Pulmonary Absorption of Recombinant Methionyl Human Granulocyte Colony Stimulating Factor (r-huG-CSF) After Intratracheal Instillation to the Hamster

Ralph W. Niven,[1,2] Fred D. Lott,[1] and Judith M. Cribbs[1]

Received March 1, 1993; accepted April 30, 1993

Recombinant methionyl human granulocyte colony stimulating factor (G-CSF), a molecule of 18.8 kDa, has been shown to induce a systemic response after delivery by aerosol. In this work, rate and extent of absorption as well as the response were determined after bolus administration of solutions by intratracheal instillation (IT). The protein was quantified using a specific ELISA and the biological response was assessed by monitoring the increase in numbers of circulating white blood cells (WBC). A dose–response curve was obtained after IT, subcutaneous injection (SC), and intracardiac injection (IC) of 100 μL of a nominal dose ranging from 1 to 1000 μg/kg G-CSF ($n = 5$). WBC numbers were determined 24 hr postadministration. Absorption and clearance kinetics were determined after IT and IC of 500 μg/kg protein over a 24-hr time period ($n = 5$). The response of the lung to G-CSF was monitored by WBC counts and differentials in lung lavage fluid. 73.6 ± 10.5% ($n = 7$) of the IT dose reached the lung lobes. The response to single doses of G-CSF by IT or SC was similar, with WBC numbers increasing over 4× baseline at the higher doses. Absorption from the lung was rapid and did not follow first-order kinetics. Clearance after the IC dose was described by a biexponential equation ($\alpha = 1.41, \beta = 0.24\ hr^{-1}$). Peak serum levels were obtained ≈1–2 hr after IT. The bioavailability was 45.9% of the administered dose and 62.0% of the dose reaching the lung lobes. These results indicate that G-CSF is rapidly absorbed from the lung. Pulmonary delivery via the airways has promise as an alternative to parenteral injection.

KEY WORDS: aerosol; cytokine; drug delivery; granulocyte colony stimulating factor (G-CSF); recombinant methionyl human G-CSF; intratracheal instillation; proteins; pharmacokinetics; pulmonary absorption.

INTRODUCTION

Drug delivery via the airways is an accepted route of administration to the lung but, with few exceptions, is not used for systemic delivery of drugs. For most macromolecules it would also be expected that their size should significantly retard their absorption across the pulmonary epithelium (1,2). Consequently, the delivery of larger molecules, such as proteins, to the systemic circulation via the lungs has not been thoroughly investigated.

The current revolution in the pharmaceutical industry with the arrival of DNA technology and the cloning and production of numerous therapeutic proteins has led to a search, in many instances, for noninvasive routes of administration. Oral administration of these compounds has been hampered by problems associated with enzymatic and acid digestion together with apparently poor gastrointestinal permeability. This has stimulated interest in the nasal and particularly the pulmonary routes of administration. Adjei, for example, has shown that the peptide leuprolide acetate (1.3 kDa) is readily absorbed from the lungs (3) and phase III clinical trials are presently under way. The early work of Wigley *et al.* (4), demonstrating that insulin (5.8 kDa) induces a systemic response by the pulmonary route, has recently been quantified in experimental animals by Colthorpe *et al.* (5) and further clinical evidence of its efficacy has been demonstrated (6,7). Other smaller proteins that have been shown to be absorbed to different degrees include somatostatin (1.64 kDa) (8) and calcitonin (≈4.5 kDa) (8). However, larger proteins can also be absorbed including parathyroid hormone (9.5 kDa) (8), α-interferon (≈18 kDa) (8) growth hormone (22 kDa) (9,10), α-1-antitrypsin (51 kDa) (11), albumin (68 kDa) (12,13), and even fluorescently labeled catalase (230 kDa) (14).

With the exception of insulin (5), very little information is available on the kinetics of protein transport and there is a definite need to catalog the rate and extent to which they are absorbed. By using the proteins as "probes," information on the mechanism(s) of absorption may be obtained. In this work the pulmonary absorption of recombinant methionyl human granulocyte-colony stimulating factor (18.8 kDa) (G-CSF) has been studied. G-CSF is a polypeptide hematopoietic growth factor that regulates the production of polymorphonuclear leukocytes (PMN) (15). It is presently approved for use in humans by parenteral administration for the treatment of febrile neutropenia and as an adjunct in patients receiving cancer chemotherapy. Chronic administration of this growth factor would benefit from administration by a noninvasive route. It has previously been demonstrated by Platz *et al.* (16) and Nelson *et al.* (17) that G-CSF can induce a systemic response in rodents after intrapulmonary administration. Here, we obtain quantitative information on the dose–response, time course of response, and basic pharmacokinetics of G-CSF after intratracheal instillation in the hamster.

