Systemic Absorption and Activity of Recombinant Consensus Interferons After Intratracheal Instillation and Aerosol Administration

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Purpose. The pulmonary pharmacokinetics and bioactivity of *E. coli* derived recombinant consensus interferon (CIFN) and a modified lactose-conjugated consensus interferon (LacCIFN) were evaluated in animals.

Methods. Estimated doses of 20 and 100 μg/kg of the interferons were administered to anesthetized rats by aerosol via ultrasonic nebulizer as well as intravenous injection. Rats also received nominal doses of 50 and 200 μg/kg via intratracheal instillation (IT). Hamsters were treated with interferon via various routes including IT. The effectiveness of treatment was assessed by the resistance to development of hind leg paralysis following infection with encephalomyocarditis virus.

Results. Significant amounts of CIFN and LacCIFN were found in rat plasma after aerosol administration. Peak plasma levels occurred ≈25–30 minutes with estimated bioavailabilities approaching 70%. Absorption of CIFN was rate limiting and plasma levels were detectable 12 hr post-dose. The CIFN at IT doses as low as 5 μg/kg was effective at reducing paralysis in hamsters but protection was variable and doses of up to 100 μg/kg were not 100% effective.

Conclusions. Despite the incomplete protection, the results demonstrate the feasibility of treating systemic viral infections with interferon administered directly to the lungs.

KEY WORDS: aerosols; consensus interferon; pulmonary drug delivery; lungs; proteins.

INTRODUCTION

Recombinant-methionyl interferon-consensus (CIFN) is a type I interferon produced in *E. coli* through recombinant DNA techniques developed at Amgen (1). The protein is a consensus of most known human alpha-interferon subtypes (2). CIFN is presently being developed at Amgen for the treatment of hepatitis C. For such a disease condition, chronic therapy is necessary and patients would likely benefit from the availability of a non-invasive form of treatment. Fortunately, there is some evidence that systemic absorption of interferons does occur after inhalation. Bocci and colleagues (3) have noted the pulmonary absorption and catabolism of alpha-interferon in an isolated lung preparation. While Kinnula and coworkers (4) have demonstrated that high doses of aerosolized alpha-interferon result in systemic side effects similar to those observed after injection of the interferon. Recently, Patton and coworkers (5) purposely studied the pulmonary absorption of an alpha-interferon in the rat with a view to systemic delivery of the protein. They were able to demonstrate plasma levels of alpha-interferon in the rat up to 9 hrs after intratracheal instillation.

The objective of the present investigation was to evaluate the systemic delivery of recombinant consensus interferon (CIFN) and a modified consensus interferon (LacCIFN) after intra-pulmonary administration. The pharmacokinetics of the interferons after aerosol administration to the rat and the biological activity of intratracheally instilled interferon against an encephalomyocarditis viral infection in hamsters (6) have been examined.

MATERIALS

CIFN (MW 19.5 kDa) was obtained from Amgen manufacturing (Lot #T424) at a concentration of 0.2 mg/ml (absorptivity at 280 nm = 1.14 cm^2/mg) in a phosphate buffered saline (100 mM sodium chloride and 25 mM sodium phosphate, pH 7.0). A neoglycosylated analogue of CIFN was synthesized and purified as described below. Dubellco's phosphate buffered saline (PBS) pH7.0 without calcium and magnesium (Gibco BRL, Life Technologies, Grand Island, New York) was used for the dilution of all dosing solutions. N-acetyl-L-cysteine, bacitracin (69000 units/g) and tetrasodium ethylenediamine-tetraacetic acid were all obtained from Sigma Chemical Co. (St. Louis, Missouri) and used without further modification. Encephalomyocarditis virus (EMCV) was propagated at Amgen in a murine L929 cell line and subsequently purified and diluted to a concentration of 20000 plaque forming units/ml.

