Phase I Study of Intravenously Administered Bacterially Synthesized Granulocyte-Macrophage Colony-stimulating Factor and Comparison with Subcutaneous Administration

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ABSTRACT

A Phase I study of bacterially synthesized recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF) was undertaken in 21 patients with advanced malignancy or neutropenia. rhGM-CSF was administered once daily by i.v. bolus injection (0.3 to 3 µg/kg/day) or 2-h i.v. infusion (3 to 20 µg/kg/day) for 10 days. rhGM-CSF at all i.v. doses caused an immediate transient decrease in circulating neutrophils, eosinophils, and monocytes. By 6 h after rhGM-CSF, circulating leukocyte levels were restored. Daily i.v. bolus dosing (0.3 to 3 µg/kg/day) did not elevate leukocyte levels except in one neutropenic patient. Daily 2-h i.v. infusions (10 to 20 µg/kg/day) caused a dose-dependent leukocytosis with increased levels of neutrophils (up to 4.3-fold), eosinophils (up to 18-fold), and monocytes (up to 3.5-fold). Marrow aspirates showed increased proportions of promyelocytes and myelocytes during rhGM-CSF administration. Retreatment after 10 days without rhGM-CSF resulted in a more marked leukocytosis at doses ≥ 10 µg/kg/day. Platelet levels decreased for the first 3 days and then increased during the first course of rhGM-CSF administration. Two patients with chronic lymphocytic leukemia had a transient reduction in lymphocytosis. Serum cholesterol and albumin levels decreased, and vitamin B12 levels increased during rhGM-CSF treatment. At doses of up to 15 µg/kg/day, rhGM-CSF was relatively well tolerated by the patients, but adverse effects included bone pain, lethargy, fever, rash, and weight gain. A first dose reaction characterized by hypoxia and hypotension was identified at dose levels ≥ 1 µg/kg. Dosing i.v. was less potent at inducing a leukocytosis than previously observed for equivalent s.c. doses and was associated with a higher incidence of generalized rash and first dose reactions. The maximal tolerated dose of i.v. rhGM-CSF was 15 µg/kg/day. Phase II studies in which the desired effect is to raise leukocyte levels should be undertaken at rhGM-CSF doses of 3 to 15 µg/kg/day.

INTRODUCTION

GM-CSF is a glycoprotein that influence the proliferation, differentiation, maturation, and activation of hemopoietic cells (1, 2). The gene for GM-CSF has been cloned (3, 4). rhGM-CSF produced in yeast, mammalian cell, and bacterial expression systems has been shown in vitro to have many of the activities attributed to the native glycoprotein (5–7).

The availability of sufficient quantities of biosynthesized glycosylated rhGM-CSF enabled clinical studies to commence in 1987 (8). The early clinical trials investigated the potential efficacy of rhGM-CSF in elevating leukocyte levels in neutropenic patients with the acquired immunodeficiency syndrome (8), myelodysplasia (9–11), and aplastic anemia (11), in accelerating recovery of leukopoiesis following high-dose chemotherapy and autologous marrow transplantation (12, 13) and in preventing the neutropenia following chemotherapy for sarcomas (14). In all of these studies, glycosylated rhGM-CSF was administered i.v. These early Phase I and II studies showed the physiological effects of rhGM-CSF to be more diverse than those of recombinant human granulocyte colony-stimulating factor (15, 16) (for comparative review, see Ref. 17), in both its effects on hematological parameters and its clinical adverse effects. In these early published studies the elucidation of the effects of rhGM-CSF was complicated by the nature of the underlying marrow diseases and concomitant therapies of the patients. It was not clear from these studies whether i.v. administration was the best way to deliver rhGM-CSF, and whether the best form of rhGM-CSF to use was glycosylated rhGM-CSF which in vitro was less potent than nonglycosylated, bacterially synthesized rhGM-CSF (18, 19).

In vitro studies indicated that GM-CSF was a potent monocyte activator and that monocytes stimulated by GM-CSF exhibited tumoricidal effects (20, 21). We therefore undertook a Phase I study of s.c. administered, nonglycosylated, bacterially synthesized rhGM-CSF in patients with advanced malignancy who were otherwise well (22). We showed that once daily s.c. doses of 3 to 15 µg/kg/day were effective at inducing a leukocytosis and were tolerated by the patients although, in this dose range, adverse effects included fever, bone pain, skin rash, and elevation of liver enzymes. The dose-limiting toxicity appeared to be pericarditis (22). Autoimmune thrombocytopenia was identified as a contraindication to rhGM-CSF therapy (22). No anticancer effects were observed during the short courses of rhGM-CSF administration.