MATERIALS AND METHODS

Dosing Techniques. Male golden syrian hamsters of weights ranging from 100 to 140 g were dosed by intracardiac injection (IC), subcutaneous injection (SC), or intratracheal instillation (IT). For IT, approximately 100 μL of a known concentration of G-CSF was drawn into a 1-ml syringe and weighed. A 19-G 4-in. pipetting needle was attached to the syringe and sheathed by a second 16-G needle attached to a 5-mL syringe filled with 3 mL of air (Fig. 1). Animals were anesthetized first by inhalation of metafane followed by intraperitoneal injection of 400 mg/kg Brevital (0.4 mL, 100 mg/mL, Eli Lilly, Indianapolis, IN). Next, animals were supported, face up, on a slant board at ≈60° angle and a fiber-optic lamp was focused directly on the external neck. The tongue was pulled out and back with forceps to allow direct

[1] Amgen Inc., 8-1-A 215, 1840 Dehavilland Drive, Thousand Oaks, California 91320.

[2] To whom correspondence should be addressed.

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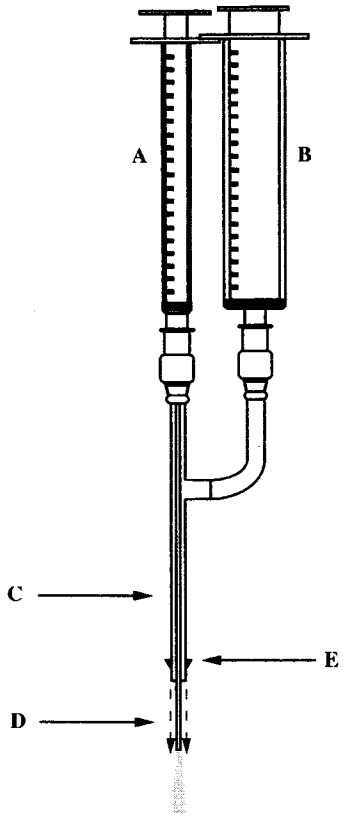

Fig. 1. The dosing mechanism used to administer intratracheal G-CSF. A plunger of a 1-mL syringe (A) was withdrawn ≈0.2 mL and then filled with 100 μL of solution. The plunger was then withdrawn a further 0.2 mL and connected to a 16-G 4-in. pipetting needle (D). The pipetting needle was surrounded by 20-G "sheath" stainless-steel tubing (C) that extended to a point 1 cm from the tip of the pipetting needle. This sheath tubing was in turn connected to silastic tubing and a "t" junction that fixed to a 5-mL empty syringe (B). A small plastic barb (E) surrounding the 20-G tube served the purpose of sealing the larynx during dosing. The plungers of the 5-mL syringe, filled with 3 mL air, and the 1-mL syringe were depressed simultaneously. The sheath air entering the trachea helped to prevent immediate backflushing of dose into the laryngopharynx.

observation of the larynx. Light from the lamp penetrated the trachea enabling the opening of the larynx to be distinguished from the surrounding tissues. The dosing needle was then immediately placed into the trachea. The entry into the trachea could be confirmed by palpating the tracheal cartilage rings as placement of the needle was made. The plungers of the dosing syringe and the 5-mL syringe were then depressed simultaneously. The introduction of "sheath" air helped to prevent backflushing of dose into the throat. Animals were allowed to recover in place for ≈1 min before being returned to cages. The Brevital is short acting and animals were typically awake and mobile 15 min after dosing. Syringes were reweighed so that an accurate determination of the administered dose could be made. IC was performed using a 28-G needle attached to a 1-mL syringe (Microfine IV insulin syringe, Becton & Dickinson, Franklin Lakes, NJ). Prior to dosing, the plunger of the syringe was withdrawn slightly. Blood flow into the hub of the syringe was an indicator that the dose would be injected into the circulation. SC was performed by injecting along the dorsal midline of the hamster.

