Inhalation Scintigraphy with Iodine-123-Labeled Interferon Gamma-1b: Pulmonary Deposition and Dose Escalation Study in Healthy Volunteers

Irene Virgolini, Amir Kurtaran, Maria Leimer, Peter Smith-Jones, Hermine Agis, Peter Angelberger, Kurt Kletter, Peter Valent, Werner Linkesch and Hans-Georg Eichler

Departments of Clinical Pharmacology, Nuclear Medicine and Internal Medicine I, University of Vienna, Vienna, and Department of Radiochemistry, Seibersdorf Research Center, Seibersdorf, Austria

Recent studies have suggested that recombinant interferon gamma (IFNg) may be useful in the treatment of various respiratory diseases, such as chronic inflammatory disease. This study was undertaken to investigate the dose response of escalating doses of inhaled ^{123}I-labeled IFNg (^{123}I-IFNg) and its safety, biodistribution and radiation absorbed doses in healthy volunteers. **Methods:** IFNg was labeled with ^{123}I to produce a specific activity of 1800 MBq/mg of IFNg. The biological activities of ^{123}I-IFNg, nebulized ^{123}I-IFNg and unlabeled IFNg were evaluated in various functional in vitro tests. Ten healthy volunteers were enrolled in the in vivo dose escalation study (180 MBq of ^{123}I-IFNg diluted with 0.1–2 mg of INFg). Inhalation scintigraphy, using a Pari-Master nebulizer, was performed for up to 37 min, during which dynamic posterior images of the lungs were obtained. Whole-body scanning was performed at various time points up to 24 hr postinjection, for biodistribution and dosimetry purposes. Blood, urine and feces were also collected over this 24-hr period. Lung perfusion scintigraphy with ^{99m}Tc-microspheres was performed at the end of the study for attenuation correction. **Results:** Inhaled nebulized IFNg showed a uniform deposition pattern in the lungs with deposition ratios of 0.74 (central-to-peripheral) and 0.78 (upper-to-lower). The lung deposition of IFNg was time-dependent, with a deposition half-time between 1 and 5 min. Despite a large interindividual variation, the total lung deposition was proportional to the nebulizer charge and was 53 ± 12% of the inhaled dose and 19 ± 7% of the initial nebulizer charge (between 0.1 and 2 mg of IFNg). The biological half-life in the lung could be fitted to a biexponential function, with resultant half-lives of 1 and 11 hr. Blood activity was maximal at 3.5 hr after inhalation and was due to free iodine. The radioactivity was excreted through both the urinary and intestinal tracts. Plasma IFNg levels did not significantly increase over time, and no significant HLA-DR induction on peripheral blood cells was detected. The highest radiation absorbed doses of 0.14 and 0.19 mGy/MBq were determined for the trachea and the lower intestines, respectively. The effective dose equivalent was 0.05 mSv/MBq. **Conclusion:** After inhalation with the Pari-Master nebulizer, IFNg deposits normally in the lungs and shows no systemic effects in healthy volunteers.

Key Words: iodine-123-IFNg; inhalation scintigraphy; dose escalation study; dosimetry; healthy volunteers

J Nucl Med 1997; 38:1475–1481

Interferon gamma 1b (IFNg) is a major cytokine involved in immunomodulation. It is produced by both T lymphocytes and natural killer cells, and its specific effects include antiviral activity, induction of antigen specific immunity, down-regulation of interleukin 4-induced IgE synthesis and a marked enhancement of monocyte macrophage cell functions (*1–5*). Recently, the potential role of IFNg in the treatment of immunologic pulmonary disease has been suggested (*6*). Alveolar macrophages are responsible for the pulmonary host defense due to their ability to phagocytose and kill microorganisms, produce oxygen radicals and process antigens. All of these effects are augmented by IFNg (*3–5,7,8*).

The clinical impact of IFNg was evidenced by the observation that patients under treatment have a significantly lower rate of serious pulmonary infections (*6*). It has also been suggested that patients with other lung diseases, such as IgE-associated asthma (*7,9*), may also benefit from IFNg treatment. Furthermore, clinical implications of IFNg as an antitumor agent have been reported, with significant results in patients with renal cell cancer (*10–13*). In these patients, the metastatic spread of the disease to the lungs may be a potential application for inhalatory IFNg treatment.

The route of IFNg administration seems to be particularly important. The clinical results reported were obtained after subcutaneous administration so that side effects (i.e., "influenza-like syndrome") are minimized (*6,10–13*). However, a recent preclinical study has shown that the transfer of IFNg is almost exclusively unidirectional from the rodent alveolar space to the plasma pool (*14*). Consequently, subcutaneously administered IFNg may not produce an activation of lung macrophages in the bronchoalveolar lavage, which may be a critical step for the successful treatment of lung disease using IFNg. In contrast, aerosolized IFNg was recently shown to locally activate mononuclear phagocytes (*7,15,16*), most probably after binding to specific high-affinity receptors (*2–4*). Therefore, local (inhalatory) application of this cytokine in patients with pulmonary disease should be more potent clinically, as well as being much more cost-effective than subcutaneous application.

The dose of inhaled IFNg and its biodistribution in the lungs and the whole body are currently not well-defined. This healthy volunteer study was undertaken to describe the pulmonary deposition pattern of escalating doses of inhaled radioiodinated IFNg and its whole-body distribution and safety.

MATERIALS AND METHODS

Preparation and Quality Control of Iodine-123-Labeled IFNg

The recombinant human IFNg used in this study was provided, and the 17-kDa IFNg, with a specific activity of 3×10^7 units/mg, was produced through recombinant expression in *Escherichia coli*. Iodine-123 labeling of IFNg was performed with a modified Iodogen method as described previously (*17*). Briefly, 500 μl of an IFNg solution (200 μg/ml), 200 μl of phosphate-buffered saline (pH 7.5) and 180 MBq of [^{123}I]NaI (100 μl) were added to a glass microvial precoated with Iodogen and equipped with a magnetic

Received Aug. 28, 1996; revision accepted Feb. 19, 1997.

