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The Effects of Dose and Route of Administration on the Pharmacokinetics of Granulocyte–Macrophage Colony-stimulating Factor

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The pharmacokinetics of granulocyte–macrophage colony stimulating factor (GM-CSF) (0.3–30 μg/kg) were studied after subcutaneous bolus ($n = 16$) or intravenous bolus ($n = 5$) injection or 2 h intravenous infusion ($n = 12$). Each method of administration gave a different GM-CSF concentration–time profile. Highest peak serum concentrations (C_{max}) followed the intravenous bolus, and the time GM-CSF persisted at a concentration greater than 1 ng/ml ($t > 1$ ng/ml) was longer after a subcutaneous than after an intravenous injection. Area under the concentration–time curve (AUC), C_{max} and $t > 1$ ng/ml all increased with dose for each method of administration. After intravenous administration, there was a two-phase decline in concentration. The half-life ($t_{1/2}$) of the terminal phase following an intravenous bolus ranged from 0.24 to 1.18 h and, following intravenous infusion, from 0.62 to 9.07 h and appeared to increase with dose. The apparent clearance was greatest following subcutaneous injection at doses below 3 μg/kg, suggesting a saturable mechanism or different bioavailability. Only 0.001%–0.2% of the injected dose appeared in the urine as immunoreactive GM-CSF.
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INTRODUCTION

GRANULOCYTE-MACROPHAGE colony-stimulating factor (GM-CSF) is a haemopoietic growth factor now in clinical trial [1–13]. GM-CSF's effects on haemopoietic cellular proliferation, such as accelerating myeloid regeneration and activating phagocytic cells, make it a potentially attractive agent for the prevention of neutropenia following cytotoxic therapy or associated with marrow disease. Phagocyte activation may also be useful in the management of infections in patients with normal neutrophil levels [14]. By stimulating antibody-dependent cell cytotoxicity, GM-CSF may have an anticancer effect [15].

The development of an immunoassay for GM-CSF [16] allows pharmacokinetics to be investigated. There are some pharmacokinetic data [8, 9, 12, 16] and part of the data reported here has been documented previously [9, 16]. We now report more extensive pharmacokinetics of GM-CSF in 33 patients. We compare the pharmacokinetics of GM-CSF administered at different doses and by different routes.

PATIENTS AND METHODS

Patients

In two phase I studies [9, 10], GM-CSF was administered to 42 patients with advanced malignancy or neutropenia. GM-CSF was not administered to patients with myeloid malignancies. In 33 of these patients, 22 male and 11 female, pharmacokinetics was studied (Table 1). In the first study patients received GM-CSF subcutaneously by bolus injection at doses between 0.3 and 30 μg/kg per day (Table 2). In the second study, GM-CSF was administered intravenously at doses from 0.3 to 20 μg/kg per day (Table 1). 26 patients were treated for 10 days and 7 received less than 10 days' treatment because adverse effects or protocol-stipulated precautions necessitated early cessation. Patients did not receive chemotherapy during this study and at least 4 weeks had elapsed since previous chemotherapy. All had a plasma bilirubin of less than 20 μmol/l and a plasma creatinine of less than 0.15 mmol/l.

All patients were admitted for the first two doses of GM-CSF. For intravenous doses of 0.3, 1 and 3 μg/kg, 9 patients received GM-CSF by once daily bolus injection (of these, pharmacokinetics was studied in 5); however, because of an acute toxic reaction, the protocol was amended and subsequent patients received

Table 1. Characteristics of 33 patients

Median age (range)	56 (30–74)
Tumour origin	
Lung	5
Bowel	5
Lymphoma	4
Melanoma	3
Other	14
Previous therapy	
Chemotherapy	13
Radiotherapy	12
Both	6
Nil	14
Neutropenia ($< 1.5 \times 10^9$/l)	4
Marrow infiltration	4

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Table 2. Doses and routes of administration

GM-CSF dose (μg/kg)	Route		
	Subcutaneous	Intravenous bolus	Intravenous infusion
0.3	1	1	0
1.0	2	2	0
3.0	3	2	1
10.0	3	0	3
15.0	2	0	4
20.0	3	0	4
30.0	2	0	0
Total	16	5	12

GM-CSF by intravenous infusion over 2 h. Daily 2 h infusions were used for 1 patient receiving 3 μg/kg and for 11 patients receiving higher doses.

Informed consent was obtained from all patients. 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 Ethics Committee of the Royal Melbourne Hospital.

