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# Phase I study of recombinant human tumor necrosis factor

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**Summary.** A phase I clinical and pharmacokinetic study of recombinant human tumor necrosis factor (rH-TNF) was conducted in a single dose schedule in 33 patients with advanced cancer. rH-TNF was given by i.v. infusion over 30 min with a starting dose of $1 \times 10^5$ units/m$^2$. The dose was escalated up to $16 \times 10^5$ units/m$^2$ according to the modified Fibonacci scheme. Toxic effects were similar but not identical to those reported with interferons and interleukin-2, and included fever, rigors, nausea and vomiting and anorexia in a non-dose-dependent manner, and hypotension, leukocytosis, thrombocytopenia and transient elevation of transaminases (SGOT and SGPT) in an approximately dose-dependent manner. DIC syndrome was observed in one patient who had received $16 \times 10^5$ units/m$^2$. The dose-limiting toxicities were hypotension, thrombocytopenia and hepatotoxicity, and the maximum tolerated dose in a single i.v. infusion of rH-TNF appeared to be $12 \times 10^5$ units/m$^2$ when thrombocytopenia and elevation of SGOT and SGPT were taken as the dose-limiting toxicities. However, if hypotension was included, the maximum safely tolerated dose appeared to be $5 \times 10^5$ units/m$^2$.

## Introduction

Tumor necrosis factor (TNF) was discovered in the sera of mice which had been primed with *Bacillus Calmette-Guérin* and subsequently challenged with endotoxins [4]. TNF has also been obtained from the sera of similarly treated rats, guinea pigs and rabbits, and is known to be associated with in vivo and in vitro killing of tumor cells [4, 12, 15, 21]. TNF is also produced by some human cell lines [30]. Human TNF shows the same pattern of biological activities as mouse TNF, and causes hemorrhagic necrosis of TNF-sensitive mouse sarcomas [15, 30]. Recently, the gene for human TNF has been cloned and expressed in *Escherichia coli* [16, 22]. The product of this expression was isolated in pure form and shown to produce hemorrhagic necrosis of mouse tumors and cause the same pattern of cytotoxicity as natural TNF on mouse and human cell lines [16, 22, 24].

We have conducted a phase I study of a recombinant human TNF (rH-TNF) in patients with advanced cancer as part of a multi-institutional cooperative study. Since the present study was the first clinical trial of TNF in the world, we initiated the study in a single-dose schedule. The purpose of the study was to evaluate the safety of clinical administration of rH-TNF and to determine the dose-limiting toxicity and the maximum tolerated dose (MTD) of rH-TNF administered as a single dose. Pharmacokinetic studies were also conducted.

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## Patients and methods

*Patients.* Patients with histologically confirmed malignancy which had become refractory to conventional therapies were considered eligible for this study after they had given informed consent. Patients had received no anticancer treatment for at least 4 weeks before entering the study. Patients were required to have a performance status (PS) of 0–3 according to the Eastern Cooperative Oncology Group criteria [31] and to have adequate blood cell counts (leukocyte $\geq 3000/mm^3$, platelets $\geq 75000/mm^3$) and adequate renal (BUN $\leq 25$ mg/dl), hapatic (SGOT and SGPT $\leq 2$-fold of normal values, bilirubin $\leq 3$ mg/dl) and cardiovascular function.

