Distribution of Radiolabeled Subvisible Microspheres after Intravenous Administration to Beagle Dogs

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Abstract □ The distribution and fate of intravenously administered microspheres in four size ranges were studied using Beagle dogs as an animal model. Administration of a dosage labeled with 200 μCi of cerium-141 permitted γ-camera observation. Animals were scanned upon dosing and again after 2 and 24 hr and 1, 2, and 4 weeks. The dogs were sacrificed after the 4-week scan to quantitate the final distribution. Whole organs were excised and homogenized, and the total radioactivity was determined by analyzing representative samples in a well counter. Image analysis revealed no distribution differences between bolus administration and 30-min infusion of doses of equal particle size and count. Spheres in the 25-, 15-, and 8-μm ranges lodged by the time necessary to secure the first image (approximately 20 min) and did not undergo any observable relocation; the 3-μm spheres appeared to be delayed in the lung. Well counting revealed that essentially all of the 25- and 15-μm doses and the major part of the 8-μm spheres were accounted for in the lungs after 4 weeks. Traces of the 8-μm spheres also were found in the liver, spleen, heart, and kidneys. Of the 3-μm spheres, over 75% were recovered in the liver, 17% in the spleen, 3% in the lung, and trace amounts in the kidneys and heart. Radioactivity in other organs and blood was negligible.

Keyphrases □ Microspheres, radiolabeled—tissue distribution after intravenous administration to dogs, bolus and infusion compared □ Distribution, tissue—radiolabeled microspheres, after intravenous administration to dogs, bolus and infusion compared □ Radiolabeled microspheres—tissue distribution after intravenous administration to dogs, bolus and infusion compared

For a quarter of a century, there has been a continual effort toward understanding the problems caused by particulate matter in injectables (1–9). Such efforts have been directed to determine the amounts as well as the origin and nature of contaminants in commonly used intravenous fluids (10–14). Removal of these contaminants with final filters significantly reduced the complications associated with intravenous fluid administration (15–19). Although a number of these reports implicated particulate matter in injectables as a major hazard, relatively little is known about distribution, relocation, elimination, modes of action, and possible acute effects. Much available information has been arrived at largely by conjecture.

The purpose of the present study was to gain further knowledge about the distribution of spheres of known size after intravenous injection. Such knowledge should permit a more rational assessment of the clinical significance of foreign particulate matter introduced into the bloodstream.

Radiolabeled microspheres have been used to study regional blood flow of the peripheral circulation (20, 21) as well as internal organs such as the kidneys (22) and the heart (23); to study cardiac output (24, 25); to study arteriovenous shunting, particularly in disease cases (26–28); to diagnose emboli (29); and to image or scan internal organs (30, 31). One review (32) traced the development and introduction of biodegradable spheres for circulation studies. The recognized advantages of these spheres, prepared by aggregation of human serum albumin, were that they could be metabolized rapidly and presented less chance of producing physiological alterations than did the inert spheres used earlier. Although the studies reported did not address the clinical consequences of particulate matter injected intravenously, they suggested an excellent means for assessing the distribution and fate of particulate matter that reaches the bloodstream.

EXPERIMENTAL

Preparation of Doses—The ^{141}Ce-labeled microspheres[1] were obtained as a suspension in physiological saline packaged in multiple-dose vials. A 20-ml dose contained the desired number of spheres as well as sufficient activity of 200 μCi found suitable for the experiment. Prior to withdrawing the precalculated dose, the vial of spheres was placed in an ultrasonic bath for 10 min to assure proper dispersion. Table I summarizes the properties of the spheres, the actual volume of suspension, and the number of spheres administered.

The actual sizes listed in Table I were obtained by a microscopic technique, and the size distribution and particle counts were verified by using an automatic electronic particle counter[2]. The radioactive dose intended was 200 μCi, but measurement of the specific activity of the actual dose administered revealed slight discrepancies which were corrected in all calculations. The measurement was performed by spiking nonradioactive homogenized tissue samples with a known volume of the suspension using an Eppendorf pipet. This approach was also employed to assess the efficiency of the γ-counter used in evaluating the radioactive contents of tissue samples of the dogs dosed.