Pharmacokinetics

On the first day of GM-CSF administration blood was collected from patients at time zero and at times for up to 24 h depending on the method of administration. Following subcutaneous injection, blood was collected at 5, 15, 30, 45 and 60 min after the dose, every 30 min to 6 h and at 7, 8, 10, 12, 14 and 24 h. After intravenous bolus injection, blood was sampled at the start, 1, 5, 15, 30, 45, 60, 120, 240 and 360 min and at 24 h. Following the start of the 2 h intravenous infusion blood was collected at 15, 30, 45, 60, 90 and 120 min during the infusion and then at the same time points as for the intravenous bolus and at 10 and 12 h. Minor variations to these time points occurred. Samples were stored at −20°C.

Urine samples were collected in 23 patients before the GM-CSF dose and at various times up to 24 h after the first dose.

The following pharmacokinetic variables were investigated: (1) area under the concentration–time curve (AUC) following the first dose extrapolated to infinity with the trapezoidal rule [17, 18]—AUC to infinite time was calculated from the curve by exponential regression analysis; (2) maximum serum concentration (C_{max}); (3) time over which the GM-CSF concentration exceeded 1 ng/ml ($t > 1$ ng/ml), chosen because 1 ng/ml is equivalent to 100 U, which leads to near maximum proliferation *in vitro* [19]; (5) apparent clearance (Cl) by Cl = dose/AUC [17]; and (6) the half-life of the terminal phase of elimination ($t_{1/2}$) calculated from the slope of the concentration–time curve by exponential regression analysis.

GM-CSF assay

A sandwich ELISA was used to assay serum levels of GM-CSF [16]. Two variations of the original method were used. The first was the substitution of the streptavidin–biotin detection system with an antiserum to rabbit immunoglobulin directly conjugated with horseradish peroxidase (NA.934, Amersham). The second variation was the use of Insight-GM immunoassay kits (Medical Resources Limited, Sydney) described in the original method [16]. These kits contained the same anti-GM-CSF antibodies. The assay was quantitative at concentrations as low as 0.1 ng/ml [16]. All samples were assayed by both methods. The immunoassay standards were calibrated and a calibration factor of 15% was used to correct for the difference between the standards used in the two systems. Dilutions were done to bring the GM-CSF concentration to between 0.2 and 0.6 ng/ml. Samples were assayed in triplicate over a range of dilutions and results are the mean of at least two separation measurements.

Urine samples were also assayed by ELISA. Immunoreactive GM-CSF was not detected in urine before GM-CSF administration.

GM-CSF

Bacterially synthesised recombinant human GM-CSF was supplied by Schering–Plough (Kenilworth, New Jersey) as a lyophilised powder. Vials containing 50, 100, 400 or 500 μg of GM-CSF (specific activity 10^8 U/mg) were reconstituted with 0.25–1.0 ml of sterile water for injection immediately before subcutaneous administration. The final injection volume was 1.1 ml or less. For intravenous bolus injection, GM-CSF was reconstituted in 1 ml sterile water. For intravenous infusion, GM-CSF was diluted further in 100 ml normal saline. GM-CSF contained less than 12 U endotoxin per vial by the limulus amoebocyte lysate assay (Dr E. Bonnem, Schering–Plough).

Statistics

Correlation coefficients from linear regression analyses were calculated. To investigate the relations between pharmacokinetic variables and injection route, one-way analyses of variance (ANOVAs) were used. Two-way ANOVAs were used to investigate the effects of dose and route on $t_{1/2}$ and C_{max}.

RESULTS

Subcutaneous injection

GM-CSF was detectable within 30 min of a subcutaneous injection for patients receiving 1 μg/kg, and between 5 and 15 min for patients receiving higher doses. Concentrations increased over 2–7 h and then declined to undetectable levels in all patients by 24 h. Figure 1A shows concentration–time profiles for 3 patients who received 20 μg/kg. The AUC ranged from 2.9 ng h/ml for a patient receiving 1.0 μg/kg to 266 ng h/ml for a patient receiving 30 μg/kg and increased with dose (Fig. 2A). Wide variation was observed between patients at each dose level. AUC could not be calculated in 3 patients because serum concentrations were too low (at 0.3 μg/kg) or there were insufficient data to extrapolate the terminal phase reliably. The mean C_{max} was 22.14 [S.D. 6.70] ng/ml for the 7 patients who received 15 μg/kg or above. There was a significant correlation between peak levels and AUC ($R = 0.944, P = 0.0001$). Serum concentrations of GM-CSF greater than 1 ng/ml occurred in all patients receiving more than 1 μg/kg but in none of the patients receiving 1 μg/kg or less. The $t > 1$ ng/ml increased with dose (Fig. 2E).