*Treatment plans.* rH-TNF was supplied by Asahi Chemical Industry Co., Ltd, Tokyo, The rH-TNF (PAC-4D) was produced in *Escherichia coli* which expressed the gene encoding human TNF that had been identified in a human genomic DNA library by using a cloned cDNA encoding a portion of rabbit TNF as a probe [22]. The rH-TNF consists of 155 amino acids, and its molecular weight is 17000. It is over 99% pure and has a specific activity of $2.2 \times 10^6$ units/mg protein. The unit was designated as the reciprocal of dilution which killed 50% of L-M cells (ATCC CCL1.2). rH-TNF was administered in a single-dose schedule on day 0 only. rH-TNF was dissolved in 100 ml saline and infused i.v. in 30 min. The preclinical toxicology data in mice ($LD_{10} = 8.1 \times 10^5$ units/m$^2$ by a single injection) and the fact that this study was the first human trial of TNF were considered in calculation of the safe starting dose for a phase I study, which we decided was around $1 \times 10^5$ units/m$^2$. The dose was escalated to 2, 3, 5, 7, 9, 12 and $16 \times 10^5$ units/m$^2$ according to the modified Fibonacci scheme. Three patients were registered at each dose level. One or two more patients were studied when unacceptable toxicities were observed at a given dose level. A prick skin test was performed with the dissolved rH-TNF before the infusion to avoid a possible allergic

reaction to the rH-TNF preparation. Patients were removed from the study when toxic effects were unacceptable. The study was terminated when the MTD was established.

*Study parameters.* All patients were hospitalized and monitored closely for evidence of acute and delayed toxic effects. During and after the rH-TNF infusion, vital signs, including temperature, heart rate, respiration, and blood pressure, were frequently monitored at 30 min and at 1, 2, 4, 8 and 24 h after the start of infusion. Laboratory studies included blood cell counts, chemistry panels and urinalysis on day 0, day 1, day 2, day 3, day 7, day 14 and day 21. Occasional ECG and chest X-ray examinations were performed if indicated.

*Pharmacokinetic studies and assay of anti-rH-TNF antibody.* Heparinized blood was collected 0.5, 1, 2, 3, 4 and 8 h after the rH-TNF infusion. The rH-TNF level of plasma was measured by an enzyme-linked immunosorbent assay (ELISA) using two different mouse anti-rH-TNF monoclonal antibodies according to the method described by Hayashi et al. [9] with slight modification. In short, first anti-rH-TNF monoclonal antibody (II7C2) was immobilized in the wells of a 96-well microplate. After the washing away of excess antibody, test samples were added to the wells and kept at 4° C overnight. After another washing, a second peroxidase-conjugated antibody (IV3E5) was added and kept at 25° C for 6 h. Finally, a substrate solution (orthophenylenediamine and hydrogen peroxide) was added, and the plate was incubated for 15 min at 25° C. The reaction product was measured at 492 nm by an ELISA reader. The detection limit of this assay was 0.2 unit/ml of rH-TNF.

Antibody to rH-TNF was tested by a competitive ELISA method. The procedure was the same as for the above ELISA, except that 100 units/ml of rH-TNF was added for 90 min at 25° C and washed before application of the test samples. IgE levels were also measured in the same samples.

**Table 1.** Patient characteristics

| Characteristic | No. of patients |
|---|---|
| Total registered | 33 |
| Total in study | 31 |
| Total evaluated for acute toxicities | 28 |
| Total evaluated for laboratory data | 27 |
| Male/female | 16/12 |
| Age (years): Median | 54 |
| Range | 25–74 |
| PS[a]: 0 | 5 |
| 1 | 5 |
| 2 | 11 |
| 3 | 7 |
| Primary cancer | |
| Lung | 7 |
| Gastric | 6 |
| Colon | 4 |
| Breast | 3 |
| Ovarin | 3 |
| Liver | 2 |
| Esophagus | 1 |
| Metastatic cancer with primary unknown | 2 |

[a] Eastern Cooperative Oncology Group criteria [31]

## Results

During the 4-month period starting in April, 1985, in all 33 patients from 13 university and cancer hospitals in Japan were enrolled in this study. Two patients, however, did not