For correspondence or reprints contact: Irene Virgolini, MD, Department of Clinical Pharmacology, University of Vienna, Währinger Gürtel 18-20, Ebene 3L, A-1090 Vienna, Austria.

TABLE 1
Details of Volunteers' Age, Sex, Lung Function (FEV_1), TLC, FRC, IC and MV

Volunteer	Age (yr)	Sex	FEV_1 ml	FEV_1 %*	TLC Liters	TLC %*	IC Liters	IC %*	FRC Liters	FRC %*	MV Liters/min
M.B.	55	F	81	103	6.7	125	4.5	131	2.2	110	19.1
M.M.	50	M	76	93	8.1	123	5.6	121	2.5	119	28.5
F.T.	62	F	78	101	5.3	102	2.9	90	2.4	117	15.7
M.T.	65	F	79	99	4.9	119	3.2	142	1.8	99	11.0
H.K.	55	F	76	93	4.3	93	3.3	115	1.0	62	8.2
E.O.	63	F	87	111	4.1	83	2.6	89	1.5	74	11.7
A.B.	58	F	73	92	5.2	102	3.8	118	1.4	75	14.5
G.B.	60	M	71	92	7.9	108	5.3	105	3.5	146	28.7
R.K.	53	F	80	100	5.3	102	3.8	114	1.5	78	13.0
G.A.	64	F	80	102	4.7	99	3.1	112	1.6	81	14.2
Mean ± s.d.	58 ± 5		78 ± 5	99 ± 6	5.6 ± 1.4	106 ± 13	3.8 ± 1.0	114 ± 16	1.9 ± 0.7	96 ± 26	16.5 ± 7.0

*The % values are the percent of the predicted values of the parameters listed.

stirrer. This reaction mixture was stirred, at an ambient temperature, for 15 min.

An aliquot (1 μl) of the quenched reaction mixture was analyzed by paper electrophoresis (PE) using Whatman 3-mm paper and 50 m*M* barbital buffer (pH 8.6) with a potential of 300 V for 10 min. A standard of [^{123}I]NaI was run in parallel for a direct comparison. Finally, the ^{123}I-labeled IFNg (^{123}I-IFNg) was sterile-filtered through a 0.45-μm membrane filter.

In addition to PE, ^{123}I-IFNg was also analyzed by protein precipitation with 20% trichloroacetic acid and, in selected cases, by polyacrylamide gel electrophoresis (PAGE). The samples were applied on gradient gels and run under nonreducing conditions in 1% SDS/2.5 m*M* Tris buffer (pH 6.8) using a potential of 600 V (50 mA) for 90 min. The distribution of radioactivity on PE and PAGE chromatograms was determined with a thin layer radiochromatograph analyzer. The radioactivity in the precipitated protein was analyzed with an automatic NaI(Tl) gamma counter.

Biological Activity of Iodine-123-IFNg

The final radiolabeled ^{123}I-IFNg and unmodified IFNg were evaluated for binding to monocyte macrophage cells, obtained from normal subjects, as described previously (*18*). These binding assays were performed both before and after nebulization (up to 30 min) to confirm the stability of the radioligand.

To evaluate the biological effects of ^{123}I-IFNg and unmodified IFNg, expression of human leukocyte antigen DR (HLA-DR) on peripheral blood mononuclear cells (PMNCs) was analyzed. Cells were obtained from the peripheral blood of 8 of the 10 volunteers before and the day after inhalation of IFNg. PMNCs were isolated by use of Ficoll. Expression of the HLA-DR antigen on PMNC and blood monocytes was analyzed using the antibody L243 (IgG_{2a} subclass) directed against the HLA-DR antigen and flow cytometry as described (*19*). In brief, PMNCs were incubated with anti-HLA-DR antibody or isotype-matched control antibody for 30 min at 4°C, washed and then exposed to a fluorescein-labeled murine $F(ab')_2$ antimouse IgG/IgM antibody for another 30 min (4°C). After washing, cells were analyzed by flow cytometry on a fluorescence-activated cell sorter. In addition to the total PMNC fraction, blood monocytes were separately analyzed as a gated cell population.

Volunteer Selection and Handling

The pulmonary deposition of nebulized ^{123}I-IFNg was studied in 10 healthy nonsmoking volunteers (two men and eight women; age range, 54–65 yr; mean age, 58.5 ± 5.1 yr). The study protocol was approved by the University of Vienna Ethical Commission and performed according to Austrian laws as well as the Helsinki Declaration II. All subjects gave written informed consent to participate and had to undergo a routine clinical check-up before being allowed to enter the study. The lung function of the volunteers was also evaluated, and the measured parameters, such as forced expiratory volume in 1 sec (FEV_1), total lung capacity (TLC), functional residual capacity (FRC), inspiratory capacity (IC) and minute ventilation (MV), were found to be within the normal range (Table 1). The study comprised the inhalation of ^{123}I-IFNg and a perfusion scintigraphy using ^{99m}Tc-albumin microspheres. All subjects received 400 mg of sodium perchlorate, three times a day over 3 days, and 65 mg of sodium iodide, twice a day over 2 days, for thyroid blockade.

Inhalation Studies

The inhalation of ^{123}I-IFNg was performed with a Pari-Master. This nebulizer system is widely used for routine clinical application of a variety of drugs. An absolute filter was fitted to the exhalation circuit of the Pari-Master to trap all unabsorbed ^{123}I-IFNg aerosol. This system, running under standard operation conditions (20 liters/min; 20°C; 45% humidity; 1.45 bar), produced aerosols, with a median mass aerodynamic diameter of 3.1 μm (range, 1–5 μm), when analyzed with a Malvern Particle Sizer. The efficiency of the nebulizer system was 73%–88%, within 10 min (i.e., 12%–27% of all ^{123}I-IFNg was retained by the system) using an air flow of 20 liters/min and a nebulizer volume fill of 5 ml. For these studies, the initial nebulizer charge/volume fill varied from 100 μg of IFNg/5 ml to 2.0 mg of IFNg/10 ml and was achieved by adding unlabeled IFNg to the ^{123}I-IFNg. The amount of radioactivity in the nebulizer was determined by collecting a 1-min static image before and after nebulization. The absolute filter on the exhalation circuit contained up to 30% of the administered activity.

