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EFFECTS OF CAPILLARY BLOCKADE WITH ^{99m}Tc-IRON HYDROXIDE: A HISTOPATHOLOGIC STUDY

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Twenty-six mice injected with ^{99m}Tc-ferrous hydroxide macroaggregates (Tc-FHMA) were sequentially sacrificed over a 40-week period. Histologic sections of lung tissue stained for iron or with hematoxylin-eosin were prepared. Microscopic examination revealed changes in particle size and location of the FHMA with time after administration. Several mechanisms of clearance such as aggregate breakup, particle dissolution, and cellular phagocytosis were evident. The major finding was the complete absence of pathologic lesions that could be attributed to the long-term residence of Tc-FHMA in the pulmonary capillaries.

For the past 8 years iron hydroxide aggregates labeled with short-lived radionuclides have been successfully used as diagnostic agents for lung scintigraphy (*1–7*). Several studies aimed at determining the pulmonary residence time of these macroaggregates have reported a long-term component to the clearance curve with particles present weeks or months after administration (*8–11*). This finding has caused some investigators to speculate on the possibility of pathological consequences from macroaggregate administration (*8,12*). One study claimed to have found significant pathology although this has not been confirmed by others (*13*).

Because we participated in the development of one of these agents (*5,14*) and use it on a routine basis in several affiliated clinics, we felt obliged to carry out the extensive toxicologic evaluation described herein.

METHODS

Technetium-labeled ferrous hydroxide macroaggregates (^{99m}Tc-FHMA) were prepared and quality controlled according to previously published procedures (*5,14*). Twenty-six mice of equal weight were injected with 1×10^5 particles of ^{99m}Tc-FHMA. This is approximately 250 times the typical human dose (1×10^6 particles) when expressed on an equivalent weight basis. The animals were sacrificed in pairs (random selection) at prespecified times during the 40-week interval after administration. The lungs were excised, sectioned, and stained for iron using standard laboratory iron-staining techniques. The number of iron particles present in 200 microscopic lung fields were recorded for each mouse. Additional notations were made with regard to iron that was seen intracellularly or in locations other than the capillaries. Additional sections of each lung were stained with hematoxylin and eosin and both the iron and hematoxylin- and eosin-stained sections were evaluated for pathological damage. In a parallel experiment, untreated mice of the same age and weight were sacrificed at similar time intervals as a control for possible lung pathology unrelated to the administration of the iron aggregates.

RESULTS

The particle size averaged 30 microns; the smallest particles were about 8 microns and 95% of the aggregates were between 10 and 80 microns in mean diam. The labeling efficiency was more than 95% and the macroaggregates were in the ferrous state at the time of injection. More than 90% of the administered ^{99m}Tc activity was deposited in the lungs within 5 min after intravenous administration.

The particle size distribution of macroaggregates in the lung as a function of time after injection is shown in Fig. 1. The distribution at 1 hr after administration was identical to that in the serum vial before injection. At 24 hr (Day 1) the distribution

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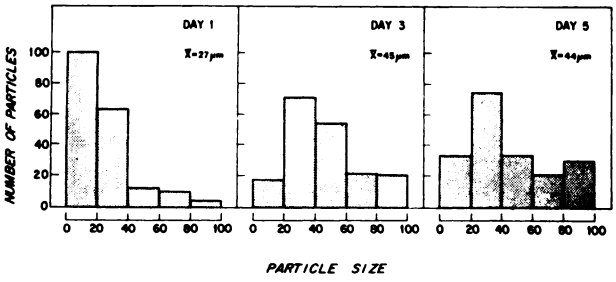

FIG. 1. Particle size distribution of ^{99m}Tc-iron hydroxide macroaggregates in mouse lung at 1, 3, and 5 days after intravenous injection.

remained essentially unchanged with an average particle size of 27 microns. On Day 3 (72 hr) a distinct shift in the particle size distribution and average particle size became apparent. A large proportion of the smaller aggregates (8–20 microns) were no longer present, giving an average particle size for those remaining of 45 microns. Some of the aggregates became fenestrated and the first signs of intracellular iron were evident. At 120 hr (Day 5), the average particle size was 44 microns (unchanged from Day 3). The fenestration of the macroaggregates was widespread at this point and a greater percentage of the iron was located more intracellularly than in the histologic sections taken on Days 1 and 3. From Day 5 through the 24th week no further alteration in the particle size distribution was apparent but the number of particles lodged in pulmonary vessels became increasingly sparse with a concomitant rise in the percentage of iron located intracellularly. From the 28th week until the completion of the investigation, the particle size decreased. Only smaller vessels were involved in these later specimens and in no instance could aggregates greater than 50 microns be located.