**Table 2.** Toxic effects of a single-dose rH-TNF administration

| | Dose of rH-TNF (x $10^5$ units/$m^2$) | | | | | | | | Total (%) |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 5 | 7 | 9 | 12 | 16 | |
| No. of evaluable cases | 2 | 2 | 3[d] | 5 | 5 | 4 | 3 | 4 | 28[d] |
| Fever (≥38° C) | 1 | 1 | 3 | 3 | 4 | 3 | 2 | 2 | 19 (67.9%) |
| Rigors | 2 | 1 | 3 | 5 | 2 | 2 | 3 | 2 | 20 (71.4%) |
| Fatigue | 1 | 0 | 1 | 3 | 3 | 2 | 1 | 2 | 13 (46.4%) |
| Anorexia | 1 | 0 | 1 | 2 | 3 | 0 | 2 | 2 | 11 (39.3%) |
| Nausea-vomiting | 0 | 1 | 1 | 2 | 2 | 1 | 0 | 2 | 9 (32.1%) |
| Hypertension[a] | 0 | 1 | 2 | 0 | 1 | 0 | 0 | 1 | 5 (17.9%) |
| Hypotension[b] | 0 | 0 | 0 | 1 | 2 | 1 | 1 | 2 | 7 (25.0%) |
| Diarrhea | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 2 (7.1%) |
| Increase of leukocyte count | | | | | | | | | |
| ≥3000/$mm^3$ | 0 | 0 | 1 | 1 | 3 | 3 | 2 | 4 | 14 (51.9%) |
| ≥10000/$mm^3$ | 0 | 0 | 0 | 0 | 3 | 2 | 1 | 2 | 8 (29.6%) |
| Thrombocytopenia[c] | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 4 (14.8%) |
| SGOT/SGPT elevation | 0 | 0 | 0 | 1 | 3 | 3 | 2 | 2 | 11 (40.7%) |
| BUN elevation | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 (3.7%) |

[a] Elevation of systolic blood pressure of more than 40 mmHg
[b] Drop of systolic blood pressure of more than 40 mmHg
[c] More than 100000/$mm^3$ decrease of platelet counts, or more than 50000/$mm^3$ decrease to below 10000/$mm^3$
[d] One patient did nor receive the full dose because of severe rigors and hypertension. This patient's data were excluded from the evaluation; laboratory data were thus evaluated for 27 patients

receive rH-TNF, one because of an asthma attack and the other because of a deterioration of performance status to 4 just prior to administration. Therefore, 31 patients in fact entered the study. The characteristics of these patients are detailed in Table 1. All patients were monitored in hospitals during the study period. Two patients were given anticancer drugs concomitantly and one patient was given three consecutive daily doses instead of a single dose on day 0. These three patients were excluded from the evaluation. In one patient, rH-TNF infusion was discontinued about 15 min after the start because of severe rigors and hypertension. This patients' data were excluded from the laboratory data evaluation, but included in the evaluation of immediate side effects. Thus, 28 patients were evaluable for immediate side effects and 27 for laboratory data. There were 16 men and 12 women, their ages ranging from 25 to 74 with a median of 54.

*Fever and rigors*

Fever of 38° C or more was seen in 19 patients, 8 of whom had fever of 39° C or more. It appeared 20–60 min after the start of infusion and lasted for 8–24 h. Low-grade fever lower than 38° C was noted in another 8 patients. Owing to severe rigors and high fever, premedication with antipyretics, such as indomethacin and aspirin, were allowed to be used with doses of $7 \times 10^5$ units/$m^2$ or more (Table 2). Therefore, the true incidence of fever might be higher than observed. Fever of 38° C or more was seen in 11 out of 16 patients who had received premedication with indomethacin and/or aspirin. Temperature elevations to over 40° C however, were not seen. The fever responded to antipyretics fairly well.

Rigors were seen in 20 patients. They appeared 15–30 min after the start of infusion and disappeared within 1 h. This usually occurred concomitantly with temperature elevation, though it preceded fever in some cases, and it was often accompanied by myalgia and arthralgia.

Nausea and vomiting were noted in 9 patients and anorexia, in 11 patients. Their symptoms resolved spontaneously within 24 h. Diarrhea was noted in 2 patients.