Safety Evaluation

Side effects were scored over the whole study period of 24 hr. Volunteers were asked to record any nausea, burning in the throat, dizziness, fever, coughing or breathlessness. The questionnaire was to be answered before, directly after the inhalation period and at 1, 2, 4 and 24 hr postinhalation.

Imaging Protocol

The volunteers were seated with their backs in front of a single-headed gamma camera, equipped with a low-energy, all-purpose collimator. An 8-cm lead screen was placed between the nebulizer and the volunteer, who breathed from the nebulizer mouthpiece. The breathing protocol, slow deep inspiration from

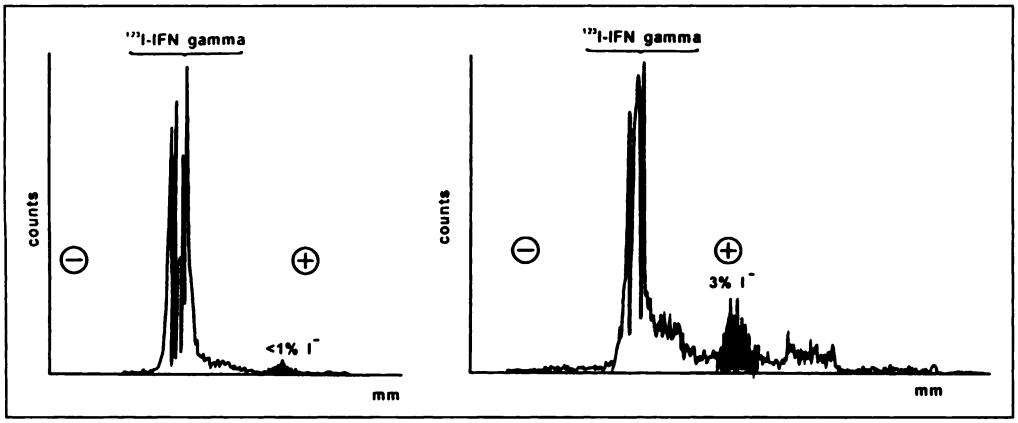

FIGURE 1. Paper electrophoresis of ^{123}I-IFNg. Paper electrophoresis was performed at the calibration time (about 20 hr after labeling). Iodine-123-labeled IFNg remains at the origin whereas the free ^{123}I (<1%) migrates towards the anode (upper). Radiochemical purity was conserved during aerosol formation (right), showing the same analytical procedure applied to ^{123}I-IFNg, which was nebulized for 15 min.

FRC, followed by short deep breath hold (3 sec), was exercised with the volunteers before the start of the study. During the first phase of the study, sequential images (128 × 128 pixels) were recorded every 30 sec for up to 37 min after the inhalation of ^{123}I-IFNg. Thereafter, static images in posterior-anterior and anterior-posterior views were accumulated for 5 min (300 kilocounts). Whole-body acquisition was then performed simultaneously in anterior and posterior views using a double-headed gamma camera. Serial whole-body images were obtained at 0.5–1, 2–4 and 18–24 hr postinjection.

Blood, Urine and Feces Collection

Blood samples were collected immediately before and 0.5, 1, 2, 4 and 24 hr postapplication of ^{123}I-IFNg. Urine was collected during the three time periods of 0–8, 8–16 and 16–24 hr. In two volunteers, feces were collected during the first 24 hr.

The amounts of radioactivity in the biological samples were determined using an automatic NaI(Tl) gamma counter. The counting efficiency was determined using a calibrated source of ^{123}I with the same geometry as the test samples. The data recorded were subjected to a decay correction, and the total amounts of activity excreted were determined by considering the volume/mass of the biological samples and the gamma counter samples.

The amount of IFNg in the blood was determined using an IFNg ELISA according to the manufacturer's description.

Gamma Camera Data Analysis

IFNg deposition in the lungs was determined by considering the specific activity of the nebulized ^{123}I-IFNg and the amount of radioactivity deposited. To determine the absolute amount of radioactivity deposited, the volunteer's individual attenuation coefficient was determined in vivo according to the established methods (*20–22*). Consequently, at the end of the study, ^{99m}Tc-albumin microspheres (about 40 MBq) were injected intravenously, and lung perfusion scintigraphy was performed in posterior and anterior views for 1 min. The ratio of the recorded lung activity and the administered activity was then used to determine the individual attenuation coefficient.

For the ^{123}I-IFNg imaging data, regions of interest (ROIs) were drawn over the lungs, and care was taken to avoid the trachea and the esophagus. These ^{123}I-IFNg counting data were converted to the physical mass of IFNg and plotted as a function of time. To calculate the effective doses reaching the lungs, ROIs were also drawn for regions covering the mouth/larynx, esophagus/trachea and the upper/lower gastrointestinal tract.

Iodine-123-IFNg Whole-Body Distribution and Radiation Absorbed Dose

For the ^{123}I-IFNg whole-body scans, ROIs were drawn at each time point. The geometric means of anterior and posterior counts were calculated for large ROIs of the lungs, upper and lower gastrointestinal tracts, mouth/larynx, esophagus/trachea and urinary bladder. All gamma camera data were corrected for background counts. Calculations were based on the MIRD concept (*23*) and the "S" absorbed dose values for the different organs (*24*). For biokinetic considerations, gamma camera, blood, urine and feces activity measurements were used and interpreted according to the ICRP report no. 66 on models of the human respiratory tract for radiological protection (*25*). Because no S value exists for the trachea, the radiation dose to this organ was calculated in accordance with the MIRD dose estimate report no. 16 (*26*), which gives a value to the trachea from a ^{99m}Tc aerosol. For our estimates, the different dose constants for ^{99m}Tc and ^{123}I, as well as the different uptake values for the trachea and the two radioligands, were considered. The effective dose, as defined by the ICRP (*25*), was also calculated.