The most rapid Cl of GM-CSF from serum was calculated in 3 patients who received the lowest subcutaneous doses (18.63 and 25.08 l/h at 1 μg/kg and 33.27 l/h at 3 μg/kg) (Fig. 2G). At the higher doses (10–30 μg/kg) the Cl for 9 patients was 8.32 [3.95] l/h. Cl was higher following the smaller doses of GM-CSF (0.3–3 μg/kg) ($n = 4$) than in those receiving 10 μg/kg or more, ($P = 0.0007, n = 9$).

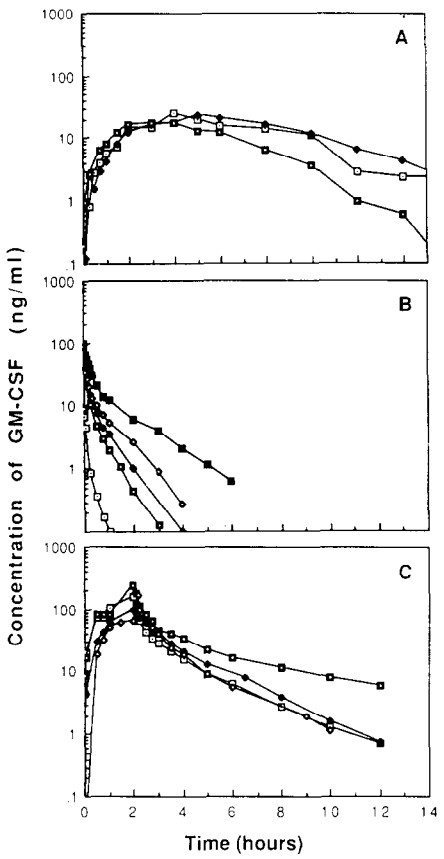

Fig. 1. Serum concentration–time profiles of GM-CSF. A = 20 μg/kg subcutaneously. B = intravenous bolus injection at, from left to right, 0.3, 1, 1, 3 and 3 μg/kg. C = 2 h intravenous infusion at 20 μg/kg. Each symbol represents a separate patient.

Intravenous bolus injection

Figure 1B shows the elimination kinetics of GM-CSF administered to patients receiving 0.3 to 3.0 μg/kg by intravenous bolus injection. A two-phase decline in concentration was observed. A rapid initial $t_{1/2}$ of about 5 min was followed by a terminal $t_{1/2}$ of 0.24–1.18 h. There was no clear relation betwen dose and $t_{1/2}$ although there was an impression of a dose dependent increase. The patient who received 0.3 μg/kg had the shortest GM-CSF $t_{1/2}$ and the highest *Cl* (16 l/h); however, since only six data points fell within the limit of sensitivity of the assay, the estimate of the terminal slope may be unreliable. There was no significant difference between the GM-CSF serum half-lives in patients treated with 1 μg/kg (0.59, 0.50 h) and 3 μg/kg (0.70, 1.18 h). *Cl* for these 4 patients was 6.23 [2.92] l/h.

C_{max} was 6.9–95.7 ng/ml and increased with dose (Fig. 2D). The $t > 1$ ng/ml appeared to increase with dose ($R = 0.852$, $P = 0.067$) as did AUC ($R = 0.840$, $P = 0.089$), but these correlations were not significant perhaps because of the small number of subjects and narrow dose range.

Intravenous infusion

For most patients, GM-CSF was detectable in serum from the first collection at 15 min (Fig. 1C). C_{max} ranged from 18.19 to 235 ng/ml and did not correlate with dose (Fig. 2D), although the highest serum concentrations were observed in patients receiving the larger doses. AUC increased in a dose-dependent manner ($R = 0.817$, $P = 0.011$). After completion of the infusion, serum GM-CSF levels appeared to follow an exponential decline and for most patients fell below 1 ng/ml within 12 h (Fig. 2F).