*Changes in blood pressure*

Transient elevation followed by a drop in blood pressure was seen in the majority of patients in an approximately dose-dependent manner (Table 2; Fig. 1). The pressure rose by 30–60 min after the start of the rH-TNF infusion and dropped gradually afterwards. The nadir was seen 2–8 h after the start of infusion, and then the pressure gradually returned to the pretreatment levels within 24 h. Elevations in systolic pressure of more than 20 mmHg and 40 mmHg were observed in 14 and 5 patients, respectively. Drops in systolic pressure by more than 20 mmHg and 40 mmHg were noted in 18 and 7 patients, respectively. Drops of more than 40 mmHg were noted in patients who received $5 \times 10^5$ units/$m^2$ or more. In 4 patients (1 receiving $9 \times 10^5$ units/$m^2$, 1 receiving $12 \times 10^5$ units/$m^2$ and 2 receiving $16 \times 10^5$ units/$m^2$), systolic pressure fell below 80 mmHg. The pressure recovered gradually usually within 12 h. Dopamine and steroid hormones did not seem to reverse the blood pressure quickly. Since most patients remained supine during the first 24 h, especially when they had severe hypotension, no accident attributable to the hypotension was encountered.

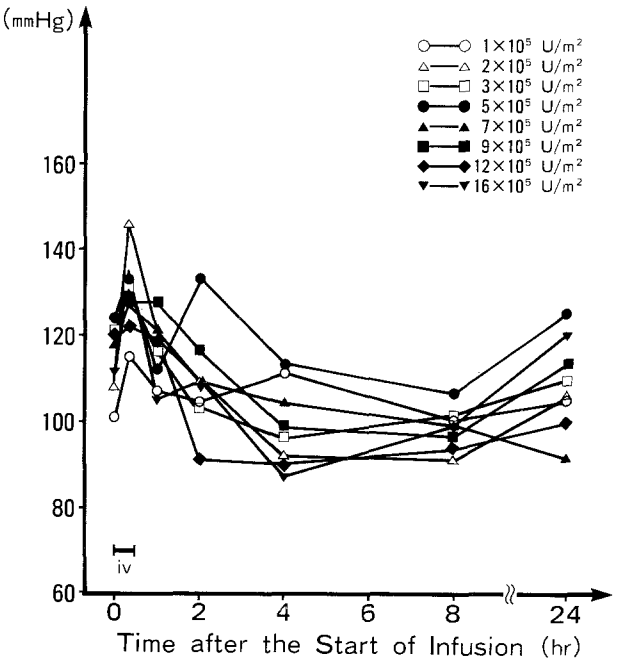


**Fig. 1.** Change of systolic blood pressure after rH-TNF infusion. Mean systolic blood pressure of the patients at each dose level is shown

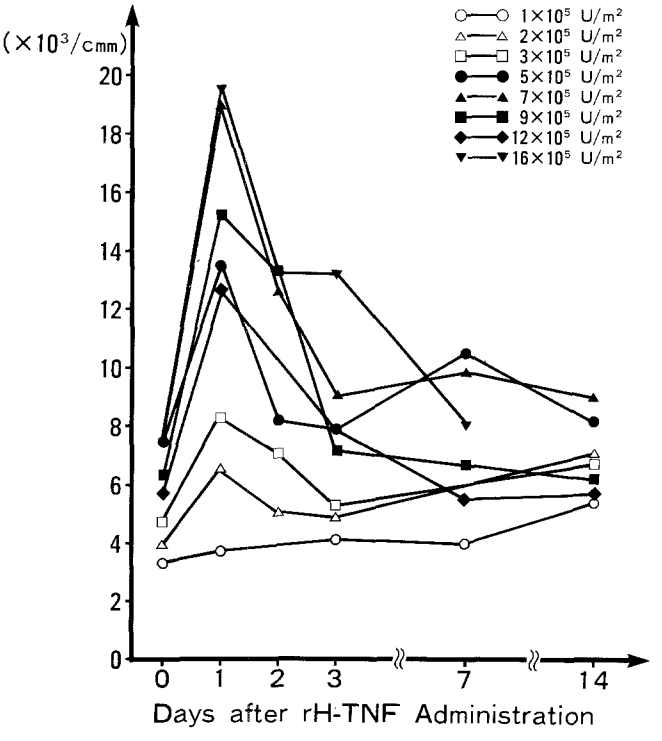


**Fig. 2.** Change of leukocyte counts after rH-TNF administration. Mean leukocyte counts of the patients at each dose level of rH-TNF are shown