METHODS

Derivatized Consensus Interferon

Details of the synthesis and characterization of the LacCIFN is to be published elsewhere (7). In brief, β-D-lactose was conjugated through the ϵ-NH_2 lysines and N-terminal amine of CIFN by reacting an optimized aqueous mixture of protein and sugar in the presence of sodium cyanoborohydrate at pH 7 for 4 days. The derivatized protein was purified by fast protein liquid chromatography (FPLC) and characterized by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), isoelectric focusing gels (IEF), size exclusion chromatography (SEC) and MALDI-TOF mass spectrometry. The derivatized product consisted of CIFN conjugated to an average of 5 lactose residues and demonstrated approximately 60% of the potency of the unmodified CIFN as determined using an *in vitro* bioassay (8).

Stability of Proteins to Aerosolization

Details of the stability of CIFN to ultrasonic nebulization are described elsewhere (8). A check of the stability of the LacCIFN to aerosolization was also performed. A volume of 5 ml modified interferon was ultrasonically nebulized for 20 minutes at a concentration of 1 mg/ml. The residual fluid in the nebulizer reservoir was then analyzed by SEC; ion exchange chromatography (IEC); SDS-PAGE and IEF.

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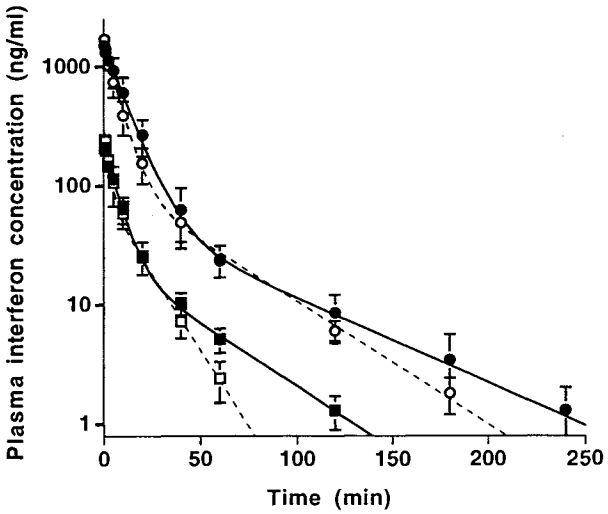

Fig. 1. Plasma interferon concentration vs. time curves after intravenous injection of 20 (□) and 100 (○) μg/kg LacCIFN and 20 (■) and 100 (●) μg/kg CIFN to the rat. The lines represent the best fits of the LacCIFN (----) and CIFN (—) data to a biexponential equation (eq. 1). Data shown is the mean ± standard deviation (n ≥ 5).

No changes in the structure of the derivatized protein were apparent after nebulization.

Animal Cannulation and Dosing

Pathogen free Sprague-Dawley rats (≈250 g) and hamsters (≈150 g) were received from Charles River Laboratories and quarantined one week before being used in studies. Food and water was provided *ad libitum*. Rats were anesthetized with ketamine (85 mg/kg) and xylazine (15 mg/kg) by intramuscular injection. A silastic cannula was then implanted into the jugular vein for blood sampling as described previously (9). Rats were allowed 24 hours to recover before initiating experiments.

Intravenous Injection. Rats were anesthetized by isoflurane and anesthesia was maintained using nose cones containing metafane. Doses of 20 or 100 μg/kg were administered by intravenous injection (IV) via the penile vein. Note that doses of LacCIFN are expressed as the amount of protein only and not protein + sugar.

Intratracheal Instillation and Insufflation. The techniques of intratracheal instillation (IT) and insufflation (IF) used in the studies have been described elsewhere (10, 11). Rats were instilled with a volume of 250 μl and hamsters with 150 μl. Estimates of the dose reaching the lung lobes via instillation have previously been determined for both species at 73.6% for the hamster (10) and 62.4% for the rat (12). Hamsters were insufflated a nominal dose of 200 μg/kg CIFN (≈30 μg pure CIFN mixed with 2 mg spray-dried mannitol).