Quantitation of Intratracheal Instillation. The dose to the lung was quantified by using ^{125}I-labeled human serum albumin (I-125 HSA Isoject, Mallinckrodt Specialty Chemical Co., Paris, KY). Animals (n = 7) were dosed with ≈100 μL of diluted radioactive marker (≈50,000 cpm) in 0.5% (w/v) bovine serum albumin (BSA; Fraction V, Sigma, St. Louis, MO) as described above. Syringes were accurately weighed before and after dosing. Approximately 1 min after dosing animals were sacrificed by CO_2 asphyxiation. The thorax was opened and the lungs were removed. The trachea and esophagus were dissected out and each component was placed in tubes suitable for a gamma counter (Cobra Auto-Gamma, Packard Inst. Co., Downers Grove, IL). In addition, the dosing needle and the mouth of each animal were flushed with 5-mL aliquots of saline (3 × 5 and 2 × 5 mL, respectively) into counting tubes. The amount of free I-125 in solution was assessed using trichloroacetic acid (TCA) precipitation. It was also important to use a dilute solution of BSA with the I-125 HSA marker. Since the filled syringe could not be placed in the gamma counter directly, the dose was determined by the difference in weight before and after dosing. If BSA was absent from the solutions, the I-125 HSA would adhere to the syringe as evidenced by low recovery of dose. This was rectified by the addition of BSA.

Dose–Response. Hamsters were first weighed, anesthetized, and then administered ≈100 μL of either 1, 10, 100, 500, and 1000 μg/kg r-huG-CSF (G-CSF) in aqueous solution by SC (n = 4), IC (n = 5), or IT (n = 5) as described above. The concentration of the unformulated G-CSF stock solution in 1 m*M* HCl was 4 mg/mL (pH 3.25). All dilutions were performed with distilled and filtered water. Syringes were reweighed after use to determine the dose leaving the syringe. Animals were sacrificed 24 hr postadministration and reweighed. One-half milliliter of blood was obtained by intracardiac puncture and placed in tubes containing EDTA. Animal lungs were also lavaged with 12 × 3 mL of chilled phosphate-buffered saline to assess the response of the lung to the G-CSF. A sample of the first pooled 6 mL (2 × 3 mL) was assayed for the presence of G-CSF. Circulating white blood cell (WBC) counts were obtained using an automated blood cell analyzer (Sysmex F800, Microcell Counter, Toa Medical Electronic Co., Kobe, Japan). For differentials, blood smears were placed on slides and subsequently treated with Wright–Giemsa stain prior to microscopic differentiation. Cell counts in lavage fluid were performed using a Coulter counter [Model ZM (CC) and sample stand, Coulter Corp., Hialeah, FL]. Cell differentials in the BAL were performed microscopically after preparing cytospins (Cytospin II, Shandon Co., Pittsburgh, PA).

Time Course. Hamsters (n = 5 for each time point) were intratracheally instilled with 100 μL of a nominal dose of 500 μg/kg G-CSF in water, pH ≈4, and sacrificed at 0, 12, 24, 36, 48, 72, 96, and 120 hr postadministration. Controls (n = 6 at each time point) were dosed with an equivalent volume of vehicle (pH 4.0). Blood samples and lung lavage samples were obtained and treated as described for the dose–response study. Animal weights were also monitored throughout the time course of the study.

Pharmacokinetics. Animals were dosed by IC or by IT

of 500 μg/kg G-CSF and sacrificed at 0.1, 0.5, 1, 3, 6, 12, and 24 hr postadministration (n = 5 at each time point). Blood and lavage fluid were obtained and treated as described above. An additional 1 mL of blood was removed and placed in tubes free of EDTA. Serum, obtained after centrifuging the blood for 10 min at 10,000g, and samples of the lavage fluid were assayed for the presence of G-CSF using an enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN 55413). The pharmacokinetic parameters of clearance (Cl_s), the volume of distribution at steady state (V_{ss}), and the bioavailability [based on the dose leaving the syringe (F) and the dose reaching the lung lobes (F_{lung})] were calculated from the area under the curve vs time (AUC) and the area under the curve * time vs time (AUMC) profiles using standard methods (18). The linear trapezoid rule was used to determine the AUC and AUMC values. Rate constants were determined using a nonlinear least-squares regression program, MINSQ II. The IC kinetics were described by a biexponential function, while the postpeak concentrations following IT were described by a single-exponential function. The volume of distribution of the central compartment (V_c) was estimated from the intracardiac dose/(A + B), where A and B are the coefficients of the biexponential equation. The weighting factor used was $1/y^2$.