Preparation of Experimental Animals—Small beagle dogs, 9.1–11.3 kg, were randomly assigned to four groups corresponding to the four particle-size ranges. Initially, three dogs were selected for each group. Prior to administering a dose, the dogs were anesthetized with 20 mg of pentobarbital sodium/kg iv. An indwelling catheter equipped with a three-way stopcock was inserted in the right radial vein, and 5% dextrose in 0.2 *N* saline was administered through an intravenous administration set with a medication chamber at a rate of 0.1 ml/min to maintain access to the vein until it was no longer needed. It was felt that this approach would reduce the chance of accidentally administering the dose in the muscle or under the skin.

Two dogs in each group were given the dose directly through the stopcock over 1 min. For comparison, the dose for the third dog was diluted to 60 ml in the medication chamber and administered as a constant intravenous infusion over 30 min. For rescans on subsequent days, the dogs were again anesthetized with pentobarbital sodium by direct venipuncture.

Scanning of Dogs—After drug administration, the dog was positioned securely under the PHO-γ-camera[3], with the chest facing the collimator plates (Fig. 1). To be sure no accumulation of radioactivity went undetected, several dogs were scanned twice (dotted circles in Fig. 1), but this practice proved to be unnecessary since no radiation could be detected outside of the central view.

Each dog was scanned until the camera had accumulated 200,000 emissions. Upon completion of the scan, the scanning time was recorded and a Polaroid picture of the distribution was obtained. The dogs were scanned immediately following administration and at 2 and 24 hr and 1, 2, and 4 weeks.

Preparation of Tissue Samples for Quantitative Determination of Distribution—On the day following the 4-week scan, selected dogs were anesthetized and sacrificed by administration of an overdose of saturated potassium chloride solution by direct cardiac puncture. In

[1] Nuclear Products Division, 3M Co., St. Paul, Minn.
[2] Model PC-305SSTA (D-2-60 sensor), High Accuracy Products Corp., Montclair, Calif.
[3] Searle model HP scintillation camera.

Table I—Properties of Radiolabeled Microspheres Used and Doses Administered

Size, μm		Dog	Specific	Total Dose Administered		
Nominal	Actual	Number	Activity, μCi/ml	Volume, ml	Radioactivity, μCi	Spheres (Millions)
3	3.3 ± 0.6	892	9.53	21.0 Infusion	200	817
3		1001	9.53	21.0 Infusion	200	817
3		888	9.53	21.0 Infusion	200	817
3		982	9.53	21.0 Bolus	200	817
3		885	0.465	21.5 Bolus	10	83.0
3		995	0.465	21.5 Bolus	10	83.0
8	8.9 ± 0.6	991	7.67	27.0 Infusion	207	34.0
8		983	7.67	27.0 Bolus	207	34.0
8		997	7.67	27.0 Bolus	207	34.0
15	16.1 ± 1.7	1004	9.56	22.7 Infusion	217	9.4
15		897	9.56	22.7 Bolus	217	9.4
15		898	9.56	22.7 Bolus	217	9.4
25	25.9 ± 0.9	829	9.06	20.5 Infusion	186	1.51
25		837	9.06	20.5 Bolus	186	1.51
25		830	9.06	20.5 Bolus	186	1.51

addition to blood samples, the following whole organs were removed: lung, heart, liver, spleen, kidneys, brain, gallbladder, and neck lymph nodes.

The organs were weighed and cut into cubes of about 1 cm. Distilled water was added to double the weight to facilitate homogenization, which was accomplished with a tissue homogenizer[4]. Aliquots between 5 and 10 g were weighed to within 50 mg using a top loading balance. The samples thus prepared were analyzed using an automatic γ-scintillation deepwell counter[5].

RESULTS AND DISCUSSION

Choice of Experimental Model—Polystyrene divinylbenzene microspheres were chosen as a model for particulate matter to maximize control and reproducibility. These microspheres are commercially available in the size ranges of interest and can be manufactured with a choice of several radioactive labels without further chemical treatment. Cerium-141 was selected because it possesses a strong γ-emission that can be detected with imaging equipment used with technetium Tc 99m. In addition, the cerium-141 half-life of about 33 days made it feasible to rescan the same experimental animal for up to 4 weeks, the duration of the study.