RESULTS

Radiochemical Purity and Bioactivity of Iodine-123-IFNg

The specific activity of the radiotracer was 1800 MBq of ^{123}I/mg of IFNg. Unbound ^{123}I, as determined by PE (Fig. 1), was always less than 5% and, in most cases, was less than 1%. Therefore, a column chromatographic separation of the ^{123}I-IFNg was not required. The amount of radioactivity associated with the trichloroacetic acid-precipitable protein was 94% ± 2% (n = 15). The PAGE protein bands (IFNg) and radioactivity bands (^{123}I-IFNg) were identical and corresponded to molecular masses of about 16.5 and 33 kDa (Fig. 2). The in vitro radiochemical stability of ^{123}I-IFNg was extended through more than 20 hr and was also maintained during the nebulization of the ^{123}I-IFNg solution (Fig. 3).

Pulmonary Deposition of Iodine-123-IFNg

The time–activity curves generated over the lungs indicated saturability of the ^{123}I-IFNg deposition with a deposition half-time ($T_{1/2}$) ranging between 1 and 5 min (Table 2; Fig. 4). The maximum lung deposition was reached within 10 min, and when the inhalation was continued for up to 37 min, it did not significantly increase the pulmonary counting rate. As shown in Table 2, the maximum deposition of ^{123}I-IFNg was a function of target site, and a steady-state condition could be reached, but it was dependent on the nebulizer charge and the inhalation time. The total administered activity varied from 21% to 47% of the initial nebulizer charge, and between 39% and 70% of this whole-body activity was found in the lungs. Thus, between 12.0% and 32.3% of the nebulizer charge was delivered to the lungs.

The regional pulmonary deposition is shown in Table 3 and Figure 5. IFNg deposition favored the lower lung and the peripheral lung regions. On average, the central to peripheral deposition ratios were 0.74, and the upper-to-lower deposition ratios were 0.78, indicating the highest deposition in the central

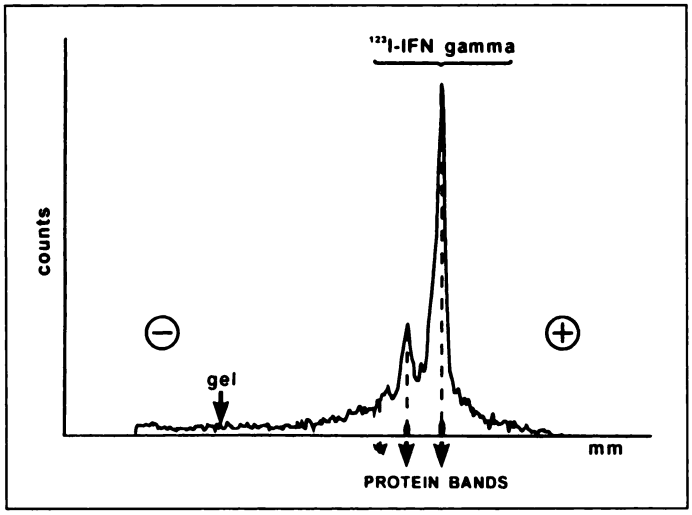

FIGURE 2. PAGE of ^{123}I-IFNg. The analysis was performed at the calibration time (about 20 hr after labeling). Iodine-123 activity was associated with the two protein bands revealed by silver staining. These bands were identical to those produced with unlabeled IFNg and corresponded to molecular masses of about 165 and 33 kDa, respectively. Most of the ^{123}I was bound to the 165-kDa protein band.

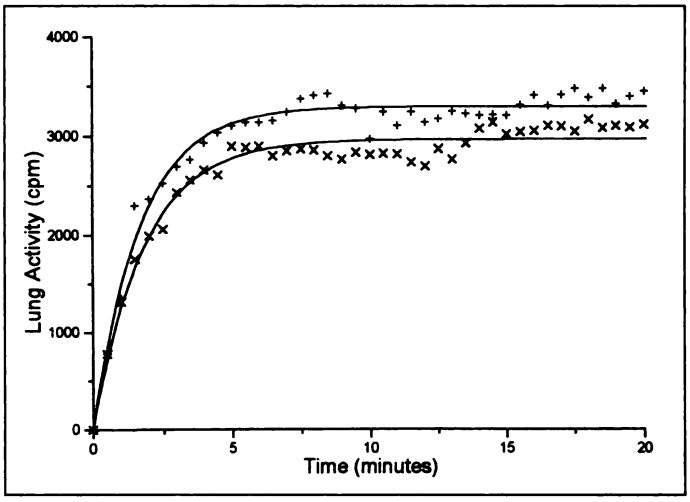

FIGURE 4. Iodine-123-IFNg inhalation in volunteer M. T. (500 μg of IFNg). The time–activity curves for both lungs indicated a deposition half time $T_{1/2}$ of 1 min for both lungs.

middle parts of the lungs, whereas a homogeneous lung deposition was found in the peripheral area.

Whole-Body Biodistribution

The kinetics of IFNg in the whole body (Fig. 6) are shown in Table 4. On average, 50% of the whole-body dose was found in the lungs at 0.5 hr after inhalation of ^{123}I-IFNg and was reduced to 6% at 24 hr. The mouth/larynx trapped 11%, the trachea/esophagus trapped 8% and the stomach/upper intestine trapped 15% of the whole-body dose. Decreasing activity over the upper intestine was associated with an increasing amount of activity over the lower intestine and an increase in the activity in the rest of the body.