The ^{99m}Tc-iron hydroxide macroaggregates appeared as brown material on routine hematoxylin-eosin stained slides and as blue staining material on sections stained for iron. A total of 200–300 particles were observed in the 200 microscopic fields from histologic sections obtained shortly after injection (first week) whereas only 10–20 aggregates could be visualized in sections from the final sacrifice groups. The particles were seen as large intravascular clumps or as finer multiple granules within the cytoplasm of cells in the alveolar wall. The vessels involved were primarily alveolar capillaries. Occasionally a vessel two to three times larger in diameter was involved; this was usually found in the earlier specimens. The involved cells appeared to be either lining the alveolar walls or within the wall but the distinction was difficult without the use of electron microscopy. No particles were seen within the lumen of the alveoli. Intracellular iron was identifiable as early as 3 days after injection. Residual intravascular particles were clearly identifiable as late as 20–24 weeks and equivocally at 28 weeks. Interestingly, clusters of neighboring cells filled with iron were seldom seen. This suggests that a single cell was usually sufficient to ingest any residual material not totally cleared by another mechanism. Intracellular iron fragments appeared to become more finely divided and diffuse with time. No iron was evident in the control mice.

Pathologic examination of the histologic sections showed a complete absence of potential chronic lesions such as fibrosis, chronic inflammation, necrosis, hyperplasia, or neoplasia. The only chronic lung abnormality seen was emphysema. The extent of the emphysema was slight to moderate and related to the age of the animals. Mice sacrificed at 1–24 weeks had less extensive disease than those sacrificed later in the study. The emphysema was not present in or adjacent to the iron-plugged capillaries. The control mice exhibited the same extent of emphysema with the same age frequency.

The only other pathology seen was focal hemorrhage or congestion which appeared to be acute changes related to the method of sacrifice (ether anesthesia followed by decapitation). These findings were uniformly present in the control animals as well as in the experimental animals.

DISCUSSION

Each lung kit prepared for human use contains 10 mg $FeSO_4 \cdot 7H_2O$ which is equivalent to 2 mg Fe. Due to the high specific activity of our agent (50–100 mCi/kit), patients generally receive only one-fifth to one-tenth of the total kit contents (200–400 μg Fe). To the best of our knowledge, the use of iron hydroxide macroaggregates has raised little or no concern for iron toxicity. The major criticism has been related to the potential pathologic consequences of long-term residence of the metallic particles in the pulmonary capillary bed. Our primary interest, therefore, was to establish whether long-term occlusion of capillaries with iron hydroxide aggregates resulted in pathologic lesions.

Initially, all of the iron was deposited within the capillary lumen. After several days a portion of the iron was located in cells lining alveolar spaces and endothelial cells of capillaries. Due to the diffuse nature of the intracellular iron, it was difficult to quantitate the amount and location of all the iron but in the later animals it appeared that a measurable amount of the iron was located in septal macrophages. Some of the very fine granules of iron near

large vessels were apparently located in the lymphatics but additional studies would be needed to confirm this suspicion.

In one of the two mice sacrificed at 40 weeks, a focus of very heavy intracellular iron deposition involved approximately 50 alveolar cells in a small section of one lobe. No intravascular aggregates were present and there were no associated pathologic changes. It seems reasonable to presume that this finding represents an area of lung tissue exposed, for some unexplained reason, to an unusually large dose of iron (perhaps a single large particle which obstructed a rather large vessel). The distribution of cells favors this explanation.

The fact that the emphysema was not seen near the iron hydroxide-occluded capillaries, was age-related, and was present to the same extent in control mice, led us to conclude that it was agonal and in no way related to the presence of the macroaggregates in the pulmonary capillary bed.

The overall impression was that there were no significant pathologic consequences due to the residence of the iron hydroxide aggregates in the lungs of mice for at least 40 weeks after administration.

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ADDENDUM

After acceptance of this manuscript, an article finding pathologic damage in mouse lung related to the administration of ferric hydroxide appeared (*15*). This work along with that of Rhodes, et al (*13*) was performed with ferric (Fe^{+3}) hydroxide. To date, no lung tissue damage has been attributed to the administration of ferrous (Fe^{+2}) hydroxide and it is quite conceivable that the oxidation state of the iron is related to the pathologic potential.