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Phase I Study of Subcutaneously-administered Bacterially-synthesised Recombinant Human Granulocyte–macrophage Colony-stimulating Factor

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A phase I study was initiated to test the effect of bacterially-synthesised recombinant human granulocyte–macrophage colony-stimulating factor (rhGM-CSF). 15 patients with advanced cancers were entered into the study and 14 were evaluable. Patients were administered a single subcutaneous injection (3.0–300 μg/m²) of rhGM-CSF. Starting at a concentration of 100 μg/m², an approximate 2-fold increase in leucocyte count was noted 24 h after the injection. By 48 h the counts had returned to baseline. The 300 μg/m² concentration also induced an approximate 2-fold increase. The leucocytosis was associated with a predominant increase in circulating neutrophils and bands. An increase in monocytes was also noted, but peak levels were recorded 48–72 h after the injection. At both the 100 μg/m² and the 300 μg/m² doses, significant levels of circulating rhGM-CSF were detected. The levels measured in the plasma of patients receiving 300 μg/m² were over 10-fold greater than those measured at 100 μg/m². There was no detectable antibody formation against the rhGM-CSF in any of the study patients. The drug was exceptionally well-tolerated. This study shows that rhGM-CSF can be safely administered by subcutaneous administration and a single injection is capable of inducing a leucocytosis with increased circulating neutrophils, bands, and monocytes when doses are used which result in significant levels of circulating rhGM-CSF.
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INTRODUCTION

GRANULOCYTE–MACROPHAGE colony-stimulating factor (GM-CSF) is a multipotential haematopoietin that stimulates the formation of granulocytes and macrophages [1], the production of eosinophils [2], and the initial division of progenitor erythroid and megakaryocyte cells [3]. The cloning of the human gene for GM-CSF [4] has led to the availability of purified recombinant GM-CSF (rhGM-CSF) protein produced by yeast [5], mammalian [6], and bacterial cells [7]. RhGM-CSFs from both yeast and mammalian cells are derived in a glycosylated form, while bacterially-synthesised rhGM-CSF is unglycosylated. The unglycosylated *Escherichia coli*-derived protein has been shown to have greater activity when compared with the glycosylated products [8]. The material from *E. coli* may also contain less endotoxin [9] and may be less immunogenic than yeast- or mammalian-derived rhGM-CSF [10].

Because of these differences, a phase I clinical trial was initiated with bacterially-synthesised rhGM-CSF administered by subcutaneous injection. The study was designed to define the effect on various haematological parameters of rhGM-CSF given at different doses, to study the pharmacokinetics of rhGM-CSF, and to assess its possible immunogenicity, as well as its toxicity, in patients with cancers who were not receiving concurrent myelosuppressive therapy.

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PATIENTS AND METHODS

Patients

All patients participating in this study had to have histologically proven evidence of carcinoma or sarcoma not amenable to therapy with curative intent. An interval of 4 weeks or longer was required from the last dose of cytoreductive, corticosteroid, radiation, or immunotherapy and at least an 8 week interval was required after treatment with nitrosoureas, mitomycin C, or mitolactol. Required baseline haematological parameters included a white blood count $>4 \times 10^9$/l (absolute neutrophil count $>2 \times 10^9$/l), platelets >100 000/μl, and haemoglobin >9 g/dl (transfusion to elevate to >9 g/dl was acceptable). This protocol was approved by the institutional review board and signed informed consent was obtained from all patients prior to initiating treatment.

Treatment and monitoring

Groups of 3 patients received a single subcutaneous injection (time 0) of rhGM-CSF at doses of 3, 10, 30, 100, and 300 μg/m². A patient treated at one dose level could not be re-entered into the study at a higher dose.

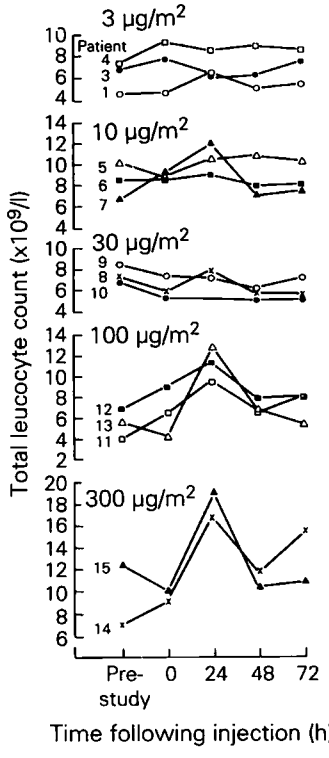

Fig. 1. Effect of different subcutaneous doses of rhGM-CSF on total leucocyte count. Results are shown for individual patients receiving rhGM-CSF at time 0 with leucocyte counts measured 24, 48, and 72 h later.