In view of the local rashes at injection sites which occurred in 40% of patients receiving s.c. rhGM-CSF (22), we also undertook a Phase II study of i.v. administered, bacterially synthesized rhGM-CSF to determine if this route of administration would be advantageous. To make daily administration feasible, we chose to study i.v. infusions of short duration. Our study was designed to define the therapeutic dose range for this route of administration, to monitor the biological effects, and to identify any anticancer effects and adverse reactions to rhGM-CSF. The similar design of our s.c. and i.v. administered rhGM-CSF studies enables us to make comparisons of these two routes of administration of rhGM-CSF.

PATIENTS AND METHODS

Patients. Patients participating in this study had either advanced malignancy and/or neutropenia for which there was no alternative appropriate treatment. At least 4 wk had elapsed since any previous chemotherapy or radiotherapy. Nonneutropenic patients had baseline neutrophil levels >1.5 × 10⁹/liter. All patients had platelet levels >100 × 10⁹/liter, an Eastern Cooperative Oncology Group performance
status of 0 to 2, and good renal and liver function (creatinine <0.15 mmol/liter, bilirubin <30 µmol/liter). Contraindications to eligibility were previous irradiation to more than 30% of marrow volume, surgery within 14 days, ongoing infection, and Eastern Cooperative Oncology Group performance status of 3 or 4. The protocol met the ethical guidelines of the National Health and Medical Research Council of Australia and was approved by the Board of Medical Research and the Ethics Committee of The Royal Melbourne Hospital. All patients gave signed informed consent.

Patient Treatment and Monitoring. Groups of 3 or 4 patients received rhGM-CSF at doses of 0.3, 1, 3, 10, 15, and 20 µg/kg/day i.v. for up to 10 days. All patients were hospitalized for the initial two doses of rhGM-CSF. For doses of 0.3, 1, and 3 µg/kg, patients received rhGM-CSF by once daily bolus i.v. injection. One patient developed hypotension with syncope 20 min after the first i.v. bolus dose of 3 µg/kg of rhGM-CSF. In view of this and evolving data regarding the efficacy and clinical effects of i.v. bolus doses of rhGM-CSF, the protocol was amended, and subsequent patients received rhGM-CSF by 2-h infusions. One further patient received 3 µg/kg of rhGM-CSF by 2-h i.v. infusion. Daily 2-h i.v. infusions were used for all patients receiving 10, 15, and 20 µg/kg/day.

Before treatment, tumor dimensions were recorded, and baseline tests were performed including biochemical parameters, full blood counts (hemoglobin, leukocyte, and platelet counts) with manual 100-cell differential leukocyte count, reticulocyte count, clotting studies, serum bilirubin, alkaline phosphatase, liver transaminases, cholesterol, triglyceride, folate and vitamin B12 levels, urinalysis, creatinine clearance and 24-h urinary protein, electrocardiograph, chest radiograph, and tests relevant to the assessment of the malignancy of the individual patients. On the first day of rhGM-CSF administration, blood was taken at frequent intervals for pharmacokinetic and blood cell studies. During treatment, patients were assessed daily for toxicity according to WHO criteria and had daily full blood counts performed immediately prior to each rhGM-CSF injection. Biochemical and other laboratory parameters were measured on the eighth treatment day and 2 and 5 days after the last treatment. Marrow was obtained by sternal aspiration prior to treatment and on the fifth or sixth treatment day and assessed by 300-cell manual differential counts.

Retreatment. Patients who did not experience significant adverse effects and whose tumor remained stable or responded were retreated with rhGM-CSF at the same dose level. They received daily i.v. injections for a further 10 days, commencing 10 days after the final dose of the first course, and if they consented then received a further ten doses of rhGM-CSF on alternate days.

Patients were reviewed clinically 1 mo after completing treatment with rhGM-CSF including blood tests and questioned regarding possible late adverse effects.

rhGM-CSF. Bacterially synthesized recombinant human GM-CSF was supplied by Schering-Plough (Kenilworth, NJ) as a lyophilized powder. Vials containing 50, 100, 400, or 500 µg of rhGM-CSF (specific activity, 10³ units/mg) were reconstituted with 1.0 ml of sterile water for injection. For i.v. bolus injections, no further dilution was made. For i.v. infusions, rhGM-CSF was further diluted in 100 ml of normal saline solution. rhGM-CSF contained less than 12 units of endotoxin per vial by the Limulus Amebocyte Lysate assay.

Statistics. Unless otherwise stated, results are expressed as the mean ± standard error. Significance values were calculated by the x² test or 2-sample t test. The Wilcoxon rank sum test was used to compare leukocyte levels during the first and second treatment cycles. Kendall's correlation coefficient was calculated to look for trend in nonparametric data.

RESULTS

Characteristics of Patients. The characteristics of 21 patients who received i.v. rhGM-CSF are shown in Table 1. Their mean age was 59 yr (range, 35 to 78 yr). Twenty of the 21 patients had histologically proven malignancy. One patient with malignancy was also neutropenic (neutrophils < 1.5 × 10⁹/liter).