RESULTS AND DISCUSSION

Quantitation of Intratracheal Instillation. A small percentage of the I-125 HSA solution was not spun down after TCA precipitation. This fraction is assumed to have remained within the syringe upon dosing by binding to the walls of the syringe. It is also assumed that the absorption of the HSA across the pulmonary barrier would be minimal between the time of dosing and the dissection of the lung. Recovery of the administered dose based on weight was 93.9 ± 7.0%. The quantity of instillate reaching the lung lobes was 73.6 ± 10.5% (44.0 ± 15.8 right lobes, 29.0 ± 7.9 in the left lobes) (Fig. 2). These results indicate that the majority of dose was distributed within the lung lobes.

Dose–Response. The dose–response curve for all three routes of administration is shown in Fig. 3. For the SC and IC data, the dose is determined from the weight of solution leaving the syringe. The IT data are adjusted to account for the dose reaching the lung lobes. The response, as a function of dose, is similar by all the routes of administration and does not plateau even after a dose 200× greater than an expected human dose (5 μg/kg). All the routes of administration cause an influx of PMN to the lung (Fig. 4). There is apparently some correlation with dose but the trend is not clear and the presence of PMN in the BAL is transitory as shown below. There is also the possibility of introducing bacteria from the upper respiratory tract during IT, and this could complicate interpretation of results. G-CSF is not directly chemotactic but will cause margination of the circulating population of PMN as well as the expected stimulation of progenitor cells in bone marrow (19). Margination would be the first step prior to diapedesis into the airways. The migration of PMN is not restricted to the lung and histopathological examination has shown that migration of PMN into most of the major organs and tissues occurs after repeated intratracheal instillation of G-CSF (data not shown). The appearance of neutrophils in tissues does not necessarily predicate an inflammatory response. Long-term exposure to G-CSF in mice induces a chronic granulocytosis in all hematopoietic tissues without obvious changes in the health of the animals (20). In fact, the initial migration of neutrophils into the lower respiratory tract may be a random effect: their increased numbers in the circulation may simply increase the probability of being sequestered within the pulmonary capillaries (21).

Time Course. The response to G-CSF after instillation of a nominal 500 μg/kg dose as a function of time is shown in Fig. 5. An increase in circulating PMN is distinguished 6 hr

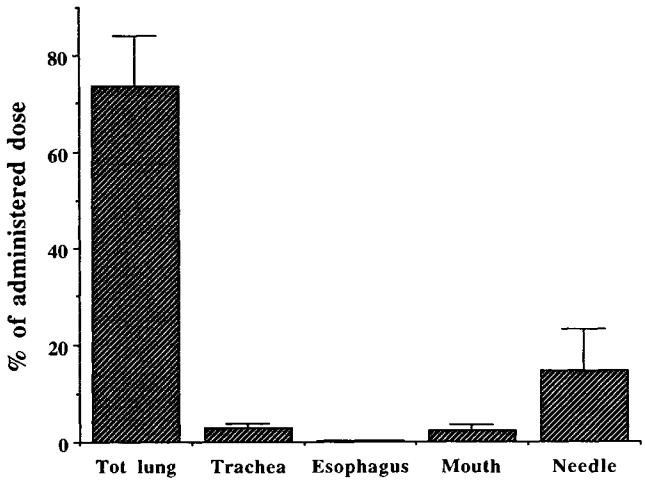

Fig. 2. The distribution of I-125 within the respiratory tract and components of the dosing mechanism after administration of instillate. Error bars are the standard deviation. n = 7.

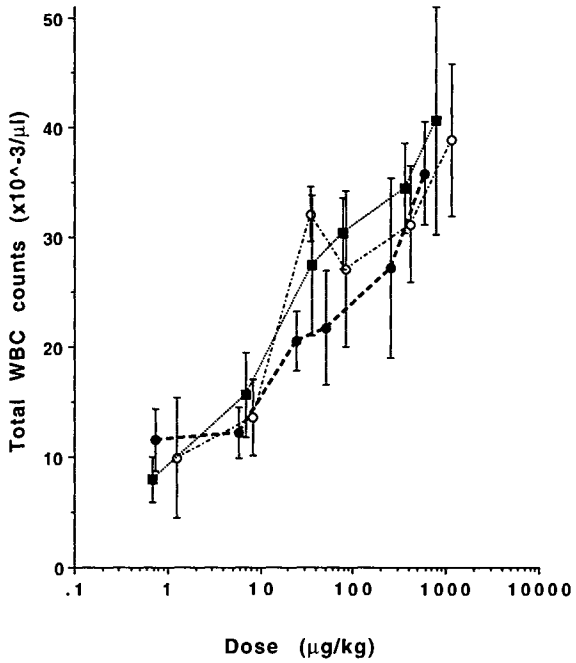

Fig. 3. Dose–response curves after administration of G-CSF by IT (--●--), SC (·····o·····), and IC (——■——). Error bars are the standard deviation. n = 5.