The $t_{1/2}$ of the terminal phase of elimination was similar in 9 of 11 patients who received doses of 10–20 μg/kg (1.66 [0.17] h, range 1.33–1.88 h). In the other 2 patients, $t_{1/2}$ was more prolonged. 1 of these patients ($t_{1/2} = 9.07$ h) had chronic lymphatic leukaemia with extensive marrow infiltration and depletion of myeloid elements, and the other ($t_{1/2} = 4.07$ h) had lymphoma and a hypoplastic marrow with neutropenia. None of the remaining patients treated by this route had neutropenia or marrow infiltration.

The mean *Cl* of GM-CSF in patients treated by intravenous infusion was 5.71 (1.17) l/h (Fig. 2H). There was no significant relation between dose and *Cl* and apparent clearances calculated for the 2 patients with long GM-CSF half-lives were similar to those for the other patients.

Comparative pharmacokinetics

For all routes of administration, AUC increased with dose and appeared larger after intravenous injection than following the same dose administered subcutaneously (Figs 2A and 2B). The ratio of the mean AUC following a subcutaneous dose relative to the mean AUC after the same dose intravenously was 0.29 at the 1 and 3 μg/kg dose levels, 0.83 at 10 and 15 μg/kg and 0.50 at 20 μg/kg.

Although there may have been a trend towards an increasing $t_{1/2}$ with dose following intravenous bolus injection (Fig. 1B), there was no similar trend observed for patients receiving GM-CSF by intravenous infusion or subcutaneous injection; however, doses were not comparable. The $t_{1/2}$ of GM-CSF after subcutaneous injection was longer than that after intravenous infusion: 3.16 (1.33) ($n = 10$) for 3–30μg/kg vs. 2.40 (2.25) ($n = 12$) for 3–20 μg/kg, respectively ($P = 0.05$) by two-way ANOVA. Excluding patients with bone marrow infiltration or neutropenia accentuated the difference in $t_{1/2}$: 7 patients treated by subcutaneous injection had a longer $t_{1/2}$ (3.14 [1.49] than 9 treated by intravenous infusion (1.54 [0.38]) ($P < 0.001$).

C_{max} increased with dose for each route of administration (Figs 2C and 2D). At 3 μg/kg, C_{max} was highest following intravenous bolus injection (80.24, 95.66 ng/ml), intermediate after intravenous infusion (24.15 ng/ml) and lowest following subcutaneous injection (4.80, 1.04, 1.65 ng/ml).

Although there was an apparent inverse relation between dose and *Cl* following a subcutaneous dose, this was not observed after intravenous injection although the highest *Cl* was calculated for the patient receiving the lowest dose (0.3 μg/kg). Nevertheless there was an inverse relation between C_{max} and *Cl* (Fig. 3).

Urinary excretion of GM-CSF

Following GM-CSF administration, immunoreactive GM-CSF was detected in the urine of 20 of 23 patients tested. The proportion of the injected dose ranged from 0.001 to 0.2%. Both the patients with the largest proportion of the administered dose appearing in urine received low doses of GM-CSF by subcutaneous injection and had both previously received cytotoxic chemotherapy with cisplatin. Figure 4 shows urine and serum concentrations of GM-CSF in 2 patients receiving 10 or 1 μg/kg subcutaneously. The patient who received 1 μg/kg (1 of the 2 who had been treated with cisplatin) had more than a 25 fold higher urinary GM-CSF concentration than the patient receiving 10 μg/kg. There was no relation between the pro-

