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A Phase I Clinical Tolerance Study of rDNA Alpha$_2$ Human Interferon in Patients with Non-reticuloendothelial System Malignancies

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Abstract—*Twenty-seven patients with non-reticuloendothelial malignancies were treated with a single intramuscular injection of recombinant leukocyte alpha$_2$ interferon (rIFN) to assess clinical tolerance and define a maximum tolerated dose. A single patient in each of six increasing dosage groups* (0.3×10^6 IU, 1×10^6 IU, 3×10^6 IU, 10×10^6 IU, 30×10^6 IU, 100×10^6 IU) *received a low dose* (0.01×10^6 IU) *and served as a control for subjective and objective toxicity measurements. Severe fatigue proved dose-limiting at* 100×10^6 IU, *and all dosages above* 3.0×10^6 IU *produced one or more signs or symptoms, which typically resembled a 'flu-like' syndrome. Objective toxicity was mild to moderate (leukopenia, thrombopenia) and no toxicities were found not already known from work with interferon obtained directly from leukocytes. Evidence of an antitumor effect was apparent in 3/19 evaluable patients.*

INTRODUCTION

INTERFERONS are naturally occurring proteins produced by virtually all nucleated cells in response to viral and non-viral stimuli [1, 2]. Non-recombinant DNA-produced interferon preparations were shown to have species-specific antiviral, antiproliferative and immunomodulatory properties [3]. From the time of their discovery in 1957 up to the present there has been considerable interest in the possible clinical use of interferons in the therapy of viral infections and cancer. Before the advent of recombinant DNA techniques the clinical evaluation of interferons was hampered by the limited quantity of human interferon. Genetic engineering has now provided clinicians with unlimited quantities of highly purified bacteria-produced human interferon [4, 5]. Human interferon prepared from leukocytes and *E. coli*-produced human alpha$_2$ interferon are comparable [6, 7], but *E. coli*-produced human alpha$_2$ interferon does not produce toxic effects when administered parenterally in non-human primates but does retain its antiviral activity [8].

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Preliminary results of clinical trials with crude leukocyte interferons and recombinant DNA alpha interferon as antitumor agents have been sufficiently encouraging to warrant further testing of the toxicities and antineoplastic effects of interferon [9–17].

This report presents the results of a phase I clinical trial conducted to evaluate the acute and limiting toxicities of a series of single doses of recombinant leukocyte alpha IFN, injected intramusculary in patients with non-reticulo-endothelial system malignancies. No toxicities were discovered that were not already known from clinical work with leucocyte interferon. Extreme fatigue proved to be the limiting toxicity.

MATERIALS AND METHODS

Interferon

Recombinant leukocyte alpha$_2$ interferon (rIFN) was prepared as previously described [4, 5], purified to 10^8 U/mg protein, dissolved in PBS, diluted to a final concentration of 100×10^6 IU/ml and frozen to −20°C. rIFN solutions were thawed once and reconstituted to the proper dilution with PBS. For each dose the final injectable volume was 2 ml.

Patients

Five parallel dose groups of four patients each and one of seven patients were enrolled, to be treated on a staggered basis at approximately weekly intervals, given the absence of any major intolerance. The patients were randomly allocated to one of the six dose groups, roughly matched for age, sex, previous cancer therapies and type and duration of tumors. In each group one patient was randomly assigned to receive a low dose of 0.01×10^6 IU. The six escalating doses were: 0.3×10^6 IU; 1×10^6 IU; 3×10^6 IU; 10×10^6 IU; 30×10^6 IU; 100×10^6 IU.

Each patient received only one intramuscular injection of IFN, was hospitalized for at least 48 hr after the injection, seen daily for the next 5 days and weekly for the following 3 weeks.

Patients in the study were 34 years of age or older, of either sex and had histological or cytological evidence of a single non-reticuloendothelial system malignancy. Criteria for entrance in the study included a Karnofsky performance level greater than or equal to 70%; less than 5% weight loss during the previous month and a life expectancy of at least 4 months. To enter into the study patients should not have received chemotherapy and radiotherapy for at least 4 weeks and should show no evidence of serious concomitant non-malignant disease. All patients signed informed consent forms.

Treatment-induced subjective adverse experiences and changes in vital signs and blood and urine chemistries were recorded during interviews scheduled at set intervals. Changes in the size of tumors and in tumoral pain were also determined during those interviews. Parameters followed included vital signs, pain at the site of injection, tumor pain, subjective 'flu-like' symptoms and physical examination plus standard hematological and clinical-chemical tests. WHO toxicity and response criteria were followed.