*Hematological changes*

Transient increases in leukocyte count of more than 3000/$mm^3$ and 10000/$mm^3$ were seen in 14 and 8 patients, respectively, in an approximately dose-dependent manner, and were prominent in patients who received $7 \times 10^5$ units/$m^2$ or more. Of the 16 patients in this group , 12 had an

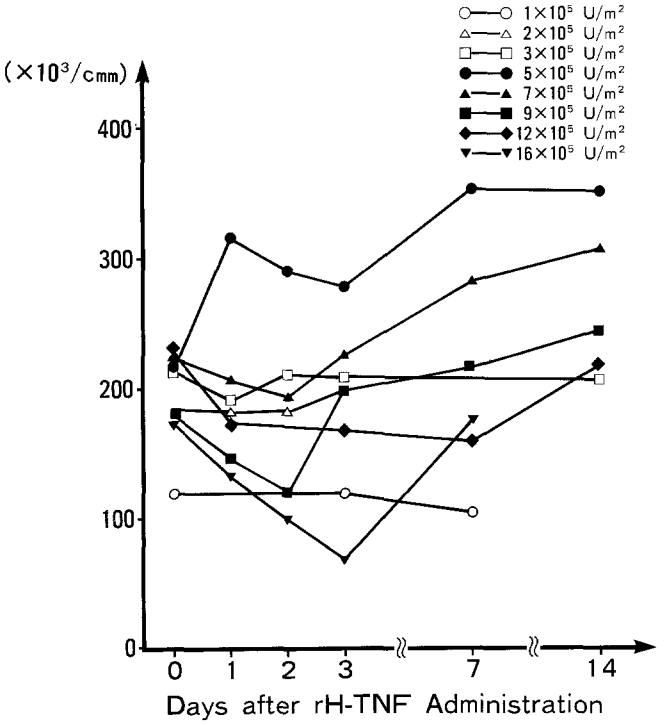


**Fig. 3.** Change of platelet counts after rH-TNF administration. Mean platelet counts of the patients at each dose level of rH-TNF are shown

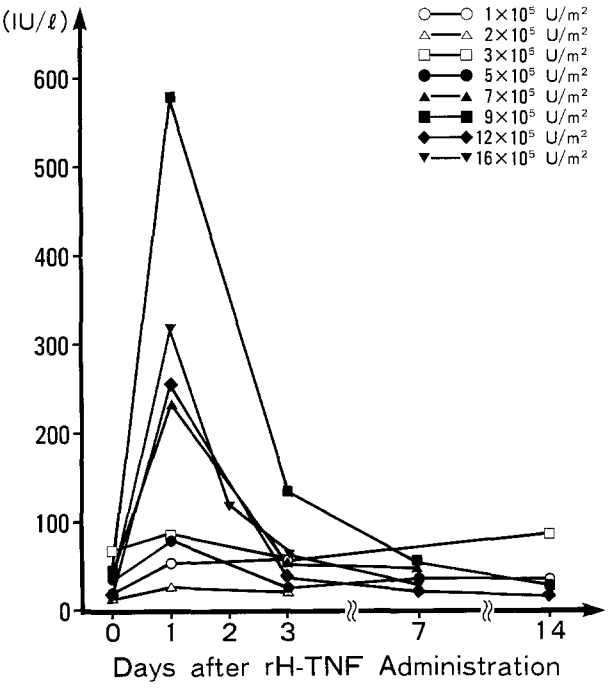


**Fig. 4.** Change of SGOT after rH-TNF administration. Mean SGOT levels of the patients at each dose level of rH-TNF are shown

increase of more than 3000/mm$^3$. The increase was greatest on day 1, and counts returned to the pretreatment levels on day 3 (Fig. 2). The highest increase was 21700/mm$^3$. Neutrophilia was the main feature.