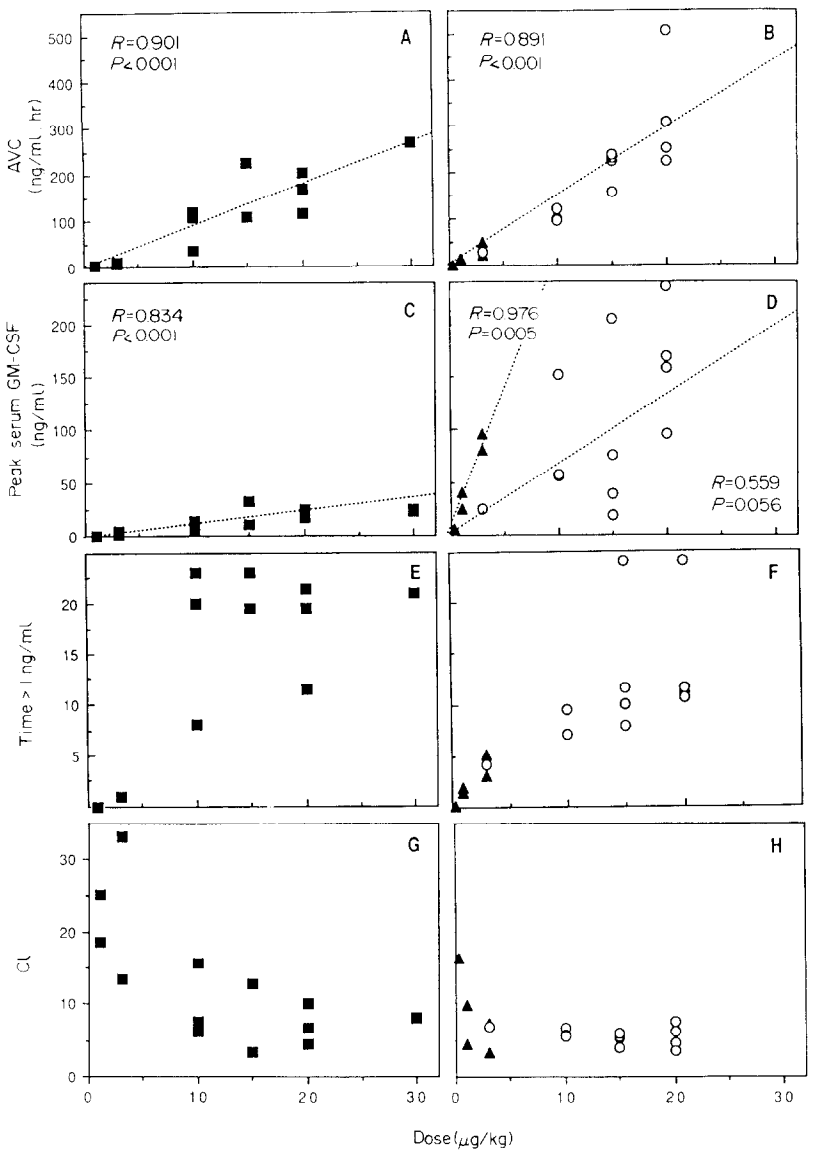

Fig. 2. Effect of route and dose on GM-CSF pharmacokinetics. Left-hand panels = subcutaneous administration. Right-hand panels = intravenous administration, either by bolus (▲) or 2 h infusion (○). In panel D, upper left line refers to intravenous bolus and lower right line refers to intravenous infusion.

portion of the dose appearing as immunoreactivity in the urine, GM-CSF dose, route of administration, C_{max} or other pharmacokinetic variables. Since all patients had normal serum creatinine concentrations, correlation between renal function and urinary GM-CSF could not be established and renal tubular damage was not evaluated.

To determine the biological activity of the urinary GM-CSF attempts were made to purify [20] GM-CSF from urine collected from a patient who received GM-CSF. Colony-stimulating activity was detectable: 34 granulocyte-macrophage and eosinophil colonies per 5×10^4 cells on day 14 (1:10 dilution). This effect was inhibited by anti-GM-CSF monoclonal antibody LMM 102 (data not shown).

DISCUSSION

Following subcutaneous injection of GM-CSF, C_{max} and AUC increased with dose but there was considerable variability between patients. This variability may relate to different rates of GM-CSF absorption from injection sites and different rates of clearance. The AUCs following the smaller subcutaneous doses (0.3–3 μg/kg) were lower than those after equivalent doses intravenously. Consequently, *Cl* was higher in patients treated with low subcutaneous doses. At higher subcutaneous doses, *Cl* was lower, and similar to that calculated for GM-CSF administered intravenously. In contrast, dose did not appear to affect *Cl* after an intravenous injection. When *Cl* was related to C_{max}, *Cl* was greatest in the patients receiving GM-CSF subcutaneously in whom the lowest C_{max} were achieved.

There are two possible explanations for the effect of route on apparent GM-CSF clearance. Firstly, bioavailability may be reduced because of reduced absorption of GM-CSF from the site of a subcutaneous dose. We know that at least some of the injected GM-CSF dose was absorbed and that even the smallest subcutaneous dose of GM-CSF was biologically active, since it caused an acute fall in neutrophil count [9] even when undetect-

able in serum by immunoassay. There is no evidence to support or exclude the possibility of the injected dose binding irreversibly to subcutaneous tissues. Studies in which radiolabelled GM-CSF has been investigated did not use the subcutaneous route but did not report the retention of label at injection sites [21, 22]. The second explanation is that GM-CSF may be rapidly cleared before being detected in venous blood. Such a rapid clearance might be observable after the lower subcutaneous dose. Following a larger subcutaneous dose or an intravenous dose, higher serum GM-CSF concentrations occurred and *Cl* was lower, which suggests that this clearance mechanism was being saturated by high GM-CSF concentrations.

