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Phase-I trial of intravenous continuous infusion of tumor necrosis factor in advanced metastatic carcinomas

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Summary. Fifteen patients with advanced metastatic adenocarcinomas were treated in a phase-I study with continuous intravenous 24 h infusion of recombinant tumor necrosis factor α (TNF-α) in order to determine the maximum tolerated dose (MTD) and associated side-effects. Patients received 40–400 μg/m² TNF-α once (arm A) or twice (arm B) weekly for a scheduled treatment period of 2 months. The observed systemic side-effects resembled those reported for interferons and included fever, chills, fatigue, headaches, myalgias, thrombocytopenia, prostration, and malaise. Dose-limiting toxicities, resulting in a median MTD of 200 μg/m² for 24 h, were fever, chills, fatique, myalgias, and thrombocytopenia. Out of 15 patients, 11 showed tumor progression, and 3 sustained in no change for over 2 months of treatment. A minor response was seen in 1 patient with a colorectal carcinoma and liver metastases. To reduce side-effects, patients were treated either with paracetamol or indomethacin. Higher MTDs were observed in patients treated with indomethacin. No detectable plasma TNF-α levels or TNF antibodies were measured under therapy (plasma TNF-α < 20 pg/ml). We conclude that TNF-α appears to have some antineoplastic activity in patients with adenocarcinomas since 4 patients remained in no change or showed a minor response.

Key words: Recombinant human tumor necrosis factor alpha – Maximum tolerated dose

Introduction

Tumor necrosis factor α (TNF-α) or cachectin is a polypeptide of 17 kDa arranged in a mainly trimeric form (Aggarwal et al. 1985a, b; Beutler et al. 1985) with various biological functions (Beutler and Cerami 1987). Using recombinant techniques, the primary structure of human TNF-α has been determined (Pennica et al. 1984; Shirai et al. 1985; Wang et al. 1985) and purified recombinant human TNF-α is now available for clinical trials. So far, preclinical studies with TNF-α have been demonstrated to induce in vivo tumor regression by hemorrhagic necroses of various tumors in animal models (Carswell et al. 1975; Haranaka et al. 1984).

Various clinical phase-I trials using different regimens of application are now in progress. So far, studies have dealt with the intravenous bolus and direct intratumoral injection as well as 5-day continuous infusion of the purified protein (Blick et al. 1987; Sherman et al. 1988). Considering the short plasma half-life of TNF-α (15–20 min), we developed a study protocol, where TNF-α was continuously infused i.v. for 24 h in patients with metastatic adenocarcinomas. The aims of this study were to determine (a) the maximum tolerated dose (MTD) of TNF-α administered by 24 h continuous infusions; (b) the quantitative and qualitative dose-limiting toxicities of TNF-α; (c) the recommended dose for phase-II studies; (d) the pharmacokinetic and immunogenic properties of TNF-α, and (e) its antineoplastic activity.

Materials and methods

Patients and eligibility. A total of 15 patients with metastatic carcinomas were included in the study. All patients had measurable and histologically confirmed disease and fulfilled the following eligibility criteria (for detailed patient characteristics see Table 1). (a) Patients had an ECOG-Zubrod performance status of 0–2. (b) The expected time of survival was at least 2 months. (c) An interval of at least 4 weeks without chemotherapy, radiation therapy or immunotherapy (interferons) and major surgery had elapsed and patients had not received nitrosoureas or mitomycin C for 6 weeks prior TNF-α therapy. (d) Patients had normal hematologic, hepatic and renal functions before treatment or grade I impairment according to WHO criteria. (e) No brain metastases and no severe non-malignant concommittant disease were evident. (f) Written informed consent of the protocol procedures, risks and benefits had been

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Table 1. Patient characteristics

Total number of patients	15
Male	10
Female	5
Median age (years)	58
Range (years)	47–67
Performance status	
ECOG 0	5
ECOG 1	4
ECOG 2	6
Previous therapy	
Surgery and chemotherapy	8
Surgery, chemo- and immunotherapy	4
Surgery, chemo- and hormone therapy	1
Surgery, chemo- and radiotherapy	1
Surgery	1

obtained. The study was performed under the guidelines of the Helsinki declaration.

TNF preparation. Lyophilized recombinant human tumor necrosis factor (TNF-α) was provided by Knoll AG, Ludwigshafen, FRG, in ampoules of 1 mg. TNF-α was dissolved in 3.0 ml distilled water and diluted in 5–20% human serum albumin to the required concentrations. After initial randomization, TNF-α infusions were started once (arm A) or twice weekly (arm B) at a starting dose of 40 μg/m² for 24 h. In the absence of major side-effects weekly dose escalations were performed according to a modified Fibonacci scale (Table 2). The treatment schedule consisted of a total of 8 or 16 treatments over a period of two months. Volumetric computed tomography determination of metastatic liver lesions was performed before and after TNF-α treatment if practicable. Pre- and post-treatment clinical examination included a complete medical history, physical examination, complete blood cell count, blood chemistry, urine analysis, electrocardiogram, chest X-ray, abdominal ultrasound, and bone scan. All patients had a once (arm A) or twice (arm B) weekly follow-up during the trial period by means of a physical examination, blood cell count, blood chemistry, and urine analysis. Toxicity was graded according to WHO criteria (Miller et al. 1981). MTD was defined as the dose in which reversible grade-3 toxicity or irreversible grade-2 toxicity was present.

