

Pulmonary Perfusion Imaging: Acute Toxicity and Safety Factors as a Function of Particle Size

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Microspheres of five sizes and of three different materials were used to estimate the safety factor in pulmonary perfusion scintigraphy as a function of particle size. Particle suspensions of varying concentrations were injected into the tail vein of unanesthetized albino CD rats and mice. The LD₅₀ (24 hr) for rats was found to range from 154,000 to 705 particles per gram body weight as particle diameter ranged conversely from 13.5 to 90.7 μ. The slope of the survival curves for rats showed the minimum lethal dose (MLD) to be about 25% lower than the LD₅₀. When extrapolated to the clinical situation, these data yield safety factors of 1,663 to 16,630 for injected doses of 1 million to 100,000 microspheres 28.0 μ in diameter. The safety factor drops dramatically to 36 with an injected dose of 1 million particles 90.7 μ in diameter.

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The use of particulate radiopharmaceuticals for pulmonary perfusion scintigraphy has become an important diagnostic technique. Since the introduction of Tc-99m labeling for macroaggregates of iron hydroxide or human serum albumin (MAA), and for microspheres of human serum albumin (HAM), investigators have been concerned with the retention and possible toxicity of these particles. Both human serum albumin and iron are present in substantial quantities in the human body. Intravenous administration of ferric ion is known to cause idiosyncratic reaction (flushing response) in a significant percentage of patients, whereas ferrous ion does not elicit this adverse side effect. Immunologic studies with denatured MAA and HAM have shown no significant antigenic properties for these agents (1-3). Taplin and MacDonald have shown that the first signs of toxicity with MAA in dogs are an increase in pulmonary-artery pressure and a concomitant decrease in femoral-artery pressure. These symptoms are directly related to the size distribution and total number of particles injected (4). Thus, hemodynamic and not chemical considerations are of primary importance in assessing acute toxicity and safety factors. Although it is generally accepted that the albumin particles have a faster rate of biodegradation than has iron hydroxide (5,6), clearance

should affect only the subacute and chronic toxicity without playing a major role in acute toxicity.

There have been several different estimates of the safety factor for injected doses of iron hydroxide macroaggregates (7) MAA (4,8) and HAM (2,9,10). In a previous paper (11), toxicity was correlated with the number of vessels of a given size blocked by the injection of particles with a given diameter. It was postulated that a pulmonary agent with a diameter of approximately 13.5 μ would have a significantly improved safety factor while still fulfilling the requisite of a high first-pass extraction.

This study was undertaken to elucidate the safety factor as a function of particle size, using acute death rather than subacute pharmacologic or hemodynamic alterations as the criterion for toxicity, and to verify the greater margin of safety that exists with a particle having a 13.5 μ diameter.

MATERIALS AND METHODS

Particle preparation. Latex microspheres of five diameters were used: polystyrene-DVB 13.5 ± 1.4 μ,

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polystyrene $15.8 \pm 5.8 \mu$, styrene-DVB $25.7 \pm 10.0 \mu$, $45.4 \pm 8.9 \mu$ and $90.7 \pm 17.7 \mu^*$. The particles were obtained in aqueous suspension (10% solid by weight) and the number of particles per milliliter was determined by counting an aliquot in a hemocytometer. Styrene-DVB $90.7\text{-}\mu$ microspheres were resuspended in 10% sucrose, and all other sizes in saline with one or two drops of Tween-80®.

Carbon microspheres $15 \pm 5 \mu$ in diameter† and human serum albumin microspheres $28 \pm 12 \mu$ in diameter† were obtained in dry form. An estimate of the number of carbon particles in a given weight was obtained by multiplying the volume ($4\pi r^3/3$) by the density (1.3 g/cm^3), this product being the weight in grams per individual microsphere. The number of albumin particles per gram was assumed to be similar to the number of $25.7\text{-}\mu$ latex particles per gram for the preparation of a stock solution. The carbon microspheres were suspended in 63% sucrose with Tween-80® and the albumin microspheres in 3M Rinsing and Suspending Solution®. Both suspensions were ultrasonicated to break up clumps.

Appropriate dilutions of all stock suspensions were made, and the number of particles verified by multiple hemocytometer counts. The mean diameter and size distribution of all microspheres, when suspended under the conditions mentioned above, were determined using an automated particle-diameter measuring system having an accuracy of $\pm 0.1\%$. This system also permitted the operator to ascertain the absence of clumps.