Sixteen patients had solid tumors, and 4 patients had hematological malignancies. One patient who received 1 µg/kg/day had an idiopathic neutropenia without associated malignancy. The other neutropenic patient who received 20 µg/kg/day of rhGM-CSF had a lymphoma and hypoplastic marrow after extensive previous treatment with chemotherapy and radiotherapy. Nine patients had Hickman's catheters which were used for blood sampling and administration of rhGM-CSF.

Seventeen patients completed the initial 10-day treatment period with rhGM-CSF. Four patients did not complete the initial treatment period because of adverse reactions (see below). Twelve patients completed the second 10-day treatment period, commencing 10 days after the last dose of the first treatment period. One patient who received 3 µg/kg/day commenced the second treatment cycle after a nontreatment interval of more than 10 days during which an intercurrent illness was treated. One patient was retreated with 10 µg/kg/day 10 days after the first cycle of 15 µg/kg/day was truncated due to an adverse effect. Four patients commenced alternate day therapy after the second treatment period, and of these, 2 received 10 doses. All patients were evaluable for toxicity.

Immediate Effect of i.v. rhGM-CSF on Circulating Cells. The immediate effects of the first dose of i.v. rhGM-CSF on circulating cell levels were examined in 6 patients at doses of 0.3 to 20 µg/kg. At all doses there was an immediate transient decrease in total circulating leucocytes to 73 ± 5% of initial levels, because of a fall in numbers of circulating neutrophils (93 ± 4% of baseline), monocytes (97 ± 7% of baseline), and eosinophils (100 ± 0% of baseline) but not lymphocytes (Fig. 1). During the first 6 h after i.v. rhGM-CSF, there was no consistent change in hemoglobin or platelet levels, which varied within 9 ± 1% and 19 ± 3% of initial levels, respectively. The nadir in polymorph and monocyte levels occurred 5 to 45 min after the start of rhGM-CSF administration for both i.v. bolus or i.v. infusion doses. Circulating neutrophil numbers returned to original levels within 4 h in all 6 patients studied and, in patients receiving doses ≥3 µg/kg, continued to increase to levels 2.5 ±

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Table 1 Characteristics of patients receiving i.v. rhGM-CSF

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* Numbers in parentheses, range. ³ ECOG, Eastern Cooperative Oncology Group.
Fig. 1. Immediate effect of i.v. rhGM-CSF on circulating mature neutrophils, neutrophil band forms, eosinophils, and monocytes. rhGM-CSF was given i.v. at time 0. Points, mean of pooled data from 6 patients receiving doses of 0.3 to 20 µg/kg of rhGM-CSF by i.v. bolus or 2-h infusion; bars, SE.

0.5-fold higher than baseline levels (range, 6.1 to 17.4 x 10⁹/liter) at 6 h after rhGM-CSF. The restoration of circulating neutrophil levels was associated with the appearance of band forms in the circulation, which comprised more than 10% of circulating neutrophils at all time points from 30 min to 6 h after rhGM-CSF, compared with less than 1% prior to rhGM-CSF administration (Fig. 1). Circulating monocytes were restored to levels of 84 ± 25% of baseline by 6 h after rhGM-CSF for all doses studied.

The transient leukopenia recurred after every daily dose of i.v. rhGM-CSF studied. This effect occurred in patients with high initial leukocyte levels due to rhGM-CSF therapy on previous days, and larger absolute numbers of leukocytes disappeared from the circulating pool.

Effect of Daily i.v. rhGM-CSF on Circulating Leukocytes and Neutrophils. The effect of continued daily i.v. rhGM-CSF administration on total circulating leukocyte levels is shown in Fig. 2. Single daily bolus i.v. injections of 0.3 to 3 µg/kg/day for 10 days did not alter the pretreatment daily circulating levels in patients with normal baseline leukocyte levels. However, the neutropenic patient treated with 1 µg/kg/day of rhGM-CSF had an increase in neutrophils from pretreatment levels of less than 0.5 x 10⁹/liter to a peak level of 2.1 x 10⁹/liter 2 days after the last dose of rhGM-CSF. In this patient, during the second 10-day treatment period, neutrophil levels were sustained above baseline values in the range of 0.8 to 1.3 x 10⁹/liter. At follow-up examination 21 days after the last dose of rhGM-CSF, the neutrophil level was 0.54 x 10⁹/liter.