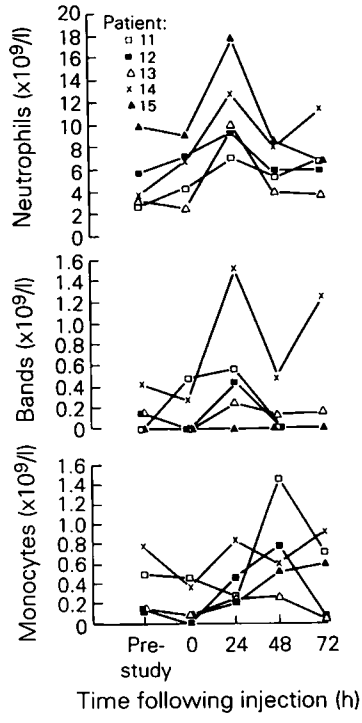

Fig. 2. Effect of 100 μg/m² and 300 μg/m² rhGM-CSF on neutrophil, band, and monocyte levels. Points represent the total counts for each patient treated at time 0 with either 100 μg/m² (patients 11–13) or 300 μg/m² (patients 14 and 15) rhGM-CSF. Cell counts were determined at 24, 48, and 72 h following injection.

GM-CSF

The bacterially-synthesised rhGM-CSF used in the present study, which has been described previously [7], was supplied by the Glaxo Institute of Molecular Biology (GIMB, Geneva, Switzerland) as a lyophilised powder. Each vial contained 1 mg of rhGM-CSF (specific activity $>1.5 \times 10^6$ units/mg) and was reconstituted with sterile water containing 0.2% human serum albumin immediately before injection.

GM-CSF antibody determinations and pharmacokinetics

Starting at the 30 μg/m² dose level of rhGM-CSF, serum antibody levels were quantified by an enzyme-linked immunosorbent assay (ELISA) utilising peroxidase-conjugated IgG and rhGM-CSF provided by GIMB. Plasma rhGM-CSF levels were determined by the InsightGM assay (Medical Resources Limited, Surry Hills, Australia), a quantitative ELISA method which can detect rhGM-CSF in serum with a sensitivity of 100 pg/ml.

RESULTS

Characteristics of the patients

15 patients with a variety of non-haematological malignancies entered the study. 1 patient enrolled in the study was not treated because of unanticipated medical problems immediately prior to the injection. There were 9 males and 6 females with a median age of 61 years (range: 21–75). All patients had a performance status of 0 to 2 by WHO criteria. All but 2 patients had received chemotherapy, radiotherapy, and/or hormonal therapy at some point before injection with rhGM-CSF.

Effects of rhGM-CSF on peripheral blood counts

rhGM-CSF at doses ≥100 μg/m² induced significant elevations in total leucocyte count (Fig. 1). An approximate doubling in total leucocytes was noted at 24 h, with a return to baseline by 48 h. At a dose of 300 μg/m² there was a higher peak leucocyte count relative to that observed at 100 μg/m². However, since, by chance, the baseline leucocyte count in the patients receiving 300 μg/m² rhGM-CSF was higher than in the 100 μg/m² patients, the overall leucocytosis was still approximately 2-fold.

The increase in total leucocyte count at 24 h for both the 100 and 300 μg/m² doses represented a predominant left shift with an increase in neutrophils and bands (Fig. 2). At the 100 μg/m² dose there was a 1.7, 1.3, and a 4.1-fold increase in circulating neutrophils for patients 11, 12, and 13, respectively (Fig. 2). At 300 μg/m² the increase was 1.9 and 2.0-fold for patients 14 and 15, respectively.

With 100 μg/m² rhGM-CSF, there was a small increase in the number of bands (Fig. 2). For patient 14, 300 μg/m² rhGM-CSF produced a marked bandemia, whereas for patient 15 no bands were produced with the same dose.