Blood activity reached a maximum at 3.5 hr after inhalation and slowly decreased over 24 hr. Plasma-associated radioactivity amounted to 3% ± 1% of the inhaled dose (whole-body dose) at 1 hr and was reduced to <1% at 24 hr. The radioactivity was excreted through both the urinary and intestinal tracts. The 24-hr urinary excretion of radioactivity was 51% ± 6% of the administered activity. The feces collected from two of the volunteers, over 24 hr, amounted to approximately 20% of the administered activity.

Effects of Inhaled Iodine-123-IFNg on Plasma IFNg Levels and Blood Cells

No consistent induction of HLA-DR expression by peripheral blood cells (PMNCs as well as monocytes) was detectable in the group of volunteers ($p < 0.1$; Student's t-test for paired data), but one subject did have unexplainable increased values (R.K.; Table 5). However, the plasma IFNg levels remained unaltered in all of the volunteers.

Dosimetry

Approximately 50% of the administered activity was deposited directly in the lungs. After suspending administration, the washout curve of this radioactivity could be described by a biexponential curve with biological half-lives of 1 and 11 hr for the fast and slow, respectively, components. Approximately one-half of this lung activity was rapidly cleared by mucociliary activity to the pharynx, where it was swallowed. The other half of the activity was taken up by the blood and ultimately excreted into the urine with a residence time of 16 hr. This slow component of the washout curve is likely to represent alveolar macrophage phagocytosis.

Approximately 20% of the activity was swallowed immediately, and the other 30% was ultimately deposited in the lungs and subsequently cleared by mucociliary clearance with a half-time of 1 hr. The residence time for the activity in the

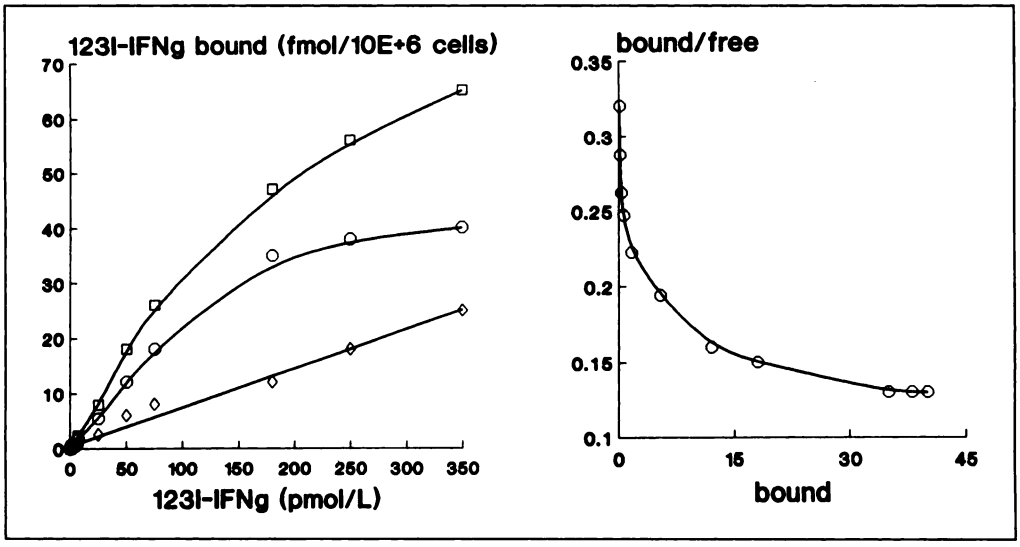

FIGURE 3. Saturation curve (left) and Scatchard plot (right) of nebulized ^{123}I-IFNg binding to PMNCs. The binding of ^{123}I-IFNg displayed two binding classes, a higher (K_d = 0.03 n*M*; 6000 sites/cell) and a lower affinity one (K_d = 0.13 n*M*; 24,000 sites/cell). □ = total binding; ◇ = nonspecific binding; ○ = specific binding.

TABLE 2
Summary of Results after Inhalation of Iodine-123-IFNg in Healthy Volunteers

Volunteer	Nebulizer charge,* IFNg (μg)	Inhalation parameters: Volume fill† (ml)	Inhalation parameters: Time (min)	Inhalation parameters: Rate (μg/min)	Inhaled dose,‡ IFNg (μg)	Lung deposition: $T_{1/2}$ (min)	Lung deposition: IFNg (μg)	% of nebulizer charge
M.B.	100	5	4	25	21 (21%)	3.5	15 (70%)	14.9
M.M.	250	5	10	25	72 (29%)	1.6	48 (67%)	19.2
F.T.	500	10	16	32	170 (34%)	4.8	66 (39%)	13.3
M.T.	500	5	25	20	139 (28%)	1.0	60 (43%)	12.0
H.K.	750	10	25	30	280 (37%)	4.8	115 (41%)	15.3
E.O.	750	5	20	37	352 (47%)	5.0	187 (53%)	24.9
A.B.	1000	5.5	25	40	450 (45%)	4.0	261 (58%)	26.1
G.B.	1000	10	30	33	408 (41%)	2.5	193 (47%)	19.3
R.K.	1500	10	37	41	705 (47%)	2.4	484 (69%)	32.3
G.A.	2000	10	15	133	649 (32%)	2.5	282 (43%)	14.1

*Nebulizer charge, amount of IFNg medication placed in the nebulizer.
†Volume fill, volume of IFN solution placed in the nebulizer.
‡Inhaled dose, quantity of actually inhaled IFNg for the given nebulizer (i.e., Pari-Master) for the defined breathing pattern, for the listed time. The inhaled dose represents the dose of both intrapulmonary and extrapulmonary deposition. Values are not corrected for nebulizer efficiency (i.e., 18%–27%).

stomach and upper intestines was estimated to be 3 hr, and for the lower intestines, a residence time of 6 hr was estimated. For the calculation of the bladder dose, a voiding frequency of 3 hr was assumed.