RESULTS

All six doses were tolerated. At and above 3.0×10^6 IU all patients experienced one or more adverse experiences. Regardless of the dose received, above this level the most frequently reported adverse experiences were: chills, fever, headache, nausea and fatigue. At the highest dose level severe fatigue was the dose-limiting factor. The spectrum of adverse effects increased with the dose. Chills were reported by 39% of the patients given a dose equal to or greater than 3.0×10^6 IU, vomiting occurred in 100% of the patients in the 30×10^6 IU and 100×10^6 IU dose groups and back pain with or without neck pain and dizziness occurred more frequently at the highest dose. Typically, the adverse experiences resembled those of a 'flu-like' syndrome and appeared in a well-defined sequence. Chill was the first symptom to appear, about 15–60 min following injection. Fever followed 1–2 hr later and was associated with myalgia, headache, nausea and vomiting. Approximately 12 hr after injection there was muscular, posterior neck pain with or without lower lumbar back pain. Twenty-four hours after dosing fatigue was felt with or without severe weakness in the legs, and lassitude was sometimes strong enough to force the patient to stay in bed. During the second day dizziness and vertigo appeared but were not related to orthostatic hypotension. The severity and duration of adverse experiences reached a maximum, in most cases, after 10×10^6 IU. All adverse experiences except fatigue were resolved within 3–4 days. Fatigue lasted up to 28 days in 50% of the patients who received 100×10^6 IU.

Temperature rose from baseline values by an average of 0.6°C in 92% of the patients who received less than 3×10^6 IU and by an average of 2.2°C in 100% of all the other patients. The mean peak temperature was dose-related up to 30×10^6 IU and above this level plateaued out. The fever was remittent in type, reaching a first peak within 8 hr after injection and a second peak 24 hr later. Temperature was usually resolved within 3 days. Rises in temperature were accompanied by increases in respiratory rates and pulse rates and a drop in systolic pressures. These changes were, however, lower than expected for the degree of pyrexia [18] and were not dose-related.

A mild–moderate drop in white blood cell counts, corresponding to WHO grades 0–3, was noted in all patients. It usually occurred within 48 hr of injection and was transient and not dose-related. The duration of the recovery period was, however, dose-related at 10×10^6 IU and above, where recovery time to baseline levels increased to 1 week. Platelet levels fell in 25 out of 27 patients, but only in 4 patients did the count fall below the lower limit of normal range, giving a thrombocytopenia WHO graded of 1–2. These changes were transient and not dose-related. The effects of rIFN on blood chemistries were minimal; further rIFN had no effect in renal and urine chemistries.

SGOT and SGPT maximally increased to grade I, alkaline phosphatase toxicity ranged from grades I to III at 4–7 days after treatment and no changes in creatinine or BUN were seen.

None of the patients reported either pain or inflammation at the site of injection lasting more than 12 hr.

Out of 20 patients with measurable tumors at the beginning of the trial, 19 were evaluable at day 28. Three patients had a regression in tumor size

>50% (PR) and in 12 other patients tumor stabilization was achieved, i.e. tumor regression <50% and growth <25%. Five patients had tumor progression. Twenty-one out of 28 patients had tumoral pain at the onset of the trial. Two patients were completely tumor pain-free by the end of the trial and 11 patients reported diminished tumoral pain.

Neutralizing antibody to interferon was not detected in pre- and post-alpha$_2$ injection sera.

DISCUSSION

Several important conclusions can be drawn from the data which are provided here.

First, there appear to be no toxicities possessed by DNA recombinant IFN not already known from work with leucocyte IFN obtained directly from human leucocytes [19]. Second, objective toxicity of the sort seen with most anticancer agents, i.e. leucopenia and thrombocytopenia, which tend to limit the dosage used, were not dose-limiting in this single injection study. No significant hepatic or renal toxicity was seen, and thus subjective complaints formed the dose-limiting factor. Most important in this regard was striking fatigue, beginning the day after treatment and lasting for 1–4 weeks thereafter. Fatigue was particularly long-lasting at the 100×10^6 units level. This was considered the dose-limiting toxicity, as also found by Gutterman *et al.* [17]. Further, high-dose ($>10 \times 10^6$) IFN was associated with nausea and vomiting, similar to that seen with standard cytotoxic agents, and with flu-like syndrome, not shared by most anticancer agents. The absence of prolonged injection site pain noted with crude interferon preparation is likely to be related to the presence of impurities. It was not observed with highly purified DNA alpha$_2$ interferon.

Human alpha$_2$ interferon is a moderate-sized protein (18,000 dalton, with 165 amino acids). It is worthy of note that no patients developed detectable levels of interferon neutralizing antibody in the serum after alpha$_2$ administration. Gutterman *et al.* [17] reported that 3 out of 16 patients developed antibody to another DNA-produced human alpha interferon.

This study had as a secondary goal the evaluation of antitumor efficacy. In this regard, 3 of 19 available patients had a partial remission and 12 had disease stabilization, despite obvious progression upon entry into the study. While the observation period is too short to say a great deal about the patients with stable disease, the 3/19 partial remissions in generally heavily pretreated patients (24/28 previous chemotherapy, 22/20 previous radiotherapy, 20/20 both chemotherapy and radiotherapy) is impressive.

For further study, 10 million units twice weekly was chosen with good acceptability [20]. Further phase II studies seem to be indicated, with the recommendation that the IFN be tested as stringently as other anticancer agents in order to objectively prove its place in the anticancer armoury.

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