RETENTION OF ^{113m}In-^{59}Fe-FERRIC HYDROXIDE IN THE MOUSE

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In 1966 Stern et al (*1*) described the use of ^{113m}In-ferric hydroxide [^{113m}In-Fe(OH)$_3$] particles for lung scanning. Recently we became interested in labeling similar particulate compounds with ^{113m}In and ^{99m}Tc for the same use. However, in our initial investigations, the lung clearance half-times of these carrier compounds were much longer than the 15–20 hr reported for the Fe(OH)$_3$ carrier (*1,2*). These results were quite puzzling because the characteristics of the preparations we studied were not totally unlike those of the ^{113m}In-Fe(OH)$_3$ suspension. Therefore we have sought to corroborate the previously published results by measuring the pulmonary retention of ^{59}Fe in mice after intravenous injection of ^{113m}In-^{59}Fe-Fe(OH)$_3$ particles.

METHODS

The ^{113m}In-^{59}Fe-Fe(OH)$_3$ particles were prepared according to the modification by Goodwin et al (*2*) of the procedure first outlined by Stern et al (*1*). The Fe^{3+} solution was labeled with approximately 20 μCi $^{59}FeCl_3$ (International Chemical and Nuclear Corp.), and the ^{113m}In was eluted from a zirconium base ^{113}Sn-^{113m}In generator (New England Nuclear). The suspension was prepared in three separate batches to conform to volumes previously used (*2*). The preparations were pooled to yield a uniform product that was used throughout the investigation. The final radioconcentrations of ^{113m}In and ^{59}Fe were 35 and 2.0 μCi/ml, respectively. The bound-to-unbound ratio for both radioactive species was approximately 99:1.

Particles were sized microscopically on a hemocytometer slide. Most particles were between 20 and 50 microns in diam, and none was larger than 70 microns.

Ninety male mice (Charles River Strain), 17–23 gm in weight, received 0.25 ml of the ^{113m}In-^{59}Fe-Fe(OH)$_3$ suspension by injection into a lateral tail vein. Sixteen groups of five mice each were killed at preselected times (from 15 min to 42 days after injection) and the lungs, liver and carcass (including tail) were assayed for ^{59}Fe. Care was taken to remove residual blood from the organs counted. In addition, whole-body retention of ^{59}Fe was determined by whole-body counting of the remaining 10 animals at most intervals studied. Conventional scintillation instrumentation and relative counting techniques were used throughout. Appropriate consideration was given to geometry and activity in the preparation of comparative standards used with each type of sample. The whole-body counting assembly

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TABLE 1. LUNG, LIVER, CARCASS AND WHOLE-BODY RETENTION OF ^{59}Fe IN MICE AFTER INTRAVENOUS INJECTION OF ^{113m}In^{59}Fe-Fe(OH)$_3$

Time after injection (days)	Lung*	Liver*	Carcass*	Whole body†
0.01	89.6 ± 2.6	2.97 ± 0.34	2.05 ± 0.81	100.0 ± 4.2
0.17	82.9 ± 5.0	5.38 ± 1.64	5.90 ± 1.97	—
0.33	78.4 ± 5.4	9.03 ± 2.38	7.18 ± 1.62	—
1.00	70.8 ± 5.3	11.7 ± 2.0	12.7 ± 3.2	—
2.00	59.8 ± 5.8	12.7 ± 5.8	17.5 ± 3.9	97.6 ± 2.4
4.00	62.9 ± 4.1	4.15 ± 1.79	16.5 ± 2.4	98.0 ± 1.7
6.00	58.2 ± 3.9	3.44 ± 0.54	16.5 ± 4.2	—
8.00	52.7 ± 4.9	4.27 ± 0.81	32.3 ± 4.9	96.1 ± 4.0
10.00	55.2 ± 3.8	4.07 ± 0.52	27.0 ± 1.9	97.2 ± 3.9
12.00	49.2 ± 3.9	4.58 ± 0.41	24.5 ± 2.4	—
14.00	46.5 ± 7.3	5.10 ± 1.07	34.3 ± 6.0	92.7 ± 4.2
16.00	47.4 ± 2.2	6.46 ± 2.44	32.5 ± 1.3	93.6 ± 4.3
21.00	44.7 ± 7.6	5.36 ± 1.48	26.5 ± 4.5	90.1 ± 3.8
28.00	42.8 ± 5.8	6.66 ± 1.47	29.9 ± 4.0	86.4 ± 4.1
35.00	42.2 ± 6.8	7.05 ± 1.50	29.2 ± 5.0	82.0 ± 1.8
42.00	45.6 ± 2.8	6.17 ± 2.57	20.9 ± 3.0	—

* Mean and standard deviation for 5 animals.
† Mean and standard deviation for 10 animals.