The radiation absorbed organ doses were 0.08 mGy/MBq for the lungs, 0.14 mGy/MBq for the trachea, 0.09 mGy/MBq for the stomach, 0.06 mGy/MBq for the small intestines, 0.19 mGy/MBq for the lower intestines and 0.02 mGy/MBq for the urinary bladder. The dose for the remainder of the body was calculated to be 0.01 mGy/MBq, and gonad doses were estimated to be 0.02 mGy/MBq for the ovaries and 0.001 mGy/MBq for the testes. The effective dose equivalent was 0.05 mSv/MBq.

Safety

All volunteers tolerated the nebulized IFNg without any notable symptoms. During the initial phase of inhalation, 6 of the 10 volunteers exhibited mild coughing. No other side effects due to IFNg occurred.

DISCUSSION

In this study, we have performed the first determination of the absolute macroscopic IFNg pulmonary deposition using a widely recognized pulmonary delivery system, the Pari-Master. Preclinical research has already established that the IFNg transfer is almost exclusively unidirectional from the rodent bronchoalveolar space to the plasma pool (*14*). This suggests

TABLE 3
Regional Pulmonary Iodine-123-IFNg Deposition

Volunteer	Nebulizer charge dose (μg)	Right lung deposition: T	U	M	L	C	P	Left lung deposition: T	U	M	L	C	P
M.B.	100	7.5*	1.6	3.4	2.5	2.5	5.0	7.5	1.6	3.7	2.2	3.7	3.8
		50.0	10.7	22.7	16.7	16.4	33.6	50.0	10.7	24.6	14.7	24.7	25.3
M.M.	250	24	4.9	10.6	8.5	8.8	15.2	24	4.8	11.1	8.1	11.2	12.8
		50	10.2	22.1	17.7	18.3	31.7	50	10	23.1	16.9	23.3	26.7
F.T.	500	36.6	9.8	15.7	11.1	14.3	22.3	30.0	8.5	14.4	7.1	10.9	19.1
		55.0	14.7	23.6	16.7	21.5	33.5	45.0	12.8	21.6	10.7	16.4	28.7
M.T.	500	30.0	5.0	15.0	10.0	12.1	17.9	30.0	5.0	15.0	10.0	13.8	16.2
		50.0	8.3	25.0	16.7	20.3	29.8	50.0	8.3	25.0	16.7	23.0	27.0
H.K.	750	65.3	11.8	30.7	22.8	27.6	37.7	49.7	9.5	25.2	15.0	21.3	28.4
		56.8	10.3	26.7	19.8	24.0	32.8	43.2	8.3	21.9	13.0	18.5	24.7
E.O.	750	103	26.2	44.9	31.6	40.2	62.5	84.3	24.5	34.0	25.8	37.0	47.4
		54.9	14.0	24.0	16.9	21.5	33.4	45.1	13.1	18.2	13.8	19.8	25.3
A.B.	1000	127	37.3	59.8	29.5	54.5	72.0	134	31.8	66.6	36.0	61.3	73.1
		48.5	14.3	22.9	11.3	20.9	27.6	51.5	12.2	25.5	13.8	23.5	28.0
G.B.	1000	105	28.6	40.1	36.3	48.6	55.4	88.0	24.9	32.4	30.7	40.7	47.3
		54.4	14.8	20.8	18.8	25.7	28.7	45.6	12.9	16.8	15.9	21.1	24.5
R.K.	1500	254	69.7	121	63.4	90	164	230	58	101	70.6	106	124
		52.5	14.4	25.0	13.1	18.6	33.9	47.6	12.0	21.0	14.6	21.9	25.7
G.A.	2000	146	38.8	62.6	44.4	57.9	88.1	136	35.5	63.6	37.2	65.4	71.0
		51.8	13.8	22.3	15.7	20.4	31.2	48.2	12.6	22.5	13.2	23.1	25.1
Mean ± s.d.		52.4	12.6	23.5	16.3	20.8	31.6	47.6	11.3	22.0	14.2	21.5	26.1
		± 2.8	± 2.4	± 1.7	± 2.5	± 2.7	± 2.8	± 1.8	± 2.8	± 1.9	± 2.6	± 1.4	

*Upper lines are the values in μg; lower lines are the % values.
T = total; U = upper; M = middle; L = lower; C = central; P = peripheral (see also ROIs in Fig. 5).

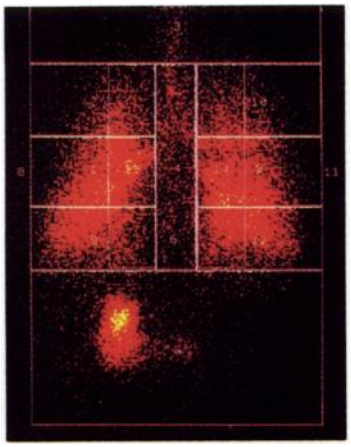

FIGURE 5. Regional pulmonary deposition of ^{123}I-IFNg (300 kilocounts, planar posterior image at 0.5 hr postinjection). The ROIs drawn represent the middle, upper, lower, central and peripheral regions (see also Table 3).

that direct pulmonary deposition is required to effectively treat IFNg-responsive pulmonary diseases.

Subcutaneous administration of IFNg has demonstrated a significant clinical benefit in immunologic lung disease (*6*) and also in tumor patients, such as patients with renal cell cancer (*10–13*). However, this mode of application can produce side effects and an immune response. In particular, one study (*27*) has demonstrated major side effects with continuous doses of 0.1 mg/day. In contrast, the nebulized cytokine has been reported to show no up-regulation of ICAM or HLA-DR expression of PMNCs in healthy volunteers who received the nebulized cytokine over a period of 2 weeks (3 mg of IFNg on days 1, 3, 5, 8, 10 and 12) (*7*). Similarly, in this study, the overall effect of inhaled IFNg on HLA-DR expression by PMNCs as well as monocytes was not significant. Furthermore, the plasma IFNg levels remained unaltered in each of the volunteers. Consequently, the plasma-associated radioactivity measured in this study was most probably due to free ^{123}I or partially metabolized ^{123}I-IFNg. One has to bear in mind, however, that in patients with inflammatory lung disease the alveolar-capillary membrane may be more permeable to inhaled molecules as compared with normal lung.