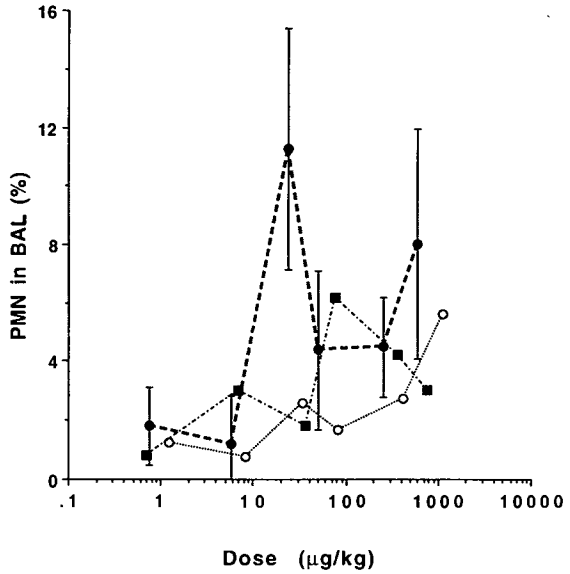

Fig. 4. The recovery of PMN from the BAL as a function of the dose after administration of G-CSF by IT (--●--), SC (·····o·····), and IC (·····■·····). Error bars are the standard deviation. $n = 5$.

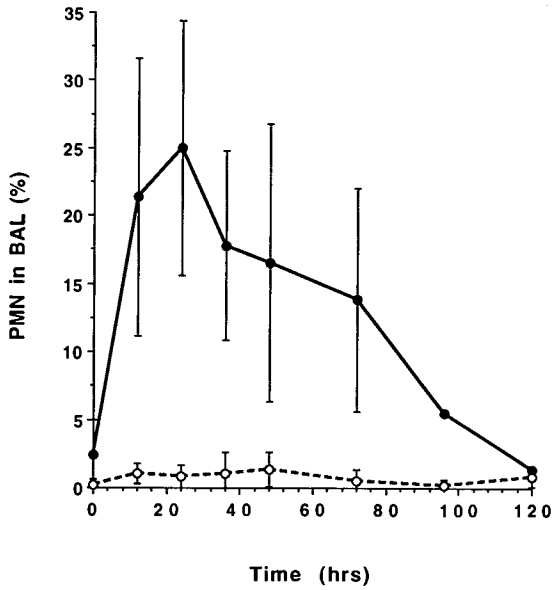

Fig. 6. The percentage of PMN in the BAL as a function of time in animals dosed with 100 μL of 500 μg/kg G-CSF (—●—) and vehicle (--o--) by IT. Error bars are the standard deviation.

postdose. Cell numbers peak at ≈24 hr and return to baseline after 2 days. The increase in cell numbers is restricted to PMN and very little, if any, increase is seen in other WBC types. The corresponding PMN fraction in the BAL is shown in Fig. 6 and the cell numbers and fractions in the blood and BAL are given in Table I. The results illustrate that infiltration of PMN into the airways occur, while PMN levels are elevated in the circulation: their corresponding profiles peaking at approximately the same time after dosing. It should be emphasized that the 500 μg/kg dose is some 100× greater than is normally administered to humans by SC and leukocyte migration at such lower doses is likely to be less significant. There is also little apparent recruitment and increase in alveolar macrophage numbers and the BAL is practically absent of eosinophils and lymphocytes. Some weight loss in the hamsters occurs 24 hr after dosing in both the control and the test animals but weight gain occurs steadily after this time (Table I).

