A phase I clinical trial of recombinant human tumor necrosis factor given daily for five days*

Patrick J. Creaven¹, Dean E. Brenner¹, J. Wayne Cowens¹, ², Robert P. Huben³, Richard M. Wolf³, Hiroshi Takita⁴, Susan G. Arbuck⁵, Mohamed S. Razack⁶, and April D. Proefrock¹

Departments of ¹ Clinical Pharmacology and Therapeutics, ² Experimental Therapeutics, ³ Urologic Oncology, ⁴ Thoracic Surgery, ⁵ Surgical Oncology and ⁶ Head and Neck Surgery and Oncology, Roswell Park Memorial Institute, New York State Department of Health, Buffalo, New York 14263, USA

Summary. A phase I trial of human recombinant tumor necrosis factor (rH-TNF) has been carried out in patients with advanced solid tumors. Sixty-six courses of the drug were given by 1 h IV infusion, daily for 5 days to 33 patients at doses of 5, 10, 20, 30, 45, 60, and 80×10⁴ U/m²/day. All patients received isotonic saline (up to 21/day) and either indomethacin or ketoprofen. Acute toxicity resembled that seen with the phase I study of a single dose (5). Dose limiting toxicity was acute, rapidly reversible, hepatic dysfunction and hypotension. Hypertension during drug infusion and dyspnea were marked in some patients. There was one complete and one minor response, both in patients with renal cell carcinoma. The dose of 80×10⁴ U/m²/day × 5 was poorly tolerated and the recommended starting dose for phase II studies is 60×10⁴ U/m²/day × 5. Caution is recommended in treating patients with pre-existing hepatic function abnormalities, hypertension, hypotension or significant obstructive airway disease.

Introduction

Tumor necrosis factor (TNF) was first demonstrated in the serum of mice which had been treated with BCG (Bacillus Calmette Guerin) and then challenged with endotoxin [2]. The serum from these mice, when injected into tumor bearing mice, produced tumor necrosis. The gene for human TNF (rH-TNF) has recently been cloned and recombinant human TNF (rH-TNF) has been produced in E. coli [16].

rH-TNF, a nonglycosylated protein with a molecular weight of 17,000 daltons, has been evaluated for antitumor activity in vitro and in vivo. Of 26 human tumor cell lines tested, 5 showed a high degree of sensitivity, with an IC₅₀ of <1 unit/ml [9]. When given i.v., it produced cures in vivo against the meth-A sarcoma and the colon 26 carcinoma but not against the B16 melanoma [9]. The recombinant TNF used in this study (rH-TNF Asahi) was clinically introduced in Japan in 1985 [12]; shortly thereafter, studies were initiated in the United States and Europe. To date, the material has been extensively evaluated on a number of different schedules, including single-dose, 24-h continuous infusion, and 5-day continuous infusion [4, 12, 15, 17, 18, 20]. Studies have also been reported of early clinical trials with TNF from other sources [1, 6, 11, 13, 19, 21]. Major dose-limiting toxicities have included hypotension, abnormalities of liver function, and thrombocytopenia. The 5-day continuous infusion demonstrated thrombocytopenia as a major dose-limiting toxicity [15].

In our initial clinical investigation of rH-TNF we studied the tolerance to single doses given every 3 weeks, with intra- and interpatient escalation. Toxicity was largely acute, with systemic toxicity resolving in 24 h. Hypotension was the dose-limiting toxicity and the maximum tolerated dose was 48×10⁴ units/m² [4]. The present study was initiated with the following objectives: (1) to determine whether higher total doses could be given if the drug was subdivided and given daily over 5 days; (2) to determine whether dose-limiting, systemic, acute toxicity could be circumvented by giving the drug in this way; (3) to characterize the dose-limiting toxicity of daily administration; (4) to attempt to evaluate the effect, if any, of ketoprofen on the acute hypotensive effect of the drug; and (5) to explore any tachyphylaxis in toxic manifestations with daily dosage. The starting total dose chosen (25×10⁴ units/m²) was approximately 50% of the highest single dose given in the previous study. This was subdivided and given as 5×10⁴ units/m² per day for 5 days.

Materials and methods

Drug. The drug was supplied by the Asahi Chemical Industry Company Limited (Tokyo, Japan) in vials containing 5×10⁴ or 5×10⁵ units. The material was purified to homogeneity by extraction and pretreatment followed by anion exchange chromatography, affinity chromatography, and gel filtration [8]. It was assayed for cytotoxicity against L-M cells. The activity (in units/ml) is defined as the reciprocal of the dilution resulting in 50% cell survival [10]; the specific activity of the material was 2.2×10⁶ units/mg [8].