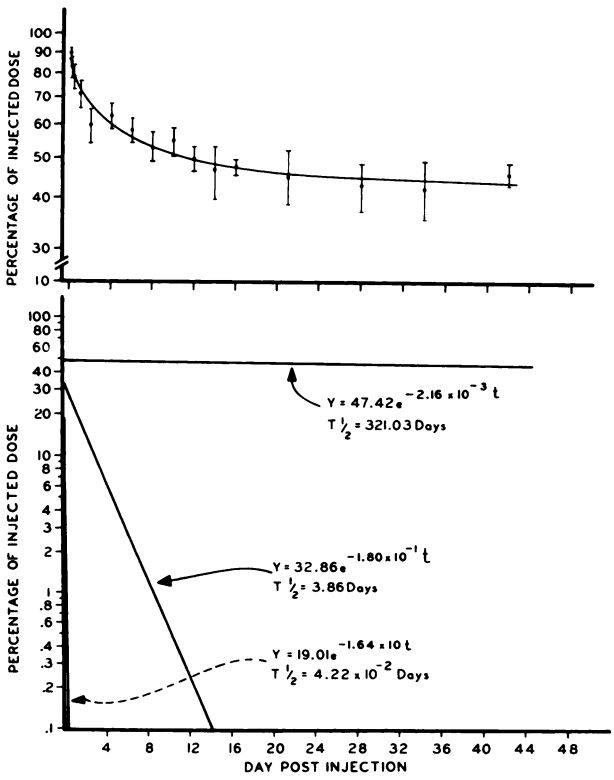

FIG. 1. Top shows pulmonary retention curve of ^{59}Fe in mice injected with ^{113m}In^{59}Fe-Fe(OH)$_3$. Bottom shows resolved components of retention curve.

consisted of a specially designed plastic animal restrainer (3 × 8 cm), a Lucite sample holder and a shielded 3 × 3-in. NaI(Tl) crystal connected to a multichannel spectrometer (Packard Instrument Co. Model 115). The mice were counted at a distance of 15 cm from the crystal. All data are expressed as a percentage of the injected dose.

Lungs excised from animals killed at 15 min, and at 35 and 42 days after injection were examined histologically. Sections were evaluated for the presence of residual iron according to the method of Lillie (*3*).

RESULTS

Lung, liver, carcass and whole-body retention data for ^{59}Fe are presented in Table 1. The amount of ^{113m}In-^{59}Fe-Fe(OH)$_3$ in the lungs 15 min (0.01 day) after injection was approximately 90% of the injected dose. From this maximum, the percentage of ^{59}Fe in the lungs decreased with time; about 45% of the dose remained in the lungs after 42 days. The radioconcentration in the liver was highest (about 13%) at 2 days, declined sharply to about 5% and remained relatively constant for the remainder of the study. Activity in the carcass reached a peak at 14 days (about 35%), then decreased gradually. Retention of the short-lived ^{113m}In in the lung, liver and carcass was also measured during the initial sampling periods. These results were very similar to those obtained with ^{59}Fe and therefore were not included.

Whole-body counting indicated a gradual decrease in total retention of ^{59}Fe to approximately 80% after 35 days. At each measurement, the total radioactivity of the samples was 5–10% less than that obtained by whole-body counting. This slight difference may be attributed to activity lost in the blood at necropsy.

The data for retention of ^{59}Fe in the lungs are given graphically in Fig. 1. A multiple exponential analysis computer program similar to that of Berman and Weiss (*4*) was used to treat the data as a sum of exponentials. The curve was found to be the sum of three exponentials having biological half-times of 1.0 hr, 3.8 days and 321 days. Of the total activity injected, 48% could be accounted for in the long-term fraction; the remaining 52% was associated with the short- (19%) and intermediate-term (33%) fractions.