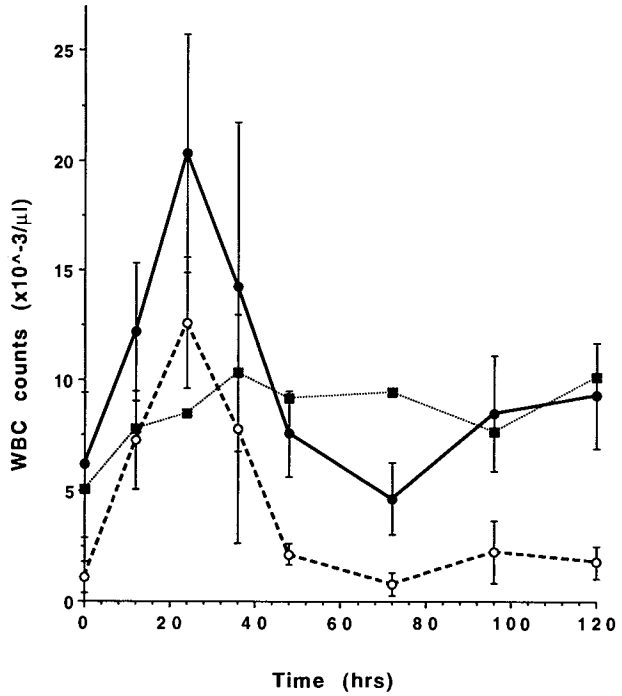

Fig. 5. The time course of response after dosing 100 μL of 500 μg/kg G-CSF by IT. Control animals were dosed with an equivalent volume of vehicle. The total WBC counts (—●—) and PMN counts (--o--) are shown together with the time course profile (·····■·····) for the control results. Error bars are the standard deviation.

Pharmacokinetics. The serum concentrations of G-CSF after pulmonary absorption and intracardiac injection of a 500 μg/kg dose are shown in Fig. 7. A two-compartmental model has been used elsewhere to describe the intravenous kinetics in rats (22) and hamsters (19) and is employed here. The line in Fig. 7 represents the best fit, by nonlinear least-squares regression through the IC data. The rate constants are 1.41 hr^{-1} ($t_{1/2}$ = 0.5 hr) and 0.24 hr^{-1} ($t_{1/2}$ = 2.9 hr) for the α and β phase, respectively. The slope of the terminal phase after intratracheal instillation is 0.27 hr^{-1} ($t_{1/2}$ = 2.6 hr) and is similar to that of the intracardiac data, indicating that the clearance kinetics of G-CSF are similar by both routes of administration. The amount of G-CSF recovered in the lavage fluid as a function of time is shown in Fig. 8 together with the corresponding serum concentrations of G-CSF. The results illustrate that G-CSF is absorbed rapidly from the airways. Some 20% of the absorbable dose is present in the serum 6 min postinstillation. Peak serum levels are obtained 1–2 hr after instillation. Other pharmacokinetic parameters are shown in Table II. From the BAL data, clearance from the lung appears not to follow first-order kinetics. However, these data may be complicated by a number of factors. Instillate is typically distributed to the more central regions of the lung (5). Consequently, some solution may not

Table I. Cell Counts and Differentials in the Blood and BAL Following IT G-CSF or Vehicle

Time (hr)	Blood[a] WtΔ (g)	WBC ($\times 10^{-3}/\mu L$)[b]	PMN (%)	Lymph (%)	BAL[a] WBC ($\times 10^{-6}$)[c]	PMN (%)	Mac (%)
500 μg/kg G-CSF							
0	—	6.2 ± 3.3	18.6	80.2	6.4 ± 3.0	2.4	97.1
12	0.2 ± 2.8	12.2 ± 3.1	59.2	40.2	5.2 ± 0.7	21.4	78.6
24	−1.3 ± 1.5	20.3 ± 5.4	62.7	36.5	7.6 ± 1.3	25.0	74.8
36	2.5 ± 3.6	14.3 ± 7.5	61.0	39.0	6.7 ± 1.9	17.8	82.2
48	2.5 ± 2.3	7.6 ± 2.0	29.4	39.8	10.4 ± 6.4	16.5	82.5
72	3.7 ± 5.3	4.6 ± 1.6	15.5	83.0	8.1 ± 2.7	13.8	85.8
96 (n = 3)	6.7 ± 5.5	8.5 ± 1.6	24.0	75.0	6.2 ± 3.7	5.5	94.5
120 (n = 3)	7.0 ± 4.6	9.3 ± 2.3	19.0	80.0	1.6 ± 0.7	1.3	98.7
Vehicle							
0	—	5.04 ± 1.32	6.0	93.3	8.0 ± 2.6	0.2	99.8
12	3.1 ± 1.5	7.8 ± 1.89	17.0	81.5	7.0 ± 1.4	1.0	98.8
24	−2.5 ± 2.0	8.5 ± 3.7	13.0	86.0	7.2 ± 1.3	0.8	99.2
36	3.7 ± 3.3	10.4 ± 1.2	17.8	81.2	8.9 ± 1.7	1.0	99.0
48	2.2 ± 1.5	9.2 ± 4.3	18.2	79.8	9.0 ± 0.9	1.3	97.0
72	2.5 ± 1.2	9.4 ± 0.3	22.3	77.5	7.8 ± 2.2	0.5	98.0
96	3.2 ± 1.5	7.7 ± 3.3	18.5	81.0	8.9 ± 1.6	0.2	98.5
120	14.2 ± 8.0	10.2 ± 2.4	17.8	81.2	7.2 ± 1.3	0.8	97.0