Since normal myeloid cells, including tissue macrophages [23], myeloid precursors [24] and endothelial cells [25] express specific, high-affinity, membrane-associated receptors for GM-CSF, binding to existing unoccupied receptors could mediate the initial clearance. Alternatively GM-CSF might bind to extracellular matrix components such as heparan sulphate or other glycosaminoglycans [26, 27]. If GM-CSF receptors are available to bind GM-CSF at the concentrations achieved after injection, the high-affinity receptors (K_d 30 pmol/l, 0.45 ng/ml) [23] would be expected to be largely occupied at the concentrations that followed an intravenous dose (6.9–235 ng/ml). However, at the concentrations achieved after a subcutaneous dose (0.6–31.8 ng/ml) receptor occupancies would be lower. Such a clearance mechanism might therefore be limiting following intravenous dose but not after a subcutaneous dose.

Studies with iodinated native murine GM-CSF injected into mice have shown that 1 h after injection, [^{125}I]-GM-CSF counts were localised in a variety of tissues, including liver, spleen and kidney [21]. Similar studies with murine multipotential colony-stimulating factor (interleukin-3) [28] also show localisation in these organs. We do not know which cells are responsible for GM-CSF clearance, or whether clearance occurs through specific receptors or by other mechanisms. The clearance of haemopoietic growth factors by specific receptors has been proposed as the main elimination mechanism for macrophage CSF [29] and has been suggested as a regulatory mechanism for circulating granulocyte CSF [30].

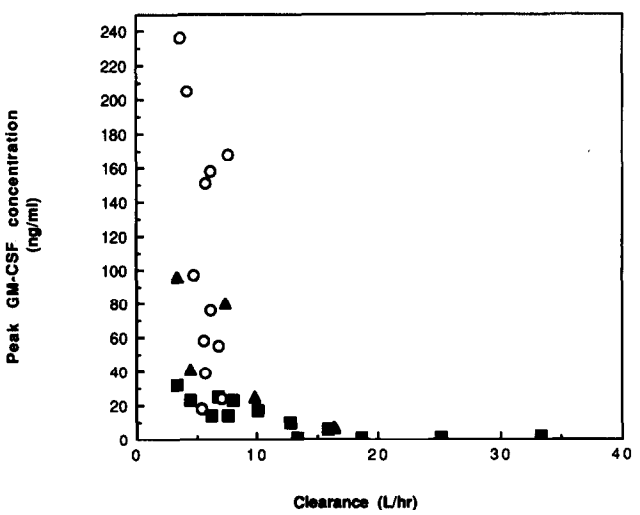

Fig. 3. Relation between route of GM-CSF administration and *Cl* and C_{max}. ■ = subcutaneous injection, ▲ = intravenous bolus injection and ○ = intravenous infusion over 2 h.

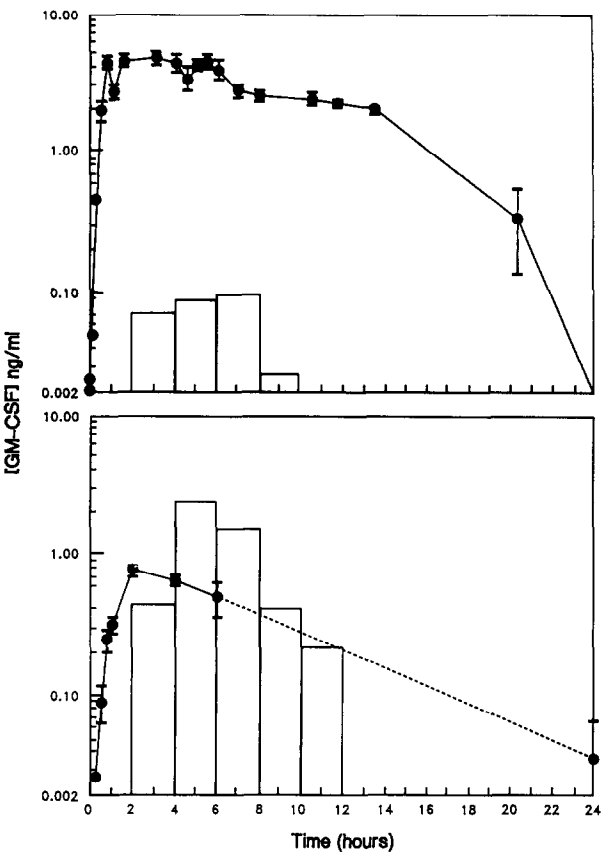

Fig. 4. Urinary (columns) and serum (lines) concentrations of GM-CSF following subcutaneous bolus administration in 2 patients. Upper = 10 μg/kg, and lower = 1.0 μg/kg.

