrhagic necrosis. Kaposi's sarcoma showed tumour regression in 15 out of 16 tumours injected with rTNF [18]. Since Kaposi's sarcoma is a neoplastic endothelial cell proliferation, the effects of rTNF on endothelium may account for the tumour responses seen. It is likely that a critical local rTNF concentration at the tumour site is important for the induction of a tumour response. This critical local rTNF concentration cannot be reached by intravenous dosage due to excessive toxicity, and the short half-life of rTNF of approximately 20 min. Other mechanisms of improving local concentration of rTNF at the site of malignancy are being investigated, such as liposomes filled with rTNF [32], and the genetic engineering of TIL to produce TNF [33].

Since future studies are designed to evaluate the effect of a combination of cytokines, namely rTNF and interferon-gamma, further dose escalation of single TNF injections was not investigated. The synergistic activities of rTNF and interferon-gamma on tumour growth inhibition may also be expressed in toxicity. Therefore we accepted the dose of rTNF found in this study as a safe range to start a new trial investigating the effects of both cytokines.

This study shows that local injection of rTNF in a liver metastasis by sonographic control is a safe procedure and is a method to obtain maximal concentration of the drug at the tumour site, while reducing systemic toxocity. Although the

scope of drug administration by intratumoral injection is limited for the treatment of disseminated malignancies, it may help to elucidate the relevance of rTNF as an antineoplastic agent.

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