Platelet counts often fell in patients who received $7 \times 10^5$ units/m$^2$ or more, in an approximately dose-dependent manner (Fig. 3). Decreases of more than 100000/mm$^3$ in platelet counts or of more than 50000/mm$^3$ to below 100000/mm$^3$ were encountered in 4 out of 7 patients who had received $12 \times 10^5$ units/m$^2$ or more. In one patient who received $12 \times 10^5$ units/m$^2$, the platelet count dropped from $334 \times 10^3$/mm$^3$ to $165 \times 10^3$/mm$^3$ on day 3. In another patient who received $16 \times 10^5$ units/m$^2$, the platelet count dropped from $108 \times 10^3$/mm$^3$ to $47 \times 10^3$/mm$^3$ on day 2. In the third patient who received $16 \times 10^5$ units/m$^2$, platelet count dropped from $279 \times 10^3$/mm$^3$ to $127 \times 10^3$/mm$^3$ on day 3. In the fourth patient who received $16 \times 10^5$ units/m$^2$, the platelet count dropped from $227 \times 10^3$/mm$^3$ to $129 \times 10^3$/mm$^3$ on day 1, $9 \times 10^3$/mm$^3$ on day 3 and $8 \times 10^3$/mm$^3$ on day 5. In the last case, fibrinogen was 355, 160 and 300 mg/dl and FDP was 12.5, 23.7 and 4.2 μg/ml on days 3, 5, and 7, respectively. Disseminated intravascular coagulation (DIC) syndrome was diagnosed in this patient, who was treated with a serine proteinase inhibitor, gabexate mesilate. No major bleeding tendency was seen in these patients. Platelets recovered to the pretreatment values on day 7 in all cases. Although they did not fall below 100000/mm$^3$, drops in platelet counts by more than 50000/mm$^3$ were noted in another 5 patients who had received $7 \times 10^5$ units/m$^2$ or more. On the other hand, increases in platelet counts by more than 50000/mm$^3$ were observed in 3 patients. No abnormality of erythrocyte counts or hemoglobin levels was observed.

*Abnormalities of hepatic and renal function tests*

Transient elevation of SGOT and SGPT was observed in an approximately dose-dependent manner in 11 and 9 patients, respectively, who received $7 \times 10^5$ units/m$^2$ or more of rH-TNF. Elevation of SGOT (Fig. 4) tended to be more pronounced than that of SGPT. The elevation was highest on day 1 and returned to the pretreatment levels between day 3 and day 7 in most cases. Mild elevation of total bilirubin was noted in 7 patients who received $7 \times 10^5$ units/m$^2$ or more. It also returned to the pretreatment levels between day 3 and day 5. Elevation of BUN was noted in 1 patient who received $16 \times 10^5$ units/m$^2$ (from 25 to 41 mg/dl) with concomitant elevation of creatinine (from 0.9 to 2.1 mg/dl). Both returned to the pretreatment levels on day 5.

*Other toxicities*

Fatigue was noted in 13 patients. Palpitations were complained of by 11 patients. Chest pain and dyspnea were noted in 1 patient. Headache was reported by 2 patients.

*Pharmacokinetic studies*

Plasma levels of rH-TNF were measured following the 30-min infusion of rH-TNF (Fig. 5). The level was highest at the end of the infusion, in a dose-dependent manner, and decreased over 8 h. The mean highest plasma levels of a group of patients who had received 1, 2, 3, 5, 7, 9, 12 and $16 \times 10^5$ units/m$^2$ rH-TNF were 4.8, 21.6, 62.4, 109.4, 181.2, 204, 527.7, and 896 units/ml, respectively. The plasma half-life tended to be longer in the cases that received the higher doses, and was approximately 10, 17, 26, 43, 86, 55, 48, and 80 min at doses of 1, 2, 3, 5, 7, 9, 12, and $16 \times 10^5$ units/m$^2$, respectively.