Although early studies with radiolabelled GM-CSF identified the kidneys as important sites of GM-CSF binding, the role of renal mechanisms in GM-CSF clearance is poorly understood. Radiolabel in the urine following GM-CSF injection into mice could not be precipitated with trichloroacetic acid [21] and so any whole GM-CSF molecules filtered by the kidney may have been degraded before renal excretion. We found that less than 0.01% of an injected dose of human GM-CSF appeared in the urine as immunoreactive protein, which is consistent with these observations. The finding that up to 10 times more GM-CSF appeared in the urine of 2 patients who had previously been treated with cisplatin is of interest. Low molecular weight proteins are filtered by the glomeruli, reabsorbed and degraded by cells of the renal proximal convoluted tubules. β_2-microglobulin is a low molecuar weight protein handled by this mechanism and its appearance in urine has been proposed as a marker for detecting cisplatin nephrotoxicity [31]. A similar mechanism for excretion may apply for GM-CSF.

The 2 patients who had the most prolonged GM-CSF serum half-lives following a 2 h infusion had haematological disorders. It is unclear why $t_{1/2}$ was prolonged in these cases since 3 other neutropenic patients and 2 other patients with marrow infiltration did not handle GM-CSF in a different way from the remaining haemopoietically normal subjects, although small patient numbers makes comparisons difficult.