Animal model. Particle suspensions from the various dilutions of stock were injected into the tail vein of unanesthetized albino CD-1 Charles River mice (both sexes, 18–40 g) and albino CD Charles River rats (male, 56–100 g). Four to 12 animals were tested with each stock dilution. The mice received a volume between 0.15 and 0.30 ml and the rats between 0.30 and 0.60 ml. The condition of the animals was monitored for 24 hr.

Toxicity. For each particle type and size, survival curves were plotted using regression analysis to show the relationship between the percentage of animals surviving and the number of particles injected per gram body weight. The LD_{50} was the number of particles per gram body weight giving 50% mortality within 24 hr. The minimum lethal dose (MLD) was obtained by extrapolation from the least-squares regression line to its intersection with the abscissa.

RESULTS

Most of the mice who died did so within a few minutes after injection of the microspheres, while the rats generally died within 2 hr. All of the ani-

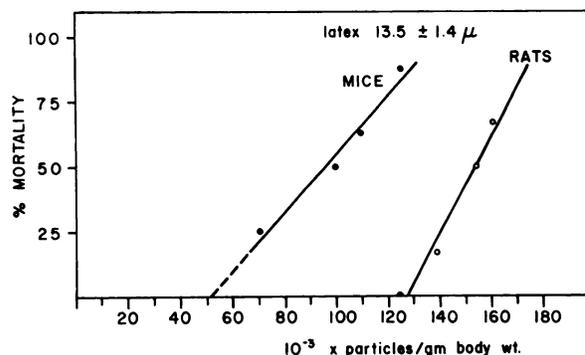


FIG. 1. Survival curves for mice and rats using latex microspheres $13.5 \pm 1.4 \mu$ in diameter ($LD_{50} = 96,000$, $r = 0.98$ for mice; $LD_{50} = 154,000$, $r = 0.99$ for rats). MLD is obtained by extrapolation of survival curves to intersect with abscissa.

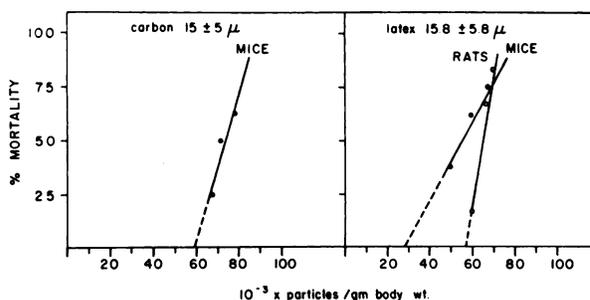


FIG. 2. Survival curves for mice using carbon microspheres $15 \pm 5 \mu$ in diameter ($LD_{50} = 73,500$, $r = 0.98$), and for mice and rats using latex microspheres $15.8 \pm 5.8 \mu$ in diameter ($LD_{50} = 56,000$, $r = 0.98$; $LD_{50} = 65,000$, $r = 1.00$, respectively).

mals that died exhibited an acute toxic response (tachycardia, dyspnea, dystaxia) immediately after administration of the microspheres. Although most animals with these symptoms died shortly after injection of the particles, a small number survived for several hours but were found dead the following morning. Since these were considered to have succumbed from the initial acute insult and not from any chronic toxic response, they were included, and thus lethality was expressed as LD_{50} (24 hr). The results would not have been significantly different if the LD_{50} (6 hr) had been used. There was no difference in the pattern of death, or in the toxic symptoms shown, between the biodegradable albumin microspheres and the nonbiodegradable latex and carbon microspheres, which clearly indicated that biodegradability is not an important factor in acute death resulting from blockade of the pulmonary capillaries.