Aerosol Administration. Paired rats were anesthetized with ketamine and xylazine as described above and intubated with a small teflon catheter (The sheath of a 1.5in, 18G Quick-Cath; Baxter Healthcare Corp. McGaw Pk., Illinois) surrounded with a short piece of silastic ≈2cm from the distal tip. The cannulae were connected to an apparatus consisting of a small animal Harvard ventilator (2.5 ml tidal volume per rat; 60 beats per minute) the outlet of which was connected directly to the vent of a DeVilbiss, "Aerosonic" ultrasonic nebulizer. The nebulizer was modified to accomodate a heat exchanger which was adjusted to maintain the dosing solution below 30°C during the 20 min dosing period. This prevented thermal denaturation of CIFN and LacCIFN (11). Aerosol emerging from the device was passed through a silica gel drying chamber before reaching the rats. The volume of dosing solution administered by aerosol has been estimated at 25.5 μl using solutions of protein spiked with Tc-99m labelled human serum albumin (Tc-99m HSA) (9).

Virus Infection. The anti-viral effects of CIFN against EMCV have previously been established after intraperitoneal injection (6). Hamsters were inoculated with 100 μl of virus (20000 plaque forming units/ml) by intraperitoneal injection (IP). Inoculation of the virus was carried out six hours after dosing interferon. Infection with virus alone results in 90 to 100% of animals developed hind leg paralysis within 2 weeks. After dosing virus, the hamsters were monitored daily for indications of infection. Once apparent, the hamsters were immediately euthanized by CO_2 gassing. Effectiveness of interferon treatment was based upon the % of a given treatment group that did not exhibit hind leg paralysis two weeks after dosing.

Sampling and Interferon Assay

Blood samples of 200 μl were removed from the jugular cannula of rats at predetermined time points after dosing with interferon. Samples were placed in EDTA coated tubes and held on ice until centrifuged at 10000 × g for 10 min. The plasma was then decanted and stored in freezer tubes at −80°C until assayed. Detection of the interferons in plasma was performed using an enzyme immuno assay developed at

Table I. Pharmacokinetic Parameters After Intravenous Bolus Doses of Interferons to the Rat[a]

Dose (μg/kg)	n	$t_{1/2}\alpha$ (min)	$t_{1/2}\beta$ (min)	Cl_p (ml/min/kg)	MRT (min)	V_{ss} (ml/kg)
CIFN						
20	6	4.9 ± 1.1	30.0 ± 6.7	6.1 ± 1.2	17.9 ± 2.2	107 ± 15
100	5	7.1 ± 1.5	43.7 ± 8.4	5.7 ± 2.0	20.1 ± 4.8	109 ± 27
LacCIFN						
20	6	2.4 ± 1.2	12.0 ± 1.6	9.4 ± 2.1	17.3 ± 1.7	161 ± 24
100	6	4.6 ± 2.0	33.6 ± 4.9	7.6 ± 1.5	18.2 ± 0.7	138 ± 25

[a] The results shown are the mean data ± standard deviation.

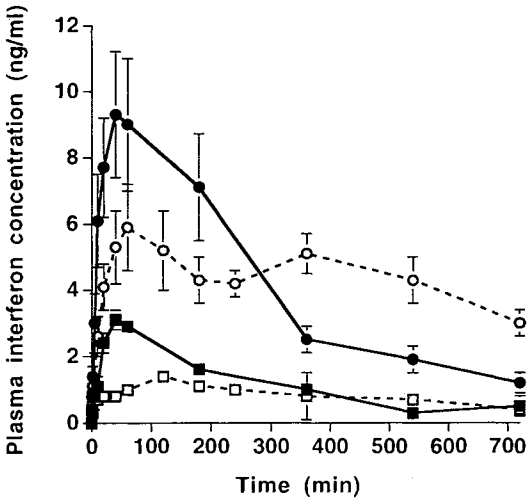

Fig. 2. Plasma interferon concentration vs. time curves resulting from the intratracheal instillation of 50 (□) and 200 (○) μg/kg CIFN + bacitracin (1 mg/kg in PBS) and 50 (■) and 200 (●) μg/kg CIFN alone to the rat. Data shown is the mean ± standard deviation (n ≥ 5).