1. Antin JH, Smith BR, Holmes W, *et al.* Phase I/II study of recombinant human granulocyte-macrophage colony-stimulating factor in aplastic anemia and myelodysplastic syndrome. *Blood* 1988, **72**, 705–713.
2. Antman K, Griffin J, Elias A, *et al.* Effect of recombinant human granulocyte-macrophage colony stimulating factor on chemotherapy-induced myelosuppression. *N Engl J Med* 1988, **319**, 593–598.
3. Blazar BR, Kersey JH, McGlave PB, *et al. In vivo* administration of recombinant human granulocyte/macrophage colony-stimulating factor in acute lymphoblastic leukemia patients receiving purged allografts. *Blood* 1989, **73**, 849–857.
4. Brandt SJ, Peters WP, Atwater SK, *et al.* Effect of recombinant human granulocyte-macrophage colony-stimulating factor on hematopoietic reconstitution after high-dose chemotherapy and autologous bone marrow transplantation. *New Engl J Med* 1988, **318**, 869–876.
5. Champlin RE, Nimer SD, Ireland P, Oette DH, Golde DW. Treatment of refractory aplastic anemia with recombinant human granulocyte-macrophage colony-stimulating factor. *Blood* 1989, **73**, 694–699.
6. Ganser A, Völkers B, Greher J, *et al.* Recombinant human granulocyte-macrophage colony-stimulating factor in patients with myelodysplastic syndromes—a phase I/II trial. *Blood* 1989, **73**, 31–37.
7. Groopman JE, Mitsuyasu RT, DeLeo MJ, Oette DH, Golde D. Effect of recombinant human granulocyte-macrophage colony-stimulating factor on myelopoiesis in the acquired immunodeficiency syndrome. *New Engl J Med* 1987, **317**, 593–598.
8. Herrmann F, Schulz G, Lindemann A, *et al.* Hematopoietic responses in patients with advanced malignancy treated with recombinant human granulocyte-macrophage colony stimulating factor. *J Clin Oncol* 1989, **7**, 159–167.
9. Lieschke GJ, Maher D, Cebon J, *et al.* Effects of subcutaneously administered bacterially synthesized recombinant human granulocyte-macrophage colony-stimulating factor in patients with advanced malignancy. *Ann Intern Med* 1989, **110**, 357–364.
10. Lieschke GJ, Maher D, O'Connor M, *et al.* Phase I study of intravenously administered bacterially synthesized granulocyte-macrophage colony-stimulating factor and comparison with subcutaneous administration. *Cancer Res* 1990, **50**, 606–614.
11. Steward WP, Austin R, Scarffe JH, *et al.* Phase I study of recombinant DNA granulocyte-macrophage colony stimulating factor (rGM-CSF). *Br J Cancer* 1988, **57**, 258.
12. Thompson JA, Lee DJ, Kidd P, *et al.* Subcutaneous granulocyte macrophage colony stimulating factor in patients with myelodysplastic syndrome: toxicity, pharmacokinetics, and hematological effects. *J Clin Oncol* 1989, **7**, 629–637.
13. Vadhan-Raj S, Keating M, LeMaistre A, *et al.* Effects of recombinant human granulocyte-macrophage colony-stimulating factor in patients with myelodysplastic syndromes. *N Engl J Med* 1987, **317**, 1545.
14. Fleischmann J, Golde DW, Weisbart RH, Gasson JC. Granulocyte-macrophage colony-stimulating factor enhances phagocytosis of bacteria by human neutrophils. *Blood* 1986, **68**, 708–711.
15. Grabstein KH, Urdal DL, Tushinski RJ, *et al.* Induction of macrophage tumoricidal activity by granulocyte-macrophage colony-stimulating factor. *Science* 1986, **232**, 506–508.
16. Cebon J, Dempsey P, Kannourakis G, *et al.* Pharmacokinetics of human granulocyte-macrophage colony stimulating factor (hGM-CSF) using a sensitive immunoassay. *Blood* 1988, **72**, 1340–1347.
17. Gibaldi M. *Biopharmacokinetics and Clinical Pharmacokinetics*, 3rd edn Philadelphia, Lea and Febiger, 1984.
18. Bury RW. Area estimation in pharmacokinetic studies using a hand held programmable calculator. *Int J Bio-Med Comput* 1984, **15**, 219–224.
19. Metcalf D. *The Haemopoietic Colony Stimulating Factors*. Amsterdam, Elsevier, 1984.
20. Cebon J, Nice E, Gardner I, *et al.* Granulocyte macrophage colony stimulating factor from human lymphocytes: effect of glycosylation on receptor binding and biological activity. *J Biol Chem* 1990, **265**, 4483–4491.
21. Burgess AW, Metcalf D. Serum half-life and organ distribution of radiolabelled colony stimulating factor in mice. *Exp Hematol* 1977, **5**, 456–464.
22. Donahue RE, Wang EA, Stone DK, *et al.* Stimulation of hematopoiesis in primates by continuous infusion of recombinant human GM-CSF. *Nature* 1986, **321**, 872–875.
23. Gasson JC, Kaufman, Weisbart RH, Tomonaga M, Golde DW. High affinity binding of granulocyte-macrophage colony-stimulating factor to normal and leukemic human myeloid cells. *Proc Natl Acad Sci USA* 1986, **83**, 669–673.
24. Nicola NA. Haemopoietic growth factors and their interactions with specific receptors. *J Cell Physiol* 1987, **5**, 9–14.
25. Bussolino F, Wang JM, Defilippi P, *et al.* Granulocyte- and granulocyte-macrophage colony stimulating factors induce human endothelial cells to migrate and proliferate. *Nature* 1989, **337**, 471–473.
26. Gordon MY, Riley GP, Watt SM, Greaves MF. Compartmentalization of a hematopoietic growth factor (GM-CSF) by glycosaminoglycans in the bone marrow microenvironment. *Nature* 1987, **326**, 403–405.
27. Roberts R, Gallagher J, Spooncer E, Allen TD, Bloomfield F, Dexter TM. Heparan sulphate bound growth factors: a mechanism for stromal cell mediated haemopoiesis. *Nature* 1989, **332**, 376–378.
28. Metcalf D, Nicola NA. Tissue localisation and fate in mice of injected multipotential colony-stimulating factor. *Proc Natl Acad Sci USA* 1988, **85**, 3160–3164.
29. Tushinski RJ, Oliver IT, Guilbert LJ, Tynan PW, Warner JW, Stanley ER. Survival of mononuclear phagocytes depends on a lineage-specific growth factor that the differentiated cells selectively destroy. *Cell* 1982, **28**, 71–81.
30. Layton JE, Hockman H, Morstyn G. Evidence for a novel *in vivo* control mechanism of granulopoiesis: mature cell-related control of a regulatory growth factor. *Blood* 1989, **74**, 1303–1307.
31. Cohen AI, Harberg J, Citrin DL. Measurement of urinary β_2 microglobulin in the detection of cisplatin nephrotoxicity. *Cancer Treat Rep* 1981, **65**, 1083–1085.

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