Suppression of side-effects. For suppression of side-effects, such as chills and fever, patients received either paracetamol (1000 mg p.o. or rectally) or indomethacin (50 mg p.o. or rectally) 1 h before and in 6–8 h intervals during the 24 h TNF-α infusions.

TNF-α and TNF-α antibodies plasma determinations. Plasma samples for TNF-α levels were obtained from each patient before the infusion and at 12, 20, and 24 h after starting the infusion. Furthermore, samples were taken 5, 30, 60, and 120 min after stopping the infusion. Samples were assayed for TNF-α by two independent methods. (a) Plasma levels were measured by an enzyme-linked sandwich immunoassay using two polyclonal antibodies against TNF-α, where one of the antibodies was conjugated to biotin. Antobidies were produced with purified TNF-α as antigen in rabbits and purified from sera by protein A affinity chromatography (for details see Kist et al. 1988). The lower detection limit for TNF-α was 20 pg/ml. (b) Plasma TNF-α was also determined by an assay measuring the killing activity of TNF-α of L 929 cells, as described by Andus et al. (1987). This assay also possesses a detection limit of 20 pg/ml TNF-α. The presence of serum antibodies to TNF-α was monitored by enzyme immunoassay: TNF-α (1 μg/ml in 0.05 *M* sodium carbonate buffer, pH 9.2) was coated onto 96-well plates (Flow Laboratories, Meckenheim, FRG) for 2 h at room temperature. After blocking with 1% bovine serum albumin in phosphate-buffered saline (PBS) overnight and several washings in 0.05% Tween 20/PBS, a twofold-diluted serum sample was applied for 2 h. After three wash steps, specific immune complexes were detected by goat anti-(human IgG/M) antibodies coupled to peroxidase. After repeated washings in 0.05% Tween 20/PBS the specific immune complexes were quantified enzymatically by a colour reaction using 3,3′,5′-tetramethylbenzidine as a substrate.

Results

In the 15 patients who entered the trial the location of the primary tumor was as follows: colorectal carcinomas (8), renal cell adenocarcinomas (5), gastric adenocarcinoma (1), and maxillary adenocystic carcinoma (1). A group of 8 patients was randomized to treatment arm A, 7 patients received treatment under arm B.

In 10 out of 15 patients who entered the study (4 patients arm A; 6 patients arm B), dose escalations were stopped because of side-effects leading to a determination of the MTD for these patients (see Table 2). The remaining 5 patients (4 patients arm A, 1 patient arm B) were treated for 2 months with complete dose escalation without the establishment of a MTD.

Table 2. Adverse clinical effects of TNF-α: the number of patients associated with adverse effects (percentage in parentheses)

Symptom	Dose in 24 h (μg/m²)							
	40	80	140	200	250	300	350	400
Fever	11 (73%)	9 (69%)	5 (33%)	4 (50%)	4 (57%)	3 (50%)	3 (60%)	3 (60%)
Chills	14 (93%)	12 (92%)	11 (100%)	8 (100%)	7 (100%)	6 (100%)	5 (100%)	5 (100%)
Headache	7 (47%)	4 (31%)	2 (18%)	1 (13%)	2 (29%)	2 (33%)	1 (20%)	1 (20%)
Fatigue	9 (60%)	9 (69%)	6 (55%)	5 (63%)	5 (71%)	3 (50%)	3 (60%)	2 (40%)
Anorexia	1 (7%)	2 (15%)	1 (9%)	1 (13%)	1 (14%)	1 (17%)	1 (20%)	0
Nausea	4 (26%)	3 (23%)	3 (27%)	4 (50%)	3 (43%)	2 (33%)	3 (60%)	2 (40%)
Vomiting	1 (7%)	1 (8%)	1 (9%)	1 (13%)	3 (43%)	3 (50%)	3 (60%)	3 (60%)
Diarrhoea	2 (13%)	1 (8%)	1 (9%)	1 (13%)	2 (29%)	2 (33%)	1 (20%)	2 (40%)
Myalgia	2 (13%)	2 (15%)	0	0	0	0	1 (20%)	1 (20%)
Total No. of patients	15 (100%)	13 (100%)	11 (100%)	8 (100%)	7 (100%)	6 (100%)	5 (100%)	5 (100%)
Total No. of treatments	39	29	12	11	9	7	6	6

Four of these patients received indomethacin for suppression of major side-effects. Patients who did not receive full dose escalation were treated for a period of 1–7 weeks. One patient (arm B), who died within the first week of treatment of a cause unrelated to TNF-α therapy, was regarded as a drop-out.