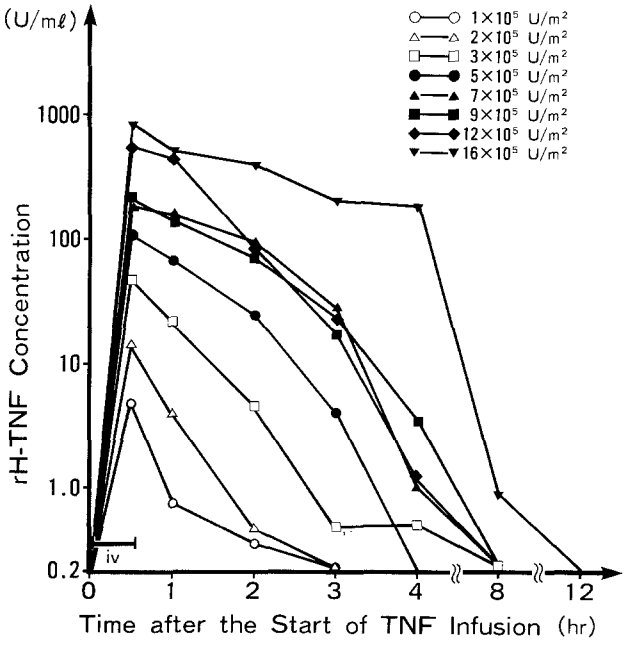


**Fig. 5.** Plasma rH-TNF levels after rH-TNF infusion. Mean plasma levels of rH-TNF in the patients after 30-min infusion at each dose level of rH-TNF are shown

*Antibodies to rH-TNF and IgE level*

Antibody to rH-TNF was tested in all patients weekly for 4 weeks. Antibody to rH-TNF was not detected in any patient. The levels of IgE showed no change during this period.

*Antitumor effect*

No antitumor effect was seen in the present study.

## Discussion

The cloning of the gene for human TNF and resultant recombinant technology have permitted the production of rH-TNF in large quantities for clinical use [16, 22]. This paper has described the first phase I clinical study of one of the rH-TNF thus produced. rH-TNF has been shown to exert the same biological activities as natural TNF [16, 22, 24]. Initially TNF was reported not to affect normal or non-transformed cells. Recently, however, several investigators have reported that TNF shows cytotoxic activities against certain subpopulations of normal mouse and human lymphocytes [17, 28], and that TNF reveals suppressive effects on human hematopoietic stem cells [7, 13]. Thus, clinical administration of rH-TNF should have been conducted with close monitoring for possible effects on normal cells. Moreover, since, to the best of our knowledge, this study was the first clinical trial of TNF when we started, the greatest care was taken to evaluate the safety of this rH-TNF. Therefore, we initiated this phase I study in a single-dose schedule, although we were aware of that other cytokines, such as interferons, require a considerable period of administration to show their effectiveness.

The clinical and laboratory toxic effects of rH-TNF were similar but not identical to those previously reported with other cytokines, such as interferons [8, 14, 23] and interleukin-2 [20, 26]. The most frequently observed side effects were such flu-like symptoms as rigors, fever, fatigue and myalgia. These side effects were observed in virtually all patients and were largely not dose-dependent. As rigor was frequent and severe, antipyretics such as indomethacin and aspirin were allowed to be used as premedication soon after the initiation of study, and all patients given $7 \times 10^5$ units/m$^2$ rH-TNF or more received this premedication. Therefore, the incidence of fever with body temperature of 38° C or more might have been higher had the premedications not been given. This may also be true for the incidence of rigors. Hypotension was another common side effect. Although this side effect has occasionally been observed during studies of interferons [6, 18, 19], and especially of gamma-interferon [29], it was apparently more common and severe in this study of rH-TNF, occurring in an approximately dose-dependent manner, and was one of the dose-limiting toxicities of this drug. A fall in systolic pressure by more than 40 mmHg was seen in 7 patients, 6 of whom received $7 \times 10^5$ units/m$^2$ or more of rH-TNF. In 4 patients, systolic pressure fell below 80 mmHg. Transient hypertension usually preceded hypotension. Therefore, vasoconstriction probably occurred prior to vasodilatation. The hypotension was maximal 2–8 h after the start of infusion, and pressure recovered to pretreatment levels within 24 h.