Amgen. Dosing solutions of each interferon were used to generate calibration curves by spiking known amounts of CIFN or LacCIFN into rat plasma. A known standard (lot #T414 CIFN) was included in all assays.

Acute Toxicity

To assess whether CIFN might cause acute inflammatory effects within the lungs of the hamsters, groups of six animals were administered a range of doses (0, 1, 20, 200 and 1000 μ/kg) of CIFN in PBS by intratracheal instillation. As a positive control, hamsters were also administered 200 μg/kg via IP injection. At 24 hr post-dose, hamsters were sacrificed by CO_2 exposure. The lungs of half the hamsters in each dosing group were immediately lavaged with 8 × 2 ml of PBS. The bronchoalveolar lavage fluid (BAL) was pooled and then centrifuged (300 × g, 10 min, ×2). After the first centrifugation the cell pellet was reconstituted and washed with 10 ml PBS. After the next centrifugation, the pellets were resuspended in 0.5 ml PBS. Cytospins were then prepared by diluting 20 μl of sample 20 × with PBS and then adding 100 μl of the mixture to cytospin holders (Cytospin II; Shandon Co., Pittsburgh, Pennsylvania). The solutions were then centrifuged for 5 minutes at 800 × g. Cells retained on the slides were fixed and then stained with Diff-Quick solutions I and II (Baxter Healthcare Corp., Miami, Florida). Differential cell counts were based upon the mean of duplicate counts of 100 cells on the slides. Total cell counts were obtained from the resuspended pellet using an electrozone particle counter (Elzone 282PC, Particle Data Inc., Elmhurst, Illinois). A total of three counts were made for each sample after diluting the resuspended samples by 1000 × in 20 ml PBS containing a red blood cell lysing reagent.

The remaining hamster lungs were fixed via airway perfusion. Tissue samples were removed from various regions of the lungs and slides were subsequently prepared and stained with hematoxylin and eosin. Slides were microscopically analyzed for histological abnormalities.

Pharmacokinetic Analysis

Calculations were performed using the computer program Scientist (Micromath Software, Salt Lake City, Utah). Plasma protein concentration vs. time curves after IV were fit to a two-compartment model by nonlinear least-squares regression using weighting of 1/(mean variance of the 'y' observation). Pharmacokinetic parameters were obtained by curve fitting the data for each rat using the biexponential equation,

$$C(t) = (D/V_c)^*[Ae^{-\alpha t} + Be^{-\beta t}] \quad (1)$$

where D is the dose, V_c is the volume of the central compartment, A and B are the zero-time intercepts corresponding to the first and second exponential terms respectively, and α and β are the first order rate constants for the initial and secondary phases of elimination respectively. The half-lives for the α and β phases were determined using $t_{1/2}$ α =