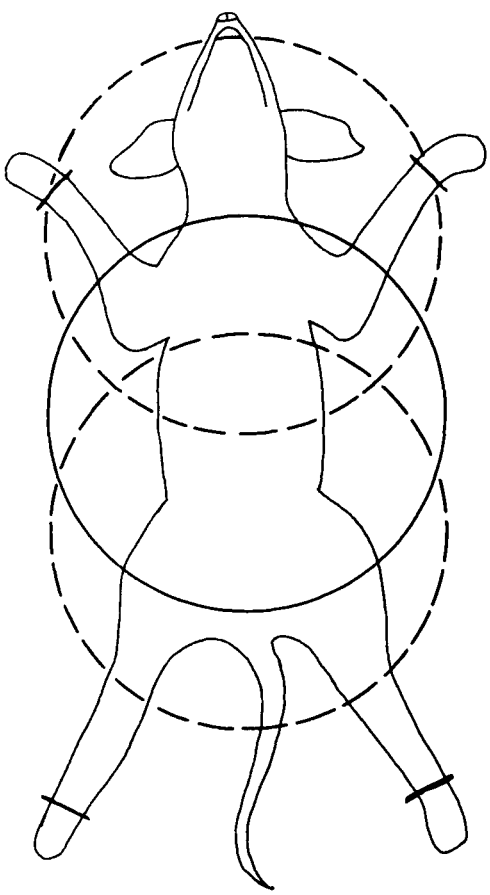

Figure 1—*Schematic representation of the field of view of the PHO-γ-camera.*

The choice of experimental animal was made on the basis of several considerations. The relatively limited resolution of about 10 mm for the camera available required the use of a large animal for imaging purposes. However, since the field of view was limited to a circle of about 30 cm in diameter, the animal could not be too large if imaging was to be accomplished in one exposure. Small dogs weighing 9.1–11.3 kg appeared to satisfy the geometric requirements. To minimize intraspecies variations, beagle dogs in that size range were chosen.

Qualitative Distribution Studies of 3-μm Spheres—Attempts to dose the full 200 μCi in the 3-μm range resulted in death by respiratory failure of three experimental animals. Although the amount of barbiturate was reduced for subsequent dogs after the first death and the dogs were placed on a respirator before starting the infusion, two additional dogs died of respiratory failure during the first scan. In an attempt to gather at least a minimum of information, a fourth dog was given the same dose of spheres. This dog survived the bolus of spheres and was scanned upon administration and 2 and 24 hr later.

Since the 3-μm spheres were the most likely to distribute over a wider area, the head and chest were scanned first, followed by a scan of the lower thorax and abdomen (Fig. 2). From the initial and 2-hr scans, it can be observed that initially a large portion of the spheres was retained in the lung. The initial scan of the lower body, which actually was started about 20 min after dosing because of the duration of the scan of the upper body, already shows a much heavier accumulation of particles in the area of the liver; the 2-hr scan reveals that the lung is rather clear of particles at this time.

Although the dog appeared to be in relatively good condition after the 2-hr scan, its condition deteriorated the following day. The 24-hr scan had to be done in a lateral position since the dog was unable to breathe in the supine position. This scan again shows that the spheres had almost entirely cleared the lungs after 24 hr. Since the dog's condition had deteriorated, it was sacrificed at this time.

Because of the death of the animals in the 3-μm group, two additional

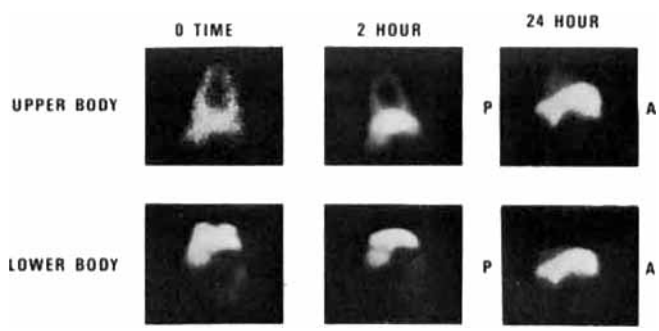

Figure 2—*Distribution of 3-μm spheres after intravenous administration. The 24-hr scan was done with the dog (No. 982) in a lateral position.*

[4] Tissumizer, model SDT, Tekmar Co., Cincinnati, Ohio.
[5] Bio-gamma, model 310, Beckman Instruments, Fullerton, Calif.