Decreases in platelet counts were encountered in patients who received $7 \times 10^5$ units/m$^2$ or more of rH-TNF; these occurred in an approximately dose-dependent manner, and were marked in patients who had received $16 \times 10^5$ units/m$^2$. Thrombocytopenia appeared on day 1, with the nadir on days 3–5, and counts recovered to the pretreatment levels on day 7. In 1 patient who received $16 \times 10^5$ units/m$^2$, the platelet count fell from $227 \times 10^3$ /mm$^3$ to $8 \times 10^3$/mm$^3$ on day 5, with a concomitant drop in fibrinogen and the appearance of FDP. In this case DIC syndrome was suspected and a serine proteinase inhibitor was given successfully. This episode, and especially its abrupt development, suggested that the thrombocytopenia was probably due to consumption of platelets rather than to myelosuppression.

Transient elevation of SGOT and SGPT was observed in 11 and 9 patients, respectively, who had received $7 \times 10^5$ units/m$^2$ or more of rH-TNF, and it arose in an almost dose-dependent manner. Since mild elevation of total bilirubin was noted in 7 of these patients, the transaminases were thought to derive from liver. However, since elevation of SGOT tended to be more pronounced than that of SGPT, they might have also derived from other organs.

All these toxic effects of rH-TNF have also been reported by other clinical study groups, whether they have used rH-TNF from a different source or from the same source as ourselves [3, 5, 11, 25, 27]. In the present study, the dose-limiting toxicities of rH-TNF were hypotension, thrombocytopenia, and elevation of SGOT and SGPT. The maximum tolerated dose of a single 30-min infusion of rH-TNF appears to be $12 \times 10^5$ units/m$^2$ if thrombocytopenia and the elevation of SGOT and SGPT are taken as the dose-limiting toxicities. However, if hypotension is also considered to be dose-limiting, the maximum safely tolerated dose seems to be $5 \times 10^5$ units/m$^2$. Since our recent experience suggests that hypotension can be prevented by premedication with ketoprofen, this side effect may not become a dose-limiting toxicity if appropriate preventive medication is applied.

Pharmacokinetic study revealed relatively slow plasma clearance of rH-TNF, especially at the higher doses. The peak plasma level of rH-TNF attained after the administration of $1 \times 10^5$ units/$m^2$ or more of rH-TNF was high enough to be cytotoxic to TNF-sensitive human tumor cell lines such as PC-10, THP-1, KYM-1 and BT-20 in vitro, in which 50% growth inhibition was seen at less than 1 unit/ml of the rH-TNF used in the present study [10]. Since other cytokines, such as interferons, are effective only when administered for a considerable period, further phase I study of rH-TNF should be carried out with daily, every-other-day, or weekly schedules, and with other administration methods, such as continuous or 3- to 5-h i.v. infusion. We are currently conducting another phase I study of rH-TNF with administration daily for 5 days.

Recently, Beutler et al. reported an identity of TNF and a monokine, cachectin, which caused cachexia and was one of the principal mediators of the lethal effects of endotoxins [1, 2]. Therefore, on the basis of these authors' findings and the results of the present phase I study, further clinical administration of rH-TNF should be conducted with great care, in spite of the promising effects of rH-TNF as an anticancer drug.

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