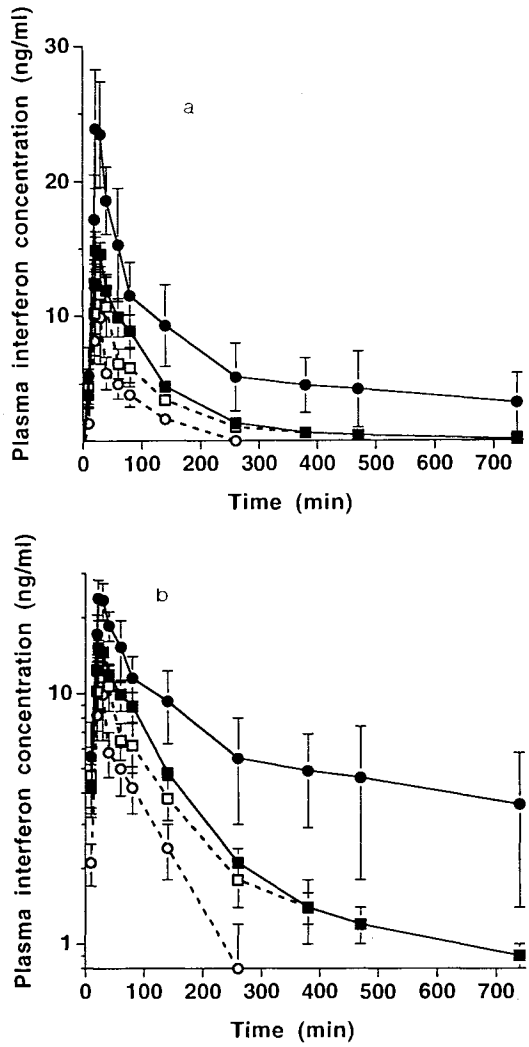

Fig. 3. (a) The plasma interferon concentration vs. time curves obtained after aerosol administration of 20 (□) and 100 (○) μg/kg LacCIFN and 20 (■) and 100 (●) μg/kg CIFN to the rat. (b) The same plots shown on semi-log coordinates to illustrate the nonlinearity of the CIFN post-peak absorptive phases. Data shown is the mean ± standard deviation.

$(\ln 2)/\alpha$, and $t_{1/2}\ \beta = (\ln 2)/\beta$. The area under the plasma protein concentration vs. time curves (AUC) and the area under the first moment curves (AUMC) were calculated using the log-linear trapezoid rule for the intravenous, aerosol and IT data. The clearance, Cl_p, volume of distribution at steady state V_{ss}, and mean residence time were determined using noncompartmental analysis using

$$Cl_p = D/AUC \quad (2)$$

$$MRT = AUMC/AUC \quad (3)$$

and

$$V_{ss} = MRT^*Cl_p \quad (4)$$

The mean absorption time (MAT) for the aerosol data were calculated using the equation

$$MAT = MRT(X) - MRT(IV) - \tau/2 \quad (5)$$

where MRT(X) is the mean residence times after aerosol or IT delivery, MRT(IV) is the mean residence time after intravenous injection and τ is the dosing period ($\tau = 0$ for bolus IT administration). Bioavailabilities, F, were calculated using the dose normalized area under the curves

$$F = AUC(X)/AUC(IV)^*D(IV)/D(X) \quad (6)$$

where AUC(X) and D(X) refer to the area under the curves and dose respectively after aerosol or IT delivery.

RESULTS

Intravenous Pharmacokinetics. Plasma concentration vs. time curves are shown in Figure 1 after intravenous doses of 20 and 100 μg/kg of CIFN and LacCIFN to the rat. Results for the pharmacokinetic analysis of the data are shown in Table 1. The clearance of the LacCIFN is significantly faster than that of CIFN in the rat at the lower dose ($\alpha = 0.05$; $p = 0.101$, 100 μg/kg; $p < 0.01$, 20 μg/kg dose). The V_{ss} of the derivatized interferon is also greater than that of CIFN, a trend that is even more pronounced in the hamster (10).

Pulmonary Pharmacokinetics. Intratracheal instillation of CIFN results in detectable protein in the circulation with peak levels being observed between 37 and 76 minutes after dosing (Figure 2). As there is some evidence to suggest that catabolism of alpha-interferons occurs within the lungs (3), IT experiments with CIFN were repeated with the addition of 1 mg/kg bacitracin to the dosing solutions. Bacitracin is a known cytosolic and membrane bound protease inhibitor at this dose level (13). However, the results do not demonstrate any significant difference in bioavailabilities (Table 2) although the presence of the bacitracin may be influencing the absorption of the CIFN as shown in Figure 2. It is not possible to draw further conclusions given the variable absorption that is observed after instillation.