From the survival curves (Figs. 1–4) the acute LD_{50} (24 hr) was determined to range in rats from 154,000 to 705 particles per gram body weight and in mice from 96,000 to 688 particles per gram body weight for particles with diameters $13.5\text{--}90.7 \mu$

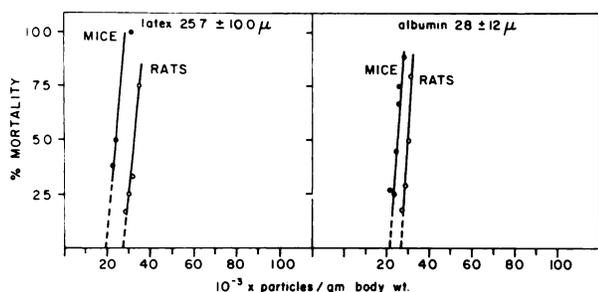


FIG. 3. Survival curves for mice and rats using latex microspheres $25.7 \pm 10.0 \mu$ in diameter ($LD_{50} = 24,000$, $r = 0.99$ for mice; $LD_{50} = 33,000$, $r = 0.97$, for rats), and albumin microspheres $28 \pm 12 \mu$ in diameter ($LD_{50} = 25,000$, $r = 0.94$; $LD_{50} = 30,000$, $r = 0.98$, respectively).

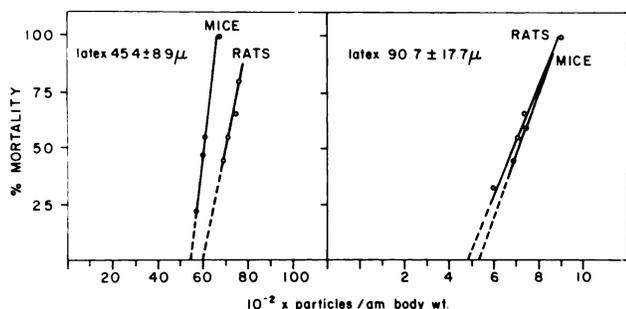


FIG. 4. Survival curves for mice and rats using latex microspheres $45.4 \pm 8.9 \mu$ in diameter ($LD_{50} = 6,000$, $r = 1.00$ for mice; $LD_{50} = 7,000$, $r = 1.00$ for rats) and latex microspheres $90.7 \pm 17.7 \mu$ in diameter ($LD_{50} = 688$, $r = 0.99$; $LD_{50} = 705$, $r = 1.00$, respectively).

(Table 1, Fig. 5). The LD_{50} values for spheres of different chemical composition but of similar size are almost identical: for example 30,000 and 33,000 particles per gram body weight in mice for albumin ($28.0 \pm 12.0 \mu$) and latex ($25.7 \pm 10.0 \mu$) spheres, respectively. The MLD in rats was approximately 20–25% less than the LD_{50} value, whereas in mice it was more variable. Using the average MLD for

mice and rats and basing calculations on a dose of 1 million particles per 70-kg man, the clinical safety factors were obtained for the different particle sizes (Table 2).

DISCUSSION

The toxicity of particulate radiopharmaceuticals and the ideal number of particles to be injected are two aspects of pulmonary perfusion scintigraphy that still require clarification (12–14). In an earlier paper, studies aimed at determining the clearance rate were discussed (11). It was pointed out that the two approaches used in the estimation of particle clearance rates—the multiple-sacrifice technique with subsequent counting of the excised lungs, and the in vivo counting of the lung field with an external scintillation probe—rely on the validity of the following two assumptions: (a) that the activity present in the lungs at any particular time is proportional to the number of particles lodged in the arteriolar capillary bed; and (b) that the values obtained in normal animals or man are unrelated to the disease state of the lungs. These may not be valid.

This investigation was designed to explore the hypothesis that the acute toxicity of the particles is directly related to their size and to the number of pulmonary blood vessels with diameters smaller than or equal to this size. Although the composition of the particles would be expected to influence their clearance rate from the lungs, it would not affect acute death in which hemodynamic considerations predominate. Changes in toxicity would occur only when the particles were dissimilar in size, thus blocking pulmonary blood vessels of different diameters. The size of the lung capillary in mice and rats is only slightly smaller than that in humans. Initially, carbon (15.0μ), albumin (28.0μ), and latex microspheres of comparable sizes were used (Table 1). Since the LD_{50} (24 hr) was determined to be similar for microspheres of the same size but of different material, latex microspheres—which are available in a range of sizes—were used to obtain a series of survival curves.