At doses of 10 to 20 µg/kg/day, rhGM-CSF administration by 2-h infusion resulted in a dose-related increase in total circulating leukocyte levels in all patients (Fig. 3A). At these doses, the rhGM-CSF-driven leukocytosis was biphasic. There was an initial increase in circulating leukocytes during the first 2 days of treatment, followed by a period when cell levels fell (8 of 9 patients). Then there was a second more sustained increase in circulating leukocytes (6 of the patients who continued to receive rhGM-CSF) which was most marked at the highest dose (20 µg/kg/day). During rhGM-CSF treatment, circulating neutrophils showed a marked left shift. At doses of 15 and 20 µg/kg/day, respectively, 39% and 38% of circulating neutrophils were band forms, 2% and 7% were metamyelocytes, and 2% and 4% were myelocytes. On cessation of rhGM-CSF therapy, the neutrophilia resolved, but 14 of 15 patients had neutrophil levels higher than pretreatment levels at the end of the 10-day nontreatment interval (pretreatment = 3.8 ± 0.4 x 10⁹/liter; end of nontreatment interval = 5.7 ± 0.6 x 10⁹/liter; n = 15; P < 0.01, t test).

Effect of i.v. rhGM-CSF on Circulating Eosinophils, Monocytes, and Lymphocytes. An eosinophilia was a major component of the leukocytosis at doses of >3 µg/kg/day (Fig. 4A). At 3 µg/kg/day, eosinophils comprised 5 ± 1% of leucocytes after 10 days of rhGM-CSF administration compared with 1 ± 1% before treatment. At higher doses increases in circulating eosin-
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There was an increase in circulating monocytes by the tenth day of treatment at doses of 10 μg/kg/day or greater (Fig. 4B), which represented a 3.5 ± 1.6-fold increase over baseline levels for the three patients treated with 20 μg/kg/day of rhGM-CSF (Fig. 3B) (P < 0.01, t test). There was no consistent change in lymphocyte levels which varied between 60% and 240% of baseline levels on Day 5 and between 40% and 250% of baseline levels on Day 10 regardless of dose.

Effect of i.v. rhGM-CSF on Hemoglobin, Platelet, and Basophil Levels. Hemoglobin levels decreased by 9 ± 2% and 8 ± 3% of baseline levels on Days 5 and 10, respectively. Reticulocyte percentages increased from a mean value for all patients before either rhGM-CSF treatment cycle of 0.9 ± 0.1% to 1.4 ± 0.2% (P < 0.01, t test) during rhGM-CSF treatment. There was no consistent effect on basophil levels.

The effect of platelet levels was variable and small. Platelet levels at Day 5 were 104 ± 3% and on Day 10 were 110 ± 4% of baseline levels for the 20 and 17 patients completing these periods of treatment. During the first rhGM-CSF treatment cycle, the minimum platelet count occurred in the first 5 days of treatment in 15 of 20 patients, and the maximum platelet count occurred in the second 5 days of treatment in 19 of 20 patients (P < 0.001, χ² test). There was a significant trend for platelet levels to increase during the first cycle of rhGM-CSF administration (r = 0.91, P < 0.001). However, for the 14 patients completing a second treatment cycle and in whom platelet levels had increased during the first rhGM-CSF treatment cycle, platelet levels decreased from 321 ± 35 x 10⁹/liter before Cycle two to 292 ± 31 x 10⁹/liter on the tenth day of cycle two (r = -0.74, P = 0.03).

Effect of i.v. rhGM-CSF on Marrow Hemopoiesis. Aspirates of sternal marrow were obtained from each patient before treatment and after 5 days of i.v. rhGM-CSF administration. Fig. 5 shows that there was a dose-dependent increase in the proportion of promyelocytes after 5 days of rhGM-CSF administration (r = 0.62, P = 0.003). There was also a dose-dependent

![Fig. 3. Dose-response curves of neutrophil (A) and eosinophil and monocyte (B) levels in patients administered i.v. rhGM-CSF. Points, mean of groups of 2 to 4 patients at each dose level.](image1)

![Fig. 4. Effect of different doses of i.v. rhGM-CSF on eosinophil (A) and monocyte (B) levels. Points, mean of groups of 3 or 4 patients receiving i.v. rhGM-CSF at the four doses indicated. rhGM-CSF was administered i.v. on Days 1 to 10. Patients received rhGM-CSF by i.v. bolus (0.3 μg/kg/day and 2 patients at 3 μg/kg/day) and by i.v. 2-h infusion (one patient at 3 μg/kg/day and all patients at 15 and 20 μg/kg/day).](image2)
increase in myelocytes (r = 0.45, P = 0.022). The proportion of segmented neutrophils in the marrow decreased after 5 days of rhGM-CSF administration. There was an increase in the proportion of eosinophil precursors at doses of 0.3 to 15 μg/kg/day but not at 20 μg/kg/day (r = −0.23, P = 0.15). There appeared to be no consistent effect on the proportion of marrow monocytes. An increase in granulopoiesis was indicated by a rise in the leukopoiesis: erythropoiesis ratio at doses of 10 μg/kg/day (1.2 ± 0.4-fold); 15 μg/kg/day (1.7 ± 0.4-fold); and 20 μg/kg/day (3.1 ± 1.2-fold). Marrow cellularity appeared to increase during rhGM-CSF administration, but this was difficult to quantify for marrow aspirates.