The concentration vs. time profiles of the instilled interferons differ substantially from those observed after aerosol administration (Figure 3). The T_{max} values are reached earlier and C_{max} values are higher for the interferons administered via aerosol (Table 2). Bioavailabilities are also higher suggesting that the rate and extent of absorption of CIFN is greater via aerosol than after IT (Table 2). This result is not unexpected and increased bioavailabilities after aerosol administration have been observed with several proteins including granulocyte colony-stimulating factor (9), growth hormone (14) and insulin (15).

Aerosol administration of the LacCIFN is also compared to that of the CIFN (Table 2). The plasma levels of LacCIFN after both the 20 and 100 μg/kg dose do not reach those obtained for similar doses of CIFN and the bioavailability for LacCIFN at the high dose is significantly less than that achieved with an equivalent dose of CIFN. This suggests that the lower plasma levels are not simply a reflection of the faster circulatory clearance of the glycosylated protein.

Interferon Bioactivity. To demonstrate bioactivity of CIFN against EMCV, hamsters were treated with interferon at doses ranging from 0 to 200 μg/kg by several routes of administration (IT; IP and subcutaneous injection (SC)). Protection against the virus is observed after IT signifying that CIFN has been absorbed and retains biological activity (Figure 4). There is significant variability in the response for each route of administration making it difficult to compare

Table II. Pulmonary Pharmacokinetic Parameters for Rats Dosed Interferon by IT and Aerosol[a]

Dose (μg/kg)	Route	n	C_{max} (ng/ml)	T_{max} (min)	MRT (min)	MAT (min)	F[b]
CIFN							
50	IT	7	3.3 ± 0.8	37.1 ± 13.8	219 ± 72	201	18.9 ± 3.7
200	IT	8	10.3 ± 5.7	60.0 ± 4.9	280 ± 101	263	12.6 ± 3.0
50 + bac[c]	IT	5	2.1 ± 1.3	40.0 ± 16.3	311 ± 33	294	14.3 ± 3.7
200 + bac	IT	5	6.6 ± 2.4	76.0 ± 60.7	366 ± 29	348	18.2 ± 5.1
20	aerosol	6	15.3 ± 2.8	27.7 ± 7.2	249 ± 31	252	69.5 ± 10.4
100	aerosol	6	26.6 ± 10.1	24.7 ± 4.1	252 ± 125	224	30.9 ± 29.6
LacCIFN							
20	aerosol	6	15.3 ± 6.9	27.7 ± 7.2	158 ± 51	130	53.7 ± 30.0
100	aerosol	7	12.8 ± 8.3	31.4 ± 21.6	109 ± 41	81	6.0 ± 2.9

[a] The results shown are the mean data ± standard deviation.
[b] Bioavailability estimates were calculated from the dose normalized AUC values using eq. 6. The AUC of the 20 μg/kg IV dose was used as the reference. The F values shown are adjusted to reflect the estimated dose to the lung lobes.
[c] Interferon was administered simultaneously with 1 mg/kg bacitracin in PBS.

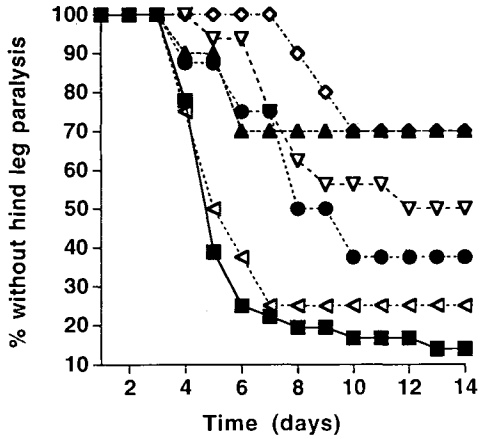

Fig. 4. Plots illustrating the influence of intratracheally instilled CIFN on the development of hind leg paralysis in hamsters after infection with 2000 pfu of EMCV. [0 (■), 1 (◁), 5 (●), 10 (▲), 20 (▽), 100 (◇) μg/kg CIFN).

one method against the other. However, the trend follows that IP > SC >= IT (Table 3). The results shown in Table 3 are presented as a) the percentage of animals not showing paralysis after 2 weeks of infection and b) the percentage of the area under the 'paralysis' curve for the treated hamsters with reference to the maximum area under the curve possible.