Using the average LD_{50} value of 28,000 particles per gram body weight for albumin and for latex particles of a size comparable to MAA (25.7μ) and HAM (28.0μ), the clinical safety factor based on the LD_{50} for the agents in current clinical use was calculated assuming an injected dose of 1 million particles per 70-kg man as shown:

$$\frac{28 \times 10^3 \times 70 \times 10^3}{10^6} = \frac{1960 \times 10^6}{10^6} = 1960.$$

The MLD extrapolated from the survival curves is 23,750, giving a safety factor of 1663. This indi-

TABLE 1. ACUTE LD_{50} FOR PARTICULATE LUNG-IMAGING AGENTS IN MICE AND RATS AS A FUNCTION OF PARTICLE SIZE

| Particle type | Particle size | LD_{50} (24 hr)* | |
|-------------------------|---------------------|--------------------|--------|
| | | rat | mouse |
| Latex (polystyrene DVB) | $13.5 \pm 1.4 \mu$ | 154,000 | 96,000 |
| Carbon | $15.0 \pm 5.0 \mu$ | — | 73,500 |
| Latex (polystyrene) | $15.8 \pm 5.8 \mu$ | 65,000 | 56,000 |
| Latex (styrene DVB) | $25.7 \pm 10.0 \mu$ | 33,000 | 24,000 |
| Albumin (HAM) | $28.0 \pm 12.0 \mu$ | 30,000 | 25,000 |
| Latex (styrene DVB) | $45.4 \pm 8.9 \mu$ | 7,000 | 6,000 |
| Latex (styrene DVB) | $90.7 \pm 17.7 \mu$ | 705 | 688 |

* Particles/gram body weight.

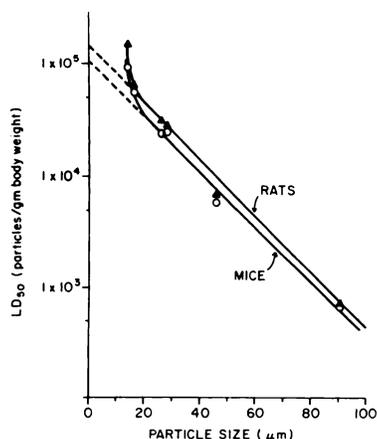


FIG. 5. Plot showing relationship between particle size (μ) and LD_{50} (particles per gram body weight) in mice and rats.

cates a wide margin of safety in the number of particles injected routinely. The safety factor for a typical clinical dose of only 100,000 HAM particles would be an order of magnitude higher than that cited in Table 2. This result is in good agreement with the data recently obtained for acute sublethal toxicity in dogs injected with various multiples of the typical human dose (8,12). It must be remembered, however, that even if the data obtained in mice, rats, and dogs can be extrapolated to human subjects, they were obtained in normal animals. In patients with chronic obstructive lung disease or severely compromised cardiopulmonary function, the safety factor could be substantially less than that in healthy individuals, due to delayed clearance and a decrease in the number of small pulmonary vessels (15-17).

The results of this study suggest that if one follows the guidelines of the FDA with respect to particle size and number (~250,000 per patient and

TABLE 2. CLINICAL FACTORS FOR INTRAVENOUSLY ADMINISTERED MICROSPHERES AS A FUNCTION OF PARTICLE SIZE

| Particle size | Safety factor* |
|-------------------|----------------|
| 13.5 ± 1.4 μ | 6283 |
| 15.0 ± 5.0 μ | 3360 |
| 15.8 ± 5.8 μ | 3360 |
| 25.7 ± 10.0 μ | 1663 |
| 28.0 ± 12.0 μ | 1663 |
| 45.4 ± 8.9 μ | 401 |
| 90.7 ± 17.7 μ | 36 |

* Based on the MLD in mice and rats and an injected dose of 1 million microspheres per 70-kg man. A typical clinical dose of 100,000 microspheres would give safety factors 10 times those shown above.

virtually all particles in the 10-50 μ range), a very large margin of safety will exist during pulmonary perfusion scintigraphy.

FOOTNOTES

* Dow Chemical Co., Particle Information Service, Indianapolis, Indiana.

† 3M Nuclear Products, St. Paul, Minn.

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The Scientific Program Committee of the Southwestern Chapter of the Society of Nuclear Medicine solicits the submission of abstracts from members and nonmembers of the Society of Nuclear Medicine for the 24th Annual Meeting to be held March 16-18, 1979 in San Antonio, Texas. The program will include submitted papers, invited speakers, and teaching sessions relevant to current topics in nuclear medicine.

Accepted abstracts will be published and should not exceed 300 words.

The title, authors, and institutional affiliations should also be included at the top of this page. The name of the author presenting the paper must be underlined. Abstracts should contain a statement of purpose, the methods used, results, and conclusions.

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