All tissue sections stained for iron gave positive results (Fig. 2). Iron-positive material, probably of a particulate nature, was found in the pulmonary capillaries, and some fine iron-containing granules

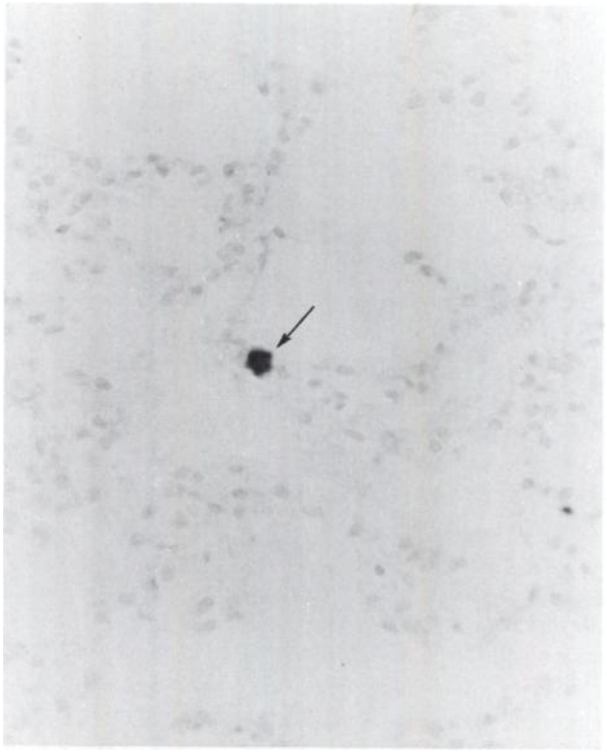

FIG. 2. Photomicrograph (X80) of section of mouse lung taken 35 days after injection of ^{113m}In^{59}Fe-Fe(OH)$_3$. Arrow indicates location of particle in capillary.

were localized in macrophages. There was no evidence of tissue damage or inflammation that could be related to the material injected.

DISCUSSION

Due to the nature and purpose of radiopharmaceuticals, major emphasis is placed upon the evaluation of acute radiation and chemical toxicity. As a result, long-term effects are sometimes overlooked. Although the earliest studies of ^{113m}In-^{59}Fe-Fe(OH)$_3$ (*1,2*) were concerned with possible long-term retention of the particles, more recent papers (*5–7*) dealing with alternative methods of preparation only discussed short-term retention.

There are some areas of close agreement between our data for lung clearance and those reported by Stern et al (*1*) and by Goodwin and his coworkers (*2*). Initial deposition in all three studies was about 80% of the injected dose. We resolved the early portion of our curve into two fractions with biological half-times of 1.0 hr and 3.8 days. If some data points were to be omitted, this portion of the curve would show only one component whose half-time would approximate the 15–20 hr previously reported (*1,2*). Although the clearance curves by these authors (*1,2*) suggest the existence of a more slowly removed fraction, neither group discussed the long-term retention of the carrier. One would assume the authors concluded that the amount of Fe(OH)$_3$ which cleared more slowly was negligible. Thus, the major disparity between our results and those previously reported is the percentage of ^{59}Fe associated with long-term retention, i.e., 48% in our study compared with less than 10% shown by Stern et al (*1*). This dissimilarity is paralleled by differing microscopic observations: Stern et al (*1*) reported no particles in lung sections 30 days after injection, whereas we saw particles after 42 days.

In our investigation a major fraction of the injected dose appeared to be retained for an extended period; by extrapolation, a significant portion of injected Fe(OH)$_3$ (approximately 22%) might still remain in the lungs after 1 year. The physiological consequences of this retention should not be overlooked.

Speculation about long-term consequences of the use of these lung-scanning particles is made difficult by the conflicting reports of the toxicity of Fe(OH)$_3$ preparations. Goodwin et al (*2*) found no histological changes in the lungs of mice sacrificed 2 weeks after an intravenous injection of ^{113m}In-Fe(OH)$_3$ particles (70 mg/kg). By contrast, Rhodes and his coworkers (*8*) reported mild to severe pathological reactions in 80% of mice injected with 10 mg ^{113m}In-Fe(OH)$_3$/kg. Nissim (*9*) reported an LD_{50} (7 days) in mice for colloidal Fe(OH)$_3$ of 25 mg Fe/kg, equivalent to 48 mg Fe(OH)$_3$/kg.

From our retention data, the presence of particles in the lungs of mice 42 days after injection and the lack of consensus regarding the toxicity of Fe(OH)$_3$ particles, it is apparent that more extensive investigation is required for a definitive evaluation of the safety of ^{113m}In-Fe(OH)$_3$ particles for lung-scanning. Such investigations are presently in progress in our laboratory.

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