It was also possible to obtain protection against the virus using a high nominal dose of insufflated spray-dried CIFN (Table 3) indicating that a solid dosage form of CIFN can retain biological activity The use of 1mg/kg bacitracin, EDTA or N-acetylcysteine simultaneously dosed with 10 μg/kg CIFN does not reduce paralysis numbers.

Acute Toxicity. The BAL and lung tissue were examined 24 hrs after instilling hamsters with CIFN doses ranging from 0 to 1 mg/kg. The results of cell differentials and counts obtained from BAL are shown in Table 4. There is no apparent direct inflammatory or immune response to the CIFN at doses of up to 1 mg/kg as indicated from the lack of leukocyte infiltration and minimal presence of lymphocytes or eosinophils in the BAL. Tissue slides concur with the BAL findings and no gross visual changes are noticeable in the lung airway and alveolar tissues compared with control animals receiving the buffer alone (data not shown).

DISCUSSION

Differences between the pharmacokinetics of LacCIFN and CIFN are not unexpected as the lactose conjugated interferon was specifically designed to target the asialoglycoprotein binding receptor present on hepatocytes with a view to targeting the interferon at the site of infection (hepatitis C). These binding receptors have known affinity for galactose-terminated triantennary complex oligosaccharides (16) and thus could contribute to the clearance of the lactose-conjugated moiety from the blood. Evidence of preferential uptake of LacCIFN by the liver has been shown in hamsters using I-125 radio-labelled material (7). Thus, the net effect of increased uptake by liver hepatocytes will be an increase in the apparent volume of distribution for a given dose as observed.

The results show marked differences in the plasma interferon concentration vs. time curves after aerosol delivery and intratracheal instillation. This is partially explained on the basis of the distribution of protein deposited in the lungs. Instillation is known to result in a more central and inhomogeneous deposition of solutions in the lungs whereas aerosol delivery results in more homogeneous and peripheral deposition pattern (17). Aerosol deposition would be expected to expose the proteins to a greater surface area for absorption as well as shorter diffusional distances between airway surface and the circulation. Thus, the higher observed flux of interferons after aerosol administration might be expected.

The pulmonary absorption of both interferons result in detectable plasma levels beyond 12 hr by IT or aerosol delivery. Whereas their levels fall below detectable levels ≈6 hr after IV injection of a 100 μg/kg dose. Thus absorption appears to be rate limiting. However, the post-peak absorptive phases do not exhibit simple first-order absorption and appear biphasic suggesting that more than one process is involved with the transfer of protein into the circulation (Figure 3B). This biphasic post-peak curvature has also been seen with recombinant human granulocyte colony-stimulating factor (rhG-CSF) after aerosol delivery using

Table III. Influence of CIFN on the Induction of Hind Leg Paralysis in Hamsters Infected with EMCV

Treatment	n	Unparalyzed (%)[a]	AUC (%)[b]
PBS + virus (2×10^3 pfu)	36	13.9	29.5
Intratracheal instillation (μg/kg)			
PBS (no virus)	10	100	100
1	8	25.0	36.4
5	8	37.5	58.5
10	10	70.0	75.0
20	16	50.0	69.9
100	10	70.0	90.5
200 (powder)[c]	8	75.0	81.8
10 + bacitracin (1 mg/kg)	10	60.0	67.3
10 + EDTA (1 mg/kg)	10	80.0	88.2
10 + N-acetylcysteine (100 mg/kg, pH 7.0)	10	70.0	73.2
Subcutaneous injection (μg/kg)			
1	10	30.0	49.5
10	10	70.0	81.4
20	10	70.0	77.7
100	10	90.0	93.2
Intraperitoneal injection (μg/kg)			
1	8	87.5	93.7
10	18	61.1	76.5
20	8	100	100

[a] The % of the treatment group that did not exhibit hind leg paralysis two weeks after being infected with 2000 pfu of EMCV via intraperitoneal injection.