Effect of Treatment with i.v. rhGM-CSF. Twelve patients received rhGM-CSF for a second 10-day period after a non-treatment interval of 10 days. A further patient was retreated after a longer interval. At bolus doses of 0.3 to 3 μg/kg/day, there was no effect of rhGM-CSF either initially or with retreatment except in one neutropenic patient. At doses of 10, 15, and 20 μg/kg/day by 2-h infusion, the second treatment period was characterized by a larger initial increment in total leukocytes after the first dose in 6 of 6 patients which averaged 2.8 times greater at 10 μg/kg (n = 2) and 4.6 times greater at 15 μg/kg (n = 2). Leukocyte levels increased to peak values at the end of the retreatment period which at 15 μg/kg/day were 30 to 54% higher than at the end of the first treatment period. The leukocytosis was significantly greater during retreatment for one of two patients receiving 10 μg/kg/day (P = 0.005 and 0.09) and for both patients receiving 15 μg/kg/day (P = 0.003 and 0.005) by the Wilcoxon rank sum test (Fig. 6).

Selected patients were treated for a further period by alternate day i.v. rhGM-CSF. Fig. 6 also shows the total leukocyte levels for two patients treated with alternate day rhGM-CSF at 10 or 15 μg/kg/dose. Alternate day rhGM-CSF at 10 μg/kg/dose did not maintain a leukocytosis above baseline, although this patient continued to have an eosinophilia (0.78 to 3.07 × 10^9/liter) during this period. At follow-up 1 mo later the eosinophilia had resolved. One patient received alternate day doses of rhGM-CSF at 15 μg/kg dose for two doses. Over this period a leukocytosis was maintained with a marked eosinophilia (1.87 to 7.81 × 10^9/liter). Dosing was ceased due to rash and pruritus. A further patient with chronic lymphocytic leukemia was treated with alternate day i.v. rhGM-CSF at 15 μg/kg/dose. In this patient, leukocyte levels did not remain above baseline, although an eosinophilia (0.47 to 2.36 × 10^9/liter) was maintained which had resolved at follow-up 1 mo later.

Effect of i.v. rhGM-CSF on Tumors. No solid tumor responses were observed during i.v. rhGM-CSF administration as assessed by tumor dimensions.

The two patients with CLL both had reductions in lymphocytosis during rhGM-CSF therapy. One patient receiving 15 μg/kg/day had Rai Stage 0 CLL with a lymphocytosis of 16 ± 2 × 10^9/liter (mean ± SD) during the preceding 8 mo. The lymphocyte level decreased progressively from 11.6 to 7.3 × 10^9/liter during the first treatment cycle and rose to 13.1 × 10^9/liter during the nontreatment interval. During the second treatment cycle, the lymphocyte level fell to 7.4 × 10^9/liter. During alternate day dosing the lymphocyte levels fluctuated in the range of 5.6 to 10.9 × 10^9/liter and at follow-up 1 mo after the last GM-CSF dose, the lymphocyte count was 9.1 × 10^9/liter. The other patient with CLL had a lymphocytosis of 27 ± 3 × 10^9/liter (mean ± SD) during the preceding 8 mo. After the first dose of 20 μg/kg/day of rhGM-CSF, the lymphocyte count decreased from 28.2 to 19.9 × 10^9/liter, but during the following 9 days of treatment, the lymphocyte level varied in the range of 24.8 to 35.0 × 10^9/liter.

Biochemical Changes. A significant decrease in serum cholesterol was observed in 25 of 30 cycles of i.v. rhGM-CSF administration. Cholesterol levels had returned to baseline levels 5 days after the first cycle of rhGM-CSF and decreased again during 11 of 13 second cycles of treatment. There was a negative correlation between leukocyte and cholesterol levels, indicating a dose-dependent effect (r = −0.58, P = 0.004, n = 28). Nonfasting serum triglycerides increased during 19 of 30 cycles of treatment, but the mean values for pooled data from 30 cycles of rhGM-CSF therapy were not significantly different before rhGM-CSF (1.8 ± 0.8 mmol/liter) and during rhGM-CSF (2.2 ± 1.2 mmol/liter). We did not measure fasting triglyceride levels. In all ten patients receiving ≥10 μg/kg/day i.v. of rhGM-CSF, a fall in serum albumin was observed during the first treatment cycle (39 ± 5 g/liter to 34 ± 7 g/liter, mean ± SD, P < 0.05, t test). Five of 6 patients receiving two 10-day courses at these dose levels became hypoalbuminemic despite...
having normal pretreatment albumin levels (P < 0.01, χ² test). The hypoalbuminemia was transient and resolved during the nontreatment interval. There was no change in 24-h urinary protein excretion during the study period in the 5 patients who became hypoalbuminemic. Only 2 patients developed an increase in liver transaminases. These patients received 15 and 20 μg/kg/day i.v. of rhGM-CSF and developed a 4- and 5-fold increase in γ-glutamyl transferase during the first treatment period which resolved during the nontreatment interval. One of these patients was retreated, and an increase in γ-glutamyl transferase did not recur during the second treatment cycle. There was no consistent effect on alkaline phosphatase levels.