Systemic toxicity

All patients exhibited systemic side-effects such as fever, chills, myalgia, and prostration (see Table 2). Fever, headache, chills, myalgia, and prostration alone or in combination were dose-limiting in 8 patients. Hypotension was not a prominent symptom (systolic blood pressure >100 mm Hg; 13 kPa) and never required any specific treatment.

Hematological toxicity

Anemia was the most prominent toxicity and was observed in 11 out of 15 patients resulting in a reduction of hemoglobin ranging from 1 g/dl to 3.5 g/dl (median 2 g/dl). However, this anemia was particularly prominent in arm B patients who received the full escalation scheme. Interestingly, no patients showed an increase in the reticulocyte count. Decreases in white blood cell count did not exceed grade 2 leukopenia and were never dose-limiting. Thrombocytopenia never exceed grade 2 except in one patient, who had received various chemotherapeutic regimens. In this case grade 4 thrombocytopenia occurred during the first course of TNF-α treatment (0.04 mg/m^2 for 24 h = MTD for this patient). Blood coagulation parameters, such as prothrombin time, partial thromboplastin time and fibrinogen before and after each course, were normal in all patients.

Other toxicities

One patient with colorectal carcinoma (treatment arm A) developed a paralytic ileus upon TNF-α treatment (0.35 mg/m^2 for 24 h) leading to a discontinuation of therapy. After cessation of therapy normal bowel function was restored. Changes in hepatic function caused by TNF-α therapy did not exceed grade 1 (Bilirubin, SGOT, SGPT, alkaline phosphatase, and γ-glutamyltransferase) in the majority of patients. Reversible grade 2 toxicity (increase in bilirubin to 3.0 mg/dl) was only observed in one patient. No impairment of renal function was noted.

Weight loss of all patients did not exceed 2.5% of the body weight as compared to pretreatment levels. No changes in serum triglycerides and cholesterol levels were observed.

Tumor response to TNF-α treatment

One patient with a colorectal carcinoma showed a minor response with a decrease of liver tumor volume as documented by computed tomography volumetric studies (460 to 278 ml) after 2 months of treatment. The volume index (metastatic over organ volume) dropped from 0.2 to 0.12 over this treatment period. Three patients with renal cell carcinomas metastasizing to bone and lung showed stable disease under treatment with TNF-α.

Plasma levels of TNF-α and anti-(TNF-α) antibodies

TNF-α plasma levels could not be measured by two independent techniques (minimum detection level: 20 pg/ml). No serum antibodies to TNF-α were detectable by enzyme immunoassay.

Discussion

Human recombinant tumor necrosis factor alpha (TNF-α), administered as a 24-h continuous intravenous infusion, possesses side-effects similar to those of other lymphokines such as interferon-α (see, for example, Queseda et al. 1983). Without the application of indomethacin a mean maximum-tolerated dose of 200 μg/m^2 in 24 h was found. Systemic side-effects, including fever, chills, prostration, anorexia, myalgia, headache, and respiratory insufficiency were severe and dose-limiting. Chills, fever (38°–41 °C), and fatigue were observed in all patients. These side-effects have been also observed in all studies published so far (see, for example, Blick et al. 1987; Sherman et al. 1988). Whereas these side-effects could be partly reduced by the administration of paracetamol or especially indomethacin, hematological toxicities (i.e. reduction of plasma hemoglobin and deficient reticulocytosis) could not be prevented by either paracetamol or indomethacin. This observation confirms and extends findings where TNF-α has been demonstrated to inhibit erythopoesis in vitro (Sassa et al. 1983). Recently, Sherman et al. (1988) reported on TNF-α induced thrombocytopenia, which was especially prominent after 5 days of continuous i.v. TNF-α infusion, indicating direct toxic effects (i.e. increased platelet consumption) rather than suppression of thrombopoiesis. Similarly, one patient in our study developed grade 4 thrombocytopenia directly after one course of TNF-α application. In contrast to Sherman et al. (1988), we observed only mild leukopenias, which are probably based on the relatively short duration of TNF-α infusion. Furthermore, interference of TNF-α with hepatic and renal function and lipid metabolism, as judged by standard laboratory blood parameters,

were only very moderate. Rigor, reported to be a common symptom (Trump et al. 1987), and very moderate hypotension were only observed in 2 patients.

In contrast to i.v. bolus injection (Blick et al. 1988) no TNF-α plasma levels were detected in this study (Sherman et al. 1988). This discrepancy, however, is not surprising considering the short plasma half-life and the amounts of TNF-α applied under 24 h continuous infusion. Since the spectrum of side-effects is similar in most studies performed so far by i.v. bolus or by i.v. continuous 24 h infusion, the effects of TNF-α are obviously achieved at very low plasma concentrations. Interestingly, no TNF-α antibodies could be detected, as also observed by others (Blick et al. 1987; Sherman et al. 1988).

Our results, obtained in a phase-I study, suggest that TNF-α may possess a moderate antineoplastic action, since three patients developed no change and 1 patient showed a minor response under the treatment period. These effects were observed despite the absence of measurable plasma levels. Further studies, however, are required to determine the optimum clinical setting and precise role of TNF-α in the treatment of cancer patients.

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