In this study, we show that IFNg can be labeled with ^{123}I to a relatively high specific activity without a postlabeling separation step to remove free iodine. As shown in Figure 3, the ^{123}I-IFNg produced had a high binding affinity for the two classes of IFNg binding sites expressed by PMNCs. This high binding affinity was also retained after nebulization of the

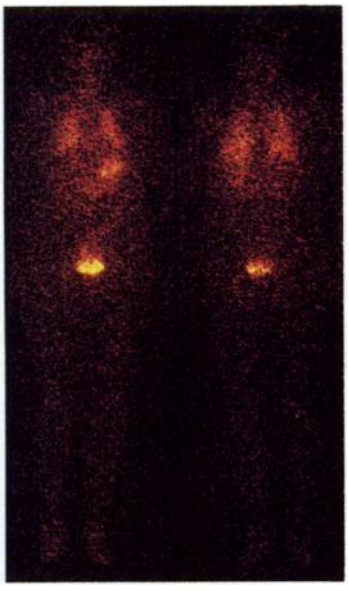

FIGURE 6. Whole-body scintigram of volunteer A. B. at 3 hr after inhalation of ^{123}I-IFNg (100 μg). Left, anterior view; right, posterior view.

TABLE 4
Whole-Body Distribution of Radioactivity in Healthy Volunteers up to 24 hr after Iodine-123-IFNg Inhalation

Organ	Time after inhalation (hr)			
	0.5	1	3.5	24
Lungs	49 ± 10	35 ± 8	21 ± 5	6 ± 2
Mouth/larynx	11 ± 5	8 ± 3	5 ± 2	<1
Trachea/esophagus	8 ± 3	6 ± 3	3 ± 2	<1
Stomach/upper intestine	15 ± 5	11 ± 5	6 ± 4	<1
Lower intestine		6 ± 2	12 ± 3	6 ± 2
Bladder		4 ± 2	5 ± 2	5 ± 2
Blood	<1	3 ± 1	7 ± 1	<1
Urine				51 ± 6
Feces (n = 2)				~20

Values are means ± s.d. (n = 10). The values listed indicate the percent dose of inhaled IFNg found in the organ listed at the time indicated.

radiolabeled cytokine. Therefore, the deposition of IFNg to the human lung can be considered to be partly due to binding of aerosolized IFNg to alveolar macrophages. This hypothesis, furthermore, is strengthened by the fact that saturation of IFNg deposition could be demonstrated within the first 10 min of inhalation (Fig. 4).

The therapeutic efficacy of nebulized IFNg, in the concomitant treatment of pulmonary disease, will also depend on the absolute deposition of this cytokine. Although the highest deposition of IFNg was found in the central middle parts of the lungs, a homogeneous lung deposition was also found in the peripheral areas. This shows that there was sufficient aerosolization of IFNg using the Pari-Master system. In general, we found that the absolute pulmonary deposition was high (19% of nebulizer charge) and did not decrease with increasing nebulizer charge (i.e., it saturated). Steady-state conditions were quickly achieved, and the pulmonary residence time of IFNg was fairly long, with about 50% of the deposited activity being cleared with a half-time of 11 hr. Table 2 shows the large

TABLE 5
Effect of IFNg Inhalation on HLA-DR Expression of PMNCs and Blood Monocytes

Volunteer	IFNg dose (μg)	Reactivity (%) of cells with monoclonal antibody to HLA-DR antigen			
		PMNCs		Monocytes	
		Before	After	Before	After
M.B.	100	N.I.	N.I.	N.I.	N.I.
M.M.	250	68	71	100	99
F.T.	500	48	52	92	97
M.T.	750	58	52	99	96
H.K.	750	N.I.	N.I.	N.I.	N.I.
E.O.	750	40	58	72	97
A.B.	1000	35	59	85	98
G.B.	1000	65	64	99	99
R.K.	1500	19	40	24	75
G.A.	2000	65	68	86	96
Mean ± s.d.		50 ± 17	58 ± 10*	82 ± 25	94 ± 8*

PMNC were isolated and analyzed with an antibody against HLA-DR as described in the text. Blood cells were drawn and prepared shortly before and 24 hr after inhalation in each volunteer. The values represent the percent of reactive cells in fluorescence-activated cell sorting analyses. Monocytes were analyzed as gated cell population. N.I. = not investigated.
*Students t-test for paired data, $p < 0.1$.

interindividual variation in the group of volunteers, which suggests that an even lower initial IFNg dose may lead to a steady-state condition for IFNg lung deposition in vivo.

The use of this tracer technique could be applied to a wide range of inhalation agents that can be radioiodinated. Because it is a tracer study, the method can be considered more reliable than another reported technique, which uses a mixture of the biologically inactive ^{99m}Tc-labeled human serum albumin and the pharmaceutical of interest (*28*). Furthermore, the use of ^{123}I-labeled inhalatory agents to determine the pulmonary deposition of pharmaceuticals appears to be a safe procedure from the point of view of the radiation dose received by the volunteers reported in this study. Further investigations in patients will demonstrate the optimal pulmonary deposition of the therapeutic dose of IFNg, allowing significant savings in terms of drug and expense.

CONCLUSION

The widely used Pari-Master nebulizer system can efficiently and homogeneously deliver IFNg to the lungs in healthy volunteers. Even at the highest doses, this cytokine shows no systemic effects. This type of study could be applied for the effective clinical dosing of IFNg for patients with lung disease undergoing IFNg inhalation treatment.

ACKNOWLEDGMENTS

We would like to thank all those who volunteered for this study.