A significant monocytosis was noted for patient 11 receiving 100 μg/m² (Fig. 2), but this effect was delayed, the peak monocyte count occurring 48 h post-injection. An increase in total monocytes was noted at 24 h for the other 2 patients treated with the 100 μg/m² dose. At 300 μg/m² an increase in monocytes was observed at 24 h and appeared to remain elevated 72 h later. However, the overall increase in total monocytes was not as great as that seen for patient 11 at the lower dose.

Eosinophilia, a major part of the leucocytosis in other studies [10–14], was observed in only one patient at 10 μg/m² (not shown). There were also no consistent changes in the levels of circulating lymphocytes, basophils, platelets, and red blood cells (not shown).

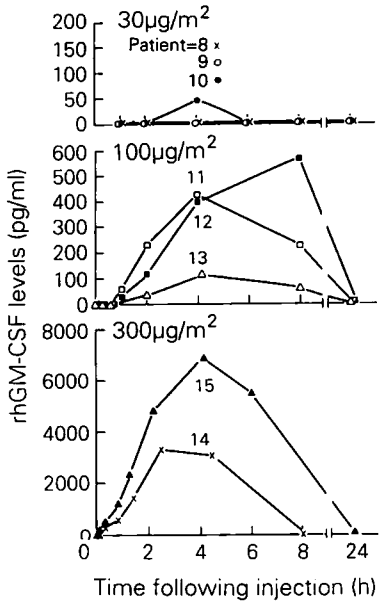

Fig. 3. Plasma levels of rhGM-CSF in individual patients following subcutaneous injection of 30 μg/m², 100 μg/m², and 300 μg/m² rhGM-CSF. These determinations were performed as described in "Patients and Methods".

GM-CSF pharmacokinetics and antibodies

1 patient at 30 μg/m² had an elevated serum level of rhGM-CSF at the limit of sensitivity of the assay (Fig. 3). Significant levels of rhGM-CSF were consistently detected in the plasma of patients receiving 100 μg/m². Ten-fold greater levels of rhGM-CSF were measured in patients treated with 300 μg/m².

Circulating levels of rhGM-CSF were first detected 1 to 2 h following the subcutaneous injection of 100 μg/m² rhGM-CSF, whereas measurable levels were present 5–15 min after the injection of 300 μg/m². The 2 h plasma level was sustained for at least an additional 4 h (or 6 h when measured) but was not detected 24 h after injection.

Within each dose level there was considerable variability in peak plasma rhGM-CSF levels. Patient 12, at 100 μg/m² rhGM-CSF, demonstrated a plasma level which continued to rise at 8 h post-injection and had a peak level that was at least 6-fold greater than that observed for patient 13 at the same dose (Fig. 3). At 300 μg/m², patient 15 had a peak plasma rhGM-CSF concentration that was double that of patient 14.

Antibodies against rhGM-CSF were not detected using the ELISA assay in the serum of any patient at either 1 or 2 weeks following injection (not shown).

Toxicities

RhGM-CSF caused minimal toxicities. Patient 3 developed left-sided parasternal chest pain and tenderness without electrocardiogram changes 24 h after the injection of 3 μg/m² rhGM-CSF. Sternal pain following intravenous rhGM-CSF has been previously reported [12, 14]. Fever, which has been reported in >50% of patients treated subcutaneously with rhGM-CSF [10], was recorded in only one patient in this study.

Twenty-four hours following injection of 300 μg/m² rhGM-CSF, patient 14 with oesophageal cancer who 2 months before the study had had an oesophagectomy with a gastric pull-up, developed a soft-tissue swelling within the neck. This was later determined to be an oesophageal–cutaneous fistula. Interestingly, this may also explain the large bandemia observed.

There was 1 death due to congestive heart failure 30 days following the injection of 10 μg/m² rhGM-CSF. This was believed to be unrelated to the drug. There was no evidence of cardiac, renal, dermatological, or neurological effects from rhGM-CSF. All biochemical parameters remained unchanged.

As expected, no tumour responses following a single subcutaneous injection of rhGM-CSF were observed.