[a] WtΔ, weight change; PMN, polymorphonuclear leukocyte; Lymph, lymphocyte; Mac, alveolar macrophage. $n \geq 5$ for each time point unless otherwise stated. Remaining fraction of cell types in blood is monocytes and eosinophils. The occasional lymphocyte or eosinophil is seen in the BAL.

[b] Total white blood cell counts in 1 μL of blood.

[c] White blood cell counts in the total recovered lavage fluid.

be available for absorption, and when lavage is carried out this "residual" G-CSF will also be collected and assayed. In addition, respiration may cause instillate that is not immediately available for absorption eventually to reach regions of the lung where absorption can take place. These factors may be partially responsible for the difficulty in interpreting these data in association with a mathematical model. Nevertheless, the G-CSF reaching the lung lobes is absorbed to a significant extent with an apparent bioavailability of 62% (F_{lung}; Table II), and from the dose–response curves it can be seen that little change in bioactivity is occurring as a consequence of pulmonary absorption. Presumably, the lack of complete absorption in this case is due to the deposition patterns of the instillate within the lung lobes and, as has

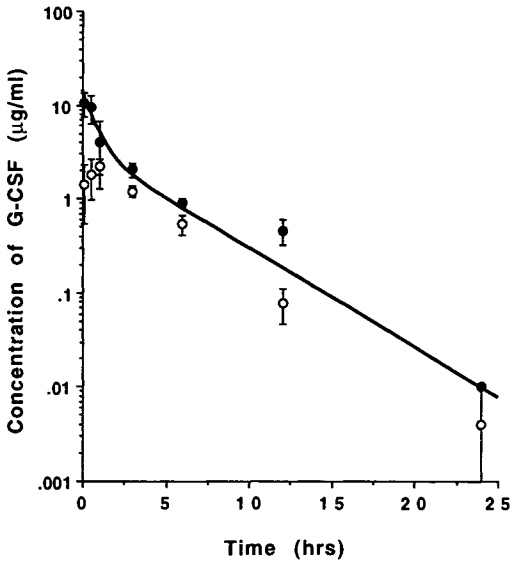

Fig. 7. The concentration of G-CSF in the serum after IT (○) and IC (●) of 500 μg/kg G-CSF. The line represents the best fit of a biexponential equation to the IC data by nonlinear least-squares regression.

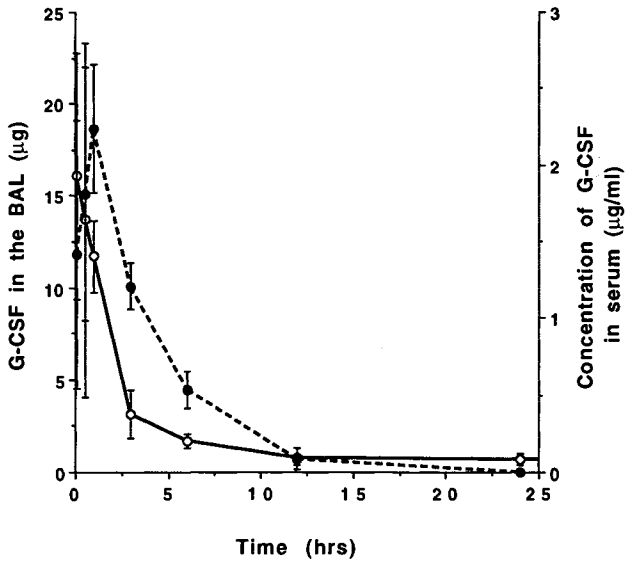

Fig. 8. The concentration of G-CSF in the serum after IT (--●--) and the corresponding amounts of G-CSF found in the BAL (—○—) as a function of time. The error bars are the standard deviation. n = 5.