Serum vitamin B₁₂ levels (normal range, 200 to 800 pg/ml) increased during rhGM-CSF therapy in 18 of 20 patients from levels of 553 ± 390 pg/ml to 1250 ± 723 pg/ml (mean ± SD, P < 0.001, t test). (Three patients had levels of >2400 pg/ml at the end of the study period.) For doses ≥10 μg/kg/day, serum vitamin B₁₂ levels increased more than 2-fold in 10 of 11 patients. There was a positive correlation between leukocyte level and vitamin B₁₂ level (r = 0.63, P = 0.005, n = 20). Serum folate levels (normal range, 3 to 17 ng/ml) decreased during the study for 13 of 20 patients and decreased in the 10 of 13 patients receiving 2 cycles of rhGM-CSF, but the change in mean folate levels was not statistically significant (from baseline levels of 5.8 ± 3.0 to levels during treatment of 4.3 ± 2.3 ng/ml, mean ± SD, P = 0.1, t test).

There was no effect on the prothrombin time or activated partial thromboplastin time.

**Adverse Effects of i.v. rhGM-CSF.** rhGM-CSF i.v. was generally well tolerated at doses of 0.3 to 15 μg/kg/day. Seventeen patients completed the initial 10-day treatment period with i.v. rhGM-CSF, and of these, 12 completed the second 10-day treatment period. The four patients who did not complete the initial treatment period stopped because of hypotension and transient loss of consciousness after the first dose (3 μg/kg/day, i.v. bolus), fluid retention with 5.8-kg weight gain (10 μg/kg/day), fever, rash, anorexia, and malaise (20 μg/kg/day), and pericardial pain (15 μg/kg/day). Three patients were not treated with rhGM-CSF following the 10-day nontreatment interval because of evidence of tumor progression. One patient developed a pulmonary embolus following a previous groin dissection for malignant melanoma. One patient discontinued treatment after the 10-day nontreatment interval because of evidence of tumor progression. One patient also developed fever which resolved when rhGM-CSF dosing was discontinued.

Several types of skin rash occurred. Two patients developed acute raised erythematous eczematous eruptions associated with pruritus which were followed by marked desquamation after rhGM-CSF was discontinued. Other patients developed diffuse erythematous areas resembling viral exanthems. Three patients had severe generalized pruritus resistant to systemic antihistamines and topical preparations, including steroids. The patient with the most marked pruritus was receiving 15 Mg/kg/day, and had marked eosinophilia but not basophilia. In this case the pruritus took 1 wk to resolve. Skin biopsies showed perivascular mononuclear cell infiltrates with occasional polymorphs. In one case the appearance resembled urticaria but in others was nonspecific.

Weight gain was associated with hypoalbuminemia but not proteinuria. The 3 patients with weight gain all had metastatic liver disease.

Two patients developed marginal keratitis within 12 h of rhGM-CSF administration, evidenced by prominent blood vessels at the corneoscleral junction of the eye, which was managed with topical steroids. One of these patients had a history of occupational conjunctivitis. In one of the 2 patients, marginal keratitis recurred within 12 h of restarting rhGM-CSF.

A recognizable clinical reaction to the first dose of i.v. rhGM-CSF occurred on 14 occasions at doses ≥1 μg/kg. Within 15 to 20 min of the first dose, patients developed all or some of: a flushing sensation; sweating; nausea; vomiting; back pain including throbbing lumbar discomfort; involuntary muscular leg spasms, and dyspnoea. During this period hypotension, tachy-
cardia, and hypoxia were documented. The reaction never occurred with subsequent doses in one course of uninterrupted treatment, but was likely to recur at the start of the second treatment if it had occurred during the first. Patients were managed with i.v. fluids, posturing, oxygen supplements, acetaminophen, nonsteroidal antiinflammatory agents, and occasionally narcotics for back pain.

**DISCUSSION**

We have studied the effects of i.v. administered, nonglycosylated rhGM-CSF in a dose escalation study and shown that i.v. rhGM-CSF induced an increase in circulating leukocyte levels and was tolerated by the patients in the dose range of 1 to 15 µg/kg/day. At these doses, adverse effects were mild to moderate. At i.v. doses ≥15 µg/kg/day by 2-h infusion, we observed fever and rigors, generalized rash, lethargy, anorexia, and two episodes of pericardial pain. While each adverse effect did not alone preclude further rhGM-CSF administration, the combination of adverse effects resulted in some patients discontinuing rhGM-CSF. In the 4 patients receiving 15 µg/kg/day six of seven 10-day courses of rhGM-CSF therapy were completed, but in the 4 patients receiving 20 µg/kg/day, 2 of 5 ten-day courses were not completed due to adverse effects, indicating that 15 µg/kg/day is the maximal tolerated dose. Nineteen of the 21 patients had stable tumors during rhGM-CSF administration, and 2 patients (both with chronic lymphocytic leukemia) had transient minor responses.