DISCUSSION

Colony-stimulating factors may have an important role in preventing the myelosuppression which can result from chemotherapy. In a phase I trial we have shown that a single subcutaneous administration of 100 μg/m² and 300 μg/m² rhGM-CSF can induce an approximately 2-fold elevation in leucocyte count with a predominant left shift.

In the present study it was not possible to define a direct relationship between rhGM-CSF dose, rhGM-CSF plasma levels and the resulting neutrophilia. Even though the peak plasma levels were greater for the 300 μg/m² dose, the relative change in neutrophils from baseline was not consistently greater than that observed for the 100 μg/m² dose.

With regard to the increase in monocytes, it appeared that this cell population was still increasing 72 h after the 300 μg/m² rhGM-CSF injection, whereas at 100 μg/m² rhGM-CSF there was a peak in monocytes at 48 h. It is interesting that in studies of other rhGM-CSFs [10, 13, 14] the increase in monocytes was not noted until after 5–10 days of daily injections (both intravenous and subcutaneous). This suggests that the formulation of rhGM-CSF used in the present study may be a more potent inducer of monocytes than other preparations previously examined.

The failure to observe an increase in platelet counts and haemoglobin levels is consistent with other studies [10–14]. GM-CSF stimulates the initial division of progenitor cells in the erythroid and megakaryocytic series, but other molecules are necessary to complete maturation of these cells [1].

In summary, this study, which was prematurely terminated because of insufficient supplies of drug, indicates that a single subcutaneous injection of an unglycosylated rhGM-CSF synthesised by bacteria is a potent inducer of leucocytosis at doses resulting in significant rhGM-CSF levels in the blood.

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Combination Chemotherapy with Vincristine, Epirubicin and Cyclophosphamide in Small Cell Lung Carcinoma

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The aim of this prospective study was to assess the activity of a combination of vincristine, epirubicin and cyclosphosphamide (VEC) in previously untreated patients with limited small cell lung carcinoma (SCLC) and to delineate the feasibility of dose escalation for epirubicin in this regimen. The chemotherapy schedule included cyclophosphamide, 1000 mg/m², vincristine, 1 mg/m² and escalating doses of epirubicin: 50 mg/m², 70 mg/m² and 90 mg/m²; respectively in three consecutive groups of patients. Drug cycles were repeated every 3 weeks. 118 patients from eight institutions were enrolled in this study between February 1986 and March 1989. Objective tumour response was observed in 81 of 116 evaluable patients (70%) including 25 patients (22%) who achieved a complete remission. Responding patients received thoracic radiation after the fourth cycle of chemotherapy. The median duration of response was 30 weeks and the median duration of survival was 52 weeks. There were no significant differences in treatment results between the consecutive groups of patients. The regimen was well tolerated for all doses of epirubicin. The main toxicities included alopecia (96%), nausea and vomiting (81%) and leukopenia (44%). Grade 4 haematological toxicity was observed in 3 patients (2.6%). No significant epirubicin dose-dependent side effects, except for mucositis were observed.
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INTRODUCTION

IN SPITE of the high sensitivity of small cell lung carcinoma (SCLC) to both cytostatic agents and chemotherapy, cure is still a rare endpoint in treatment of this tumour. Most patients experience a relapse within 2 years as a result of the emergence of drug-resistant cancer cells during chemotherapy. A large number of different cytostatic agents have been demonstrated to be active in SCLC, but there is no regimen considered to be optimal. The combination of vincristine, doxorubicin and cyclophosphamide (VAC) has been one of the most commonly used within the last decade [1]. Doxorubicin is one of the most active agents in SCLC and has probably significant impact on complete response rate and survival [2–4]. The utility of this drug in VAC combination is limited, however, due to its dose-related cardiac toxicity. It has been suggested that epirubicin, a derivative of doxorubicin, is a drug of similar activity but of less cardiotoxicity than its parent compound [5, 6]. The activity of epirubicin given as a single drug in a dose of 100–120 mg/m² was also demonstrated in SCLC [7–11]. Data pooled from these five studies on a total of 177 chemotherapy-naive SCLC patients showed a 46% remission frequency, one of the highest reported for single-agent chemotherapy of this tumour. As the mainstay of the SCLC management is multidrug chemotherapy, we have undertaken a prospective multicentre phase II study in which doxorubicin in VAC regimen was replaced by epirubicin. The purpose of this trial was to evaluate the activity of a combination