Table II. Pharmacokinetic Parameters for G-CSF in the Hamster After IC and IT Administration[a]

Parameter	IC	IT
Dose (μg)	48.7	42.4
F (%) dose[b]	100	45.9
Dose to lung (μg)	—	31.4
F_{lung} (%) lung[c]	—	62.0
$t_{1/2}$ (hr) α	0.5	—
$t_{1/2}$ (hr) β	2.9	—
$t_{1/2}$ (hr) postpeak	—	2.6
AUC (μg · hr mL^{-1})	25.3	10.1
AUMC (μg · hr^2 mL^{-1})	99.8	36.4
Cl_s (mL/hr)	1.9	—
V_{ss} (mL)	7.2	—
V_c (mL)	3.4	—

[a] n = 5 for each time point. Dose is the mean dose administered to all animals.
[b] The available percentage of the dose administered from the syringe, with the available dose by IC defined as 100%.
[c] The available percentage of the dose administered to the lung lobes.

been found after aerosolization of insulin to rabbits (5), this can be expected to improve with administration of an aqueous aerosol. The fact that a higher bioavailability (F; Table II) is achieved with the G-CSF vs insulin (45.9 vs 5.6) by intratracheal instillation is probably due to a combination of factors including the nature of the proteins, differences in dosing technique, metabolism, and, in this case, the size of the lungs. These results also illustrate the difficulty associated with assigning sensible values to describe pulmonary bioavailability which is as much technique (e.g., instillation vs aerosolization) and device dependent, as it is dependent upon the nature of the drug or formulation. This is also well illustrated by Adjei (23), who found marked differences in the absorption of leuprolide according to the depth of drug deposition within the respiratory tract.

If absolute bioavailability for the lung is defined as the fraction of drug that reaches the circulation after being deposited at a site from which it can be absorbed, then it is clear that this parameter is impractical to measure because there is no method of distinguishing between the absorbing and the nonabsorbing sites in the lung. It is, however, possible to ascertain comparative *bio*availability as long as similar dosing techniques, species, and perhaps a "standard" drug are employed. The standard, of course, should be inexpensive, easily assayable, nontoxic, and not be metabolized. Ultimately, the ratios of the AUC's for the unknown vs standard may prove more useful for investigators than attempts to ascertain individual absolute bioavailabilities.

It is worth speculating on the mechanism or mechanisms whereby proteins transfer across the alveolar epithelium into the circulation. Evidence of zonula occludens or "tight" junctions between the type I cells, that would restrict large solutes, suggests that the majority of large compounds must transfer directly across the epithelium (24,25). With the demonstration, by ultrastructural techniques, that vesicles exist within type I cells, it can be argued that their presence provides the physical means of transportation. However, very little knowledge has accumulated on how these vesicles are regulated and if they regulate the delivery of macromolecules. Some information can again be gleaned from ultrastructural studies where it has been shown that cationized ferritin (CF; >445 kDa) is endocytosed by plasmalemmal vesicles and evidence suggests that CF is released at abluminal sites (25). It is unfortunate that few proteins of therapeutic interest lend themselves to ready detection in lung tissues by electron microscopy. The immunohistochemical techniques involved in detecting the presence of proteins within specific regions of lung tissue are both difficult and tedious and antibodies may not readily be available for many proteins. However, this approach will be useful in the future. For the present, important information on the nature of the transport mechanisms can be found by carefully studying the pulmonary pharmacokinetics of the increasing number of therapeutic proteins that are available.

As well as considering how proteins are absorbed across the pulmonary barrier, it is equally intriguing to consider the physiological reason for absorption. G-CSF together with chemotactic factors derived from the macrophages and cell debris such as leukotriene B_4 (LTB_4) and interleukin-1 (IL-1) are released in response to a pulmonary bacterial infection or acute lung injury (26–28). One can speculate that there may be a pathway for one or more of these factors to penetrate the pulmonary barrier and stimulate circulating PMN so that margination and diapedesis can take place.

In summary, the pharmacological response and pharmacokinetics of G-CSF have been studied after administration of a single dose by several routes of administration. The response to G-CSF by intratracheal instillation is similar to that obtained after parenteral administration of equivalent doses indicating that the protein is absorbed to a significant extent. The transfer of G-CSF across the pulmonary barrier proceeds rapidly by an unknown mechanism. Further work is needed to elucidate the nature of the transport process and, from a clinical standpoint, to assess safety before human studies can proceed.

ACKNOWLEDGMENTS

The constructive comments of Steve Nelson and coworkers at LSU Medical Center and Colin Pitt of Amgen are gratefully acknowledged.

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