Whether i.v. and s.c. routes of rhGM-CSF administration were comparable in terms of efficacy and toxicity had not yet been determined. We were therefore interested to compare the results of this study to our previously reported Phase I study of s.c. administered rhGM-CSF (22) and to other studies which used continuous (8–10, 12, 14) or short i.v. (11, 13, 23, 24) infusions of rhGM-CSF. However, comparison of the biological effects of rhGM-CSF between the published studies is complicated by the following factors: (a) the use of short infusions of different durations (30 min, Ref. 24; 1, 2, 4, and 12 h, Refs. 11 and 13); (b) rhGM-CSF from yeast or mammalian cell expression systems has different glycosylation patterns, only a subset of the material is administered to patients, and therefore equivalent doses by mass will contain different amounts of rhGM-CSF protein; (c) doses were calculated per surface area or weight in different studies; (d) different techniques were used to determine specific activity; and (e) in other studies the effects of the underlying marrow disease or concomitant therapies of the patients would be expected to influence the response to rhGM-CSF. Nevertheless, it appears that continuous i.v. infusions are more potent than intermittent daily bolus or short i.v. infusions (25). In our study, daily bolus i.v. dosing was not effective at inducing a sustained increase in leukocytes at doses ≥3 µg/kg except in one neutropenic patient. In another study (8) a transient increase in leukocytes was seen at 6 h after single i.v. bolus doses of ≥2.6 × 10^8 units/kg. In our study, daily bolus i.v. dosing was not effective at inducing a sustained increase in leukocytes at doses ≥3 µg/kg except in one neutropenic patient. In another study (8) a transient increase in leukocytes was seen at 6 h after single i.v. bolus doses of ≥2.6 × 10^8 units/kg. In our study (14), patients with solid tumors treated with continuous infusions of 4 µg/kg of mammalian cell derived rhGM-CSF developed white cell levels of 22 to 27 × 10^9/liter after 3 to 4 days of treatment, compared with patients in our study who received 3 µg/kg/day as a bolus or short i.v. infusion and who only developed leukocyte levels of 9.1 to 12.0 × 10^9/liter after 10 days of treatment. Bolus administration resulted in hypotension in one patient on our study and has been previously associated with flushing, back pain, fever, and epigastric distress (8, 10).

While it is difficult to draw comparative conclusions from various published studies for the reasons mentioned above, the similar design of our current study and our previous study (22) allowed us to make comparisons about the relative efficacy of intermittent i.v. and s.c. dosing with the same form of bacterially synthesized nonglycosylated rhGM-CSF, even though the studies were conducted with different patients. The magnitude of the leukocytosis occurring with repeated dosing was greater for s.c. than i.v. dosing. For groups of patients at the 5 dose levels ≥1 µg/kg/day, the mean fold increase in neutrophils, eosinophils, and monocytes was greater for s.c. than i.v. dosing for 13 of 15 mean fold changes on Day 5, and for 14 of 15 mean fold changes on Day 10 (Fig. 7). The leukocytosis followed a biphasic pattern with both dosing routes. For s.c. but not i.v. dosing, we observed an apparent plateau in the fold increase of neutrophils for the dose range of 3 to 15 µg/kg/day (22). These observations are in agreement with preclinical studies which have shown that, in Rhesus monkeys, for equivalent doses of rhGM-CSF, s.c. dosing in 3 divided doses induced a greater leukocytosis than 6-h i.v. infusions (26). Similar observations have been made for interleukin 2 (27, 28) and granulocyte colony-stimulating factor (15, 17, 29).

The efficacy of rhGM-CSF administration may be related to the duration for which serum levels are maintained above 1 ng/ml, which is the concentration of the form of rhGM-CSF we were using that achieves near maximal marrow stimulation in vitro (30). A single s.c. dose of 15 µg/kg achieved these levels in vivo for 16 h (22), whereas a single i.v. dose of 15 µg/kg/day produced these levels for 8 to 22 h. Similar observations were made for interleukin 2, where maintenance of serum levels correlated with therapeutic effect (27, 28).

Adverse effects were similar following i.v. and s.c. administration, except for generalized rash and first dose reactions which appeared more common with i.v. administration (rash P < 0.01; first dose reaction, P < 0.05, χ^2 test) and an increase in liver transaminases which was more common with s.c. administration (P < 0.05, χ^2 test). An effect unique to s.c. dosing was local reactions at injection sites.