Patients. Patients with advanced cancer not amenable to other treatment or for whom other treatment had proven ineffective were entered in the study after their written, informed consent was obtained. The study protocol was reviewed and approved by the Institutional Review Board of Roswell Park Memorial Institute. The requirements for entry were an age of 18-70 years, an expected survival of

* Preliminary reports of some of these data have previously been published [3, 5]

Offprint request to: P. J. Creaven
at least 2 months, at least a 3-week interval since the last dose of potentially myelosuppressive therapy (6 weeks for nitrosourea and mitomycin C) and recovery from reversible toxicity, a 2-week interval since radiation therapy or surgery (except minor procedures), and the absence of acute intercurrent complications, pregnancy, or a history of asthma. The minimal hematologic parameters required were a WBC count of \(3.5 \times 10^3/mm^3\) and platelet count of \(10^5/mm^3\). The minimal biochemical parameters required were an SGOT level of \(< 100\) IU/l and serum bilirubin and serum creatinine levels of \(< 2\) mg/dl.

Patients were monitored as follows: temperature, pulse, and blood pressure (BP) every 2 h for 12 h and then every 4 h until they returned to baseline after each dose; complete blood count, including differential count and platelet count, daily during treatment and weekly between courses; serum chemistry (Na\(^+\), K\(^+\), Ca\(^{2+}\), PO\(_4^{3-}\), creatinine, uric acid, total protein, albumin, bilirubin, alkaline phosphatase, lactate dehydrogenase, SGOT, and blood urea nitrogen) was recorded pretreatment, on day 5, and then weekly (at higher drug doses measurements were done daily). Prothrombin time, partial thromboplastin time, serum fibrinogen, fibrin degradation products, and fibrin monomer were measured at 48 h and then weekly for 3 weeks. An electrocardiogram was recorded at 24 h after treatment.

All patients were hospitalized for the duration of the treatment. On the day before treatment blood was drawn for antibodies to TNF, and 1 h prior to infusion of the first daily drug dose, each patient was skin-tested by the i.d. injection of 0.025 ml infusion solution.

At doses of 5–45 \(\times 10^4\) units/m\(^2\) per day, patients were begun on indomethacin (50 mg orally 3 times daily) on the day before the start of therapy, and indomethacin was continued throughout the 5 days of treatment. At doses of 60 and 80 \(\times 10^4\) units/m\(^2\) per day, the patients were pretreated with ketoprofen (75 mg orally 3 times daily) instead of indomethacin, and this drug was also continued throughout the 5 days of rH-TNF treatment. In addition, 12 h before the initiation of drug therapy, patients were started on an i.v. infusion of normal saline (2 1/24 h). Hydration was continued throughout the 5 days of treatment except when an undue increase in body weight indicated that it should be discontinued.

**Results**

**Treatment**

A total of 33 patients were entered in the study; patient characteristics are listed in Table 1. In all, 85% of the patients were fully ambulatory, with a performance status (PS) of 0 or 1. A total of 66 courses were given (Table 2); 7 courses were terminated prematurely for drug-related reasons, 4 of which involved respiratory distress, 2, hepatotoxicity, and 1, hypotension. The respective doses (units/m\(^2\) per day) were 20, 30, 60 (3 patients), and 80 (2 patients). The two patients who had incomplete courses at 80 \(\times 10^4\) units/m\(^2\) per day subsequently received complete courses at 60 \(\times 10^4\) units/m\(^2\) per day.

**Toxicity**

Acute systemic toxicity was seen in all patients and did not differ markedly from that previously described [4]; the major systemic toxicities are shown in Table 3. Fever and rigors were not dose-limiting. Hypertension occurred during infusion, resolving rapidly after infusion was stopped. Pharmacologic intervention was required in 13 patients at doses of 20 (1 patient), 30 (2 patients), 60 (9 patients), and 80 (1 patient) \(\times 10^4\) units/m\(^2\) per day (pharmacologic intervention was generally considered when systolic BP > 200 mm Hg or diastolic BP > 110 mm Hg).

Hypotension occurred 2–15 h after drug administration; it was dose-related and appeared to display tachyphylaxis with repeated doses (Fig. 1). This effect was less marked at higher doses: the difference between the median nadir of systolic BP after days 1 (88 mm Hg) and 5 (97 mm Hg)

<table>
<thead>
<tr>
<th>Patients</th>
<th>Dose (units/m(^2) (\times 10^4))</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>New</td>
<td>6/5</td>
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<tr>
<td>Escalated from lower dose</td>
<td>–</td>
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<tr>
<td>Total</td>
<td>6/5</td>
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\(^a\) Number of courses/number of patients

\(^b\) Includes two patients entered at 80 and deescalated to 60 \(\times 10^4\) units/m\(^2\) per day