REFERENCES

1. Murray HW. Interferon-gamma, the activated macrophage and host defense against microbial challenge. *Ann Intern Med* 1988;108:595–608.
2. Black CM, Catterall JR, Remington JS. In vivo and in vitro activation of alveolar macrophages by recombinant interferon-γ. *J Immunol* 1987;138:491–495.
3. Nathan CF, Murray HW, Wiebe ME, Rubin BY. Identification of interferon-γ as the lymphokine that activates human macrophage oxidative metabolism and antimicrobial activity. *J Exp Med* 1983;158:870–889.
4. Murray HW, Gellene RA, Libby DM, et al. Activation of tissue macrophages from AIDS patients: in vitro response of AIDS alveolar macrophages to lymphookines and interferon-γ. *J Immunol* 1985;135:2374–2377.
5. Iwamoto I, Nakajima H, Endo H, Yoshida S. Interferon gamma regulates antigen-induced eosinophil recruitment into the mouse airways by inhibiting the infiltration of CD4+ T cells. *J Exp Med* 1993;177:573–576.
6. The International Chronic Granulomatous Disease Cooperative Study Group. A controlled trial of interferon gamma to prevent infection in chronic granulomatous disease. *N Engl J Med* 1991;324:509–516.
7. Jaffe HA, Buhl R, Mastrangeii A, et al. Organ specific cytokine therapy: local activation of mononuclear phagocytes by delivery of an aerosol of recombinant interferon-γ to the human lung. *J Clin Invest* 1991;88:297–302.
8. Halme M. Inhaled gamma-interferon gamma in humans: pharmacokinetics and effects on alveolar macrophages and blood cells [Abstract]. *Am Rev Respir Dis* 1992;145: A591.
9. Nakajima H, Iwamoto I, Yoshida S. Aerosolized recombinant interferon-gamma prevents antigen-induced eosinophil recruitment in mouse trachea. *Am Rev Respir Dis* 1993;148:1102–1104.
10. Heicappel R, Ackermann R. Rationale for immunotherapy of renal cell carcinoma. *Urol Res* 1990;18:357–372.
11. Queseda JR, Swanson DA, Trindade AV. Renal cell carcinoma: antitumor effects of leukocyte interferon. *Cancer Res* 1983;43:940–947.
12. Ernstoff MS, Nair S, Bahnson RR, et al. A Phase I trial of sequential administration recombinant DNA produced interferons: combination recombinant interferon-gamma and recombinant interferon-alpha in patients with metastatic renal cell carcinoma. *J Clin Oncol* 1989;8:1637–1649.
13. Aulitzky W, Gastl G, Aulitzky WE, et al. Successful treatment of metastatic renal cell carcinoma with a biologically active dose of recombinant interferon-gamma. *J Clin Oncol* 1989;7:1875–1884.
14. Pessina GP, Paulesu L, Corradeschi F, et al. Pulmonary catabolism of interferon-gamma evaluated by lung perfusion in both normal and smoke-exposed rats. *J Interferon Cytokine Res* 1995;15:225–230.
15. Jaffe HA, Buhl R, Mastrangeli A, et al. Aerosol delivery of recombinant interferon-γ to the lung: demonstration of upregulation of interferon-γ inducible genes in alveolar macrophages [Abstract]. *J Interferon Res* 1990;10(Suppl. 1):S12.
16. Martin RJ, Boguniewicz M, Henson JE, et al. The effects of inhaled interferon gamma in normal human airways. *Am Rev Respir Dis* 1993;148:1677–1682.
17. Virgolini I, Angelberger P, Raderer M, et al. Vasoactive intestinal peptide receptor imaging in the localization of intestinal adenocarcinomas and endocrine tumors. *N Engl J Med* 1994;331:1116–1121.
18. Virgolini I, Sillaber C, Majdic O, et al. Characterization of prostaglandin binding sites expressed on human basophils. *J Biol Chem* 1992;267:12700–12708.
19. Valent P, Bevec D, Maurer D, et al. Interleukin 4 promotes expression of mast cell ICAM-1 antigen. *Proc Natl Acad Sci USA* 1991;88:3339–3342.
20. Thomas SHL, O'Doherty MJ, Page CL, Nuan TO. Variability in the measurement of nebulized aerosol deposition in man. *Clin Sci* 1991;81:767–775.
21. Macey DJ, Marshall R. Absolute quantification of radiotracer uptake in the lungs using a gamma camera. *J Nucl Med* 1982;23:731–735.
22. Smaldone GC, O'Riordan TG. Aerosol deposition and clearance. In: Wagner H Jr, Szabo Z, Buchanan J, eds. *The principles of nuclear medicine,* 2nd ed. Philadelphia: W. B. Saunders Company 1995;895–905.
23. Loevinger R, Merman M. A revised schema for calculating the absorbed dose from biologically distributed radionuclides, MIRD pamphlet no. 1, revised. New York: Society of Nuclear Medicine; 1976.
24. Synder WS, Ford MR, Warner GG, Watson SB. "S" absorbed dose per unit cumulated activity for selected radionuclides and organs, MIRD pamphlet no. 11. New York: Society of Nuclear Medicine; 1975.
25. IRCP. Human respiratory tract: model for radiological protection, IRCP publication no. 66. (1–3). *Ann ICRP* 1994.
26. Atkins HL, Weber DA, Susskind H, Thomas SR. MIRD dose estimate report no. 16. Radiation absorbed dose from technetium-99m diethylenetriamineopentaacetic acid aerosol. *J Nucl Med* 1992;33:1717–1719.
27. Sataki I, Tari K, Nakagomi K, Ozawa K. Feasibility and pharmacokinetics of continuous subcutaneous infusion of low-dose interferon-gamma: a pilot study. *Jpn J Clin Oncol* 1993;23:356–362.
28. Ferretti PP, Annibale V, Gafa SI, et al. Pulmonary deposition of aerosolized pentamidine using a new nebulizer: efficiency measurements in vitro and in vivo. *Eur J Nucl Med* 1994;21:399–406.