[b] The % of the area under the 'paralysis' curve of the treated hamsters to that of the area under the curve for the hamsters receiving only buffer (100%). Areas were calculated between days 3 and 14 post-dose. No animals succumbed to virus beyond 14 days.

[c] A nominal dose of 200 μg/kg CIFN in a spray dried powder formulation was insufflated to the hamsters.

Table IV. Effects of CIFN on Bronchoalveolar Lavage Fluid Cell Content

CIFN dose[a] (μg/kg)	Cell differential (%)			Total counts ($\times 10^6$)
	Macrophages	Neutrophils	Lymphocytes	
0 (buffer)	4.0 ± 2.6	96.0 ± 2.6	—	8.9 ± 2.9
1	3.7 ± 2.9	96.3 ± 2.9	—	4.4 ± 0.5
20	8.7 ± 9.9	91.0 ± 9.6	0.3 ± 0.6	9.3 ± 3.9
200	3.3 ± 0.6	96.7 ± 0.6	—	7.0 ± 1.1
1000	6.0 ± 3.6	94.0 ± 3.6	—	4.4 ± 2.2
200 (IP)	1.0 ± 1.7	99.0 ± 1.7	—	5.1 ± 2.9

[a] All CIFN solution doses were administered by intratracheal instillation to the hamster unless otherwise stated. Data is presented as the mean ± standard deviation (n = 3).

similar methodology (12). In the case of rhG-CSF however, the terminal slope was similar to that observed after IV injection and the flip-flop kinetics seen with the interferons were not apparent.

It is difficult to interpret the absorption data in terms of specific mechanisms, but there is general agreement that the alveolar epithelium is the major rate limiting barrier to absorption of macromolecules (18). There is also ultrastructural evidence to suggest that vesicles exist in alveolar type I (19, 20) and type II cells (21) and that transcytosis could be mediated via these organelles. Assuming that this is true, the interferons could equally be transported across the 'thick' or 'thin' walls of the cells. Subsequent transport and diffusion could then occur directly across the capillary endothelium into the circulation providing the 'rapid' component of the absorptive phase. Or, if presenting to the interstitial matrix, hydraulic and pressure gradients could force uptake into the lymphatic system which presumably could provide a 'slow' release of protein into the circulation over time (22).

At least some of the interferon reached the circulation retains bioactivity after pulmonary absorption as evidenced from the protection endowed on animals infected with EMCV. The addition of bacitracin at the dose employed did not increase the extent of absorption after intratracheal instillation in the rat and did not change the outcome of the viral infection. If proteolytic breakdown of the protein was rapid and significant, then differences in the extent of absorption should have been observed. This was not the case and absorption of CIFN continues after 12 hrs in the absence of protease inhibitor suggesting that the protein exhibits some inherent stability in the lung microenvironment. This result is in contrast to the observed breakdown of alpha-interferon in an isolated lung preparation described by Bocci and colleagues (3). Although, no direct comparison has been made it is possible that the CIFN may be more resistant to intra-pulmonary proteolytic breakdown than alpha-interferon. Differences in the appearance of natural and recombinant alpha-interferon in the circulation of patients after inhalation has also been observed supporting the contention that differences in the pulmonary absorption of similar interferons can occur (23).

In summary, this study has provided evidence for the pulmonary absorption of CIFN and lacCIFN in rodents. Both proteins retain a significant level of bioactivity after absorption and do not cause any overt adverse effects in the lungs of hamsters at doses of up to 1 mg/kg. The results suggest that the development of an inhalation dosage form might be a feasible and useful approach where chronic treatment of a systemic viral infection is necessary. Further questions to address include the long term effects of the interferon on the lung tissue and a similar study in higher animals is warranted to provide information on bioavailability and thus the dosing requirements for possible human studies.

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