At present there is no known clinical advantage of either glycosylated or nonglycosylated rhGM-CSF. In vitro the specific activity of glycosylated rhGM-CSF is less than that of
nonglycosylated rhGM-CSF (18, 19). Interestingly, in vivo preclinical studies demonstrated equivalent potency for both forms for equivalent doses of protein (26). It is likely that different forms of rhGM-CSF will have different therapeutic dose ranges and may have different spectrums of adverse effects, particularly the development of neutralizing antibodies, but this awaits further study.

Subcutaneous or short i.v. infusions as used in our studies appeared not to be associated with as frequent episodes of thrombophlebitis as were seen in studies using continuous i.v. infusions (8, 14). We observed thrombophlebitis in only one patient at the end of treatment with 30 doses of 15 μg/kg i.v. of rhGM-CSF by a peripheral vein.

In this study, we identified occasional reactions to the first dose of rhGM-CSF including transient hypoxia and hypotension. Previous studies have reported the transient neutropenia following rhGM-CSF administration (31–33), and we have extended this observation to eosinophils and monocytes (22). The transient pulmonary sequestration of leukocytes, however, does not seem to account for the first dose reaction (34). Symptoms including dyspnoea have also been reported following the first but not subsequent doses of glycosylated forms of rhGM-CSF (8, 10, 13, 24, 32, 33).

The effect of rhGM-CSF on platelet levels has varied in reported studies. We observed only minor changes in platelet levels with a slight decrease in the first 5 days of rhGM-CSF administration and an increase in the second 5 days. Platelet counts were also observed to fall in a group of 16 sarcoma patients treated with rhGM-CSF for a mean of 4.8 days (14). Other studies have reported either no effect (8, 11, 12) or occasional apparent increases (9, 10) in some patients with myelodysplasia. We have previously identified that quiescent autoimmune thrombocytopenia may be reactivated by rhGM-CSF administration (22), and this suggests that rhGM-CSF may shorten platelet survival by promoting platelet destruction in the reticuloendothelial system.

A decrease in serum cholesterol during rhGM-CSF has previously been described (25, 35). Our study confirms this observation and suggests that there may be an associated rise in serum triglycerides. A similar change in serum lipids was seen in patients on a Phase I study of TNF-α (36) and is also seen with recombinant γ-interferon (37). The lipid changes with TNF-α administration were attributed to inhibition of the enzyme lipoprotein lipase, as this would account for the increase in serum very low density lipoproteins observed after TNF-α administration (30). A similar mechanism may apply for patients receiving rhGM-CSF, as rhGM-CSF has been shown to activate monocytes in vitro to produce TNF-α (21, 38, 39). Monocyte products such as TNF-α and interleukin-1 may also be the mediators of some of the other effects of rhGM-CSF, such as fever or bone pain.

The association of rhGM-CSF therapy with fever and leukocytosis may complicate the recognition and management of infection in patients receiving rhGM-CSF. At the moment, there is no reliable method of recognizing whether fever in a patient receiving rhGM-CSF is due to infection or the therapy, and empirical antibiotic therapy might sometimes be commenced unnecessarily. However, if fever was due to rhGM-CSF, it usually commenced with the first dose and occasionally resolved despite continuation of rhGM-CSF. A clear temporal relationship between intermittent rhGM-CSF administration and fever is sometimes discernible (40).

The doses of rhGM-CSF studied may be higher than necessary for some purposes, such as stimulation of neutrophil or monocyte function (41, 42). rhGM-CSF may have beneficial effects at doses lower than those required to stimulate cell proliferation or to maintain a sustained leukocytosis. We have shown in vivo biological effects of rhGM-CSF even at the lowest doses which were below that which caused a sustained leukocytosis. This dose caused a transient leukopenia immediately after administration either i.v. or s.c. Serum rhGM-CSF levels were identified in these patients to be less than 0.1 ng/ml at this time (30). In vitro, picomolar concentrations of rhGM-CSF promote neutrophil functions such as phagocytosis and superoxide production (5), and nanomolar concentrations appear to be required to optimally support progenitor cell proliferation (5). When rhGM-CSF was used in combination with interleukin 3 in primate studies, even relatively low doses of rhGM-CSF were associated with an augmented leukopoietic response after interleukin 3 pretreatment (43).

Our data demonstrate that bacterially synthesized rhGM-CSF is an ineffective stimulant of leukopoiesis when given as a bolus, but it is effective when administered by short i.v. infusion. For Phase II studies, where this route is preferred, doses of 3 to 15 μg/kg/day appear to be effective when an increase in neutrophil levels is the desired effect. However, our comparative analysis suggests that s.c. administration or continuous i.v. infusions of rhGM-CSF are more potent and generally clinically preferable to short i.v. infusions of rhGM-CSF.

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