Table II—Comparison of Expected and Actual Scanning Times in Seconds [a]

Nominal Size, μm	Dog Number	Initial Scan (Actual)	2-Week Scan		4-Week Scan	
			Expected [b]	Actual	Expected [b]	Actual
8	991	919	1200	1278	1650	—
	983	1124	1500	1438	2010	1964
	997	951	1260	1183	1680	1756
15	1004	1024	1350	1309	1830	1810
	897	961	1260	1150	1710	1596
	898	979	1170	1066	1740	1674
25	829	2321	3060	2861	4140	4151
	837	1671	2190	1802	2970	2736
	830	1935	2250	2369	3450	3250

[a] Time for camera to observe 200,000 counts. [b] Calculated to the nearest 30 sec.

dogs were dosed using a 10-fold decrease in the number of spheres. Since the experiment was performed at a later date, the specific activity and, thus, the total radioactive dose had decreased to the level indicated in Table I. Although the two dogs given the lower doses in this size range could not be scanned, quantitative evaluation was obtained by analyzing the tissue samples.

Qualitative Distribution of Spheres in 8-, 15-, and 25-μm Size Ranges—The dogs assigned to these size groups did not suffer any major consequences, with one exception. One dog died of barbiturate overdose prior to the 2-week rescan. The fact that the dog belonged to the 8-μm group, *i.e.*, the second highest dose, may be coincidental since there appeared to be nothing wrong with the animal otherwise.

Analysis of the scans of the dogs dosed with spheres in the 8-, 15-, and 25-μm ranges revealed that the vast majority of these spheres were filtered out and retained in the lung. Figures 3–5 show the scans obtained from dogs that had received a bolus dose of 200 μCi in the size range of 8, 15, and 25 μm, respectively. From the rescans at 2 and 24 hr and 1, 2, and 4 weeks, it also appears evident that no major relocation of the spheres occurred following the initial distribution.

Figure 6 shows a comparison of the initial scans obtained by a bolus dose to the initial scans obtained by infusing the spheres over 30 min. Qualitatively, the distributions were the same, indicating that no major physiological changes took place over the short period selected for infusion.

Table II lists the actual scanning times of selected dogs in each size range. The expected scanning times are based on the initial scanning times, taking into account isotope decay. Since exact relocation of the dogs under the camera in successive scans is practically impossible, variations in the scan times can be expected; but the absence of major discrepancies between actual and predicted scan time appears to be a good indication that no elimination of spheres had taken place throughout the experiment.

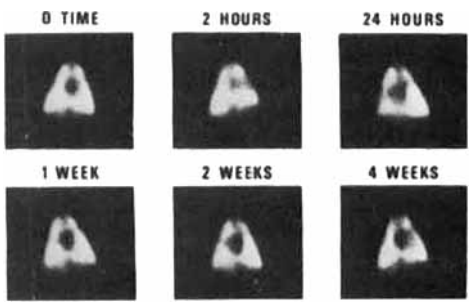

Figure 3—*Distribution of 8-μm spheres up to 4 weeks after bolus intravenous administration in Dog 997.*

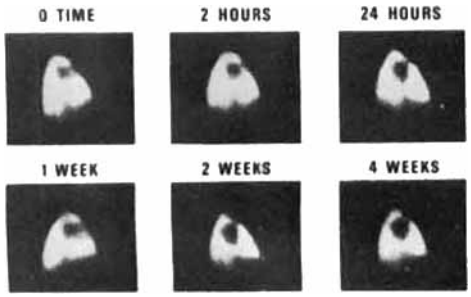

Figure 4—*Distribution of 15-μm spheres up to 4 weeks after bolus intravenous administration in Dog 897.*

The scanning time for the 25-μm treated dog was substantially longer than for the 8- and 15-μm treated dogs. Upon inspection of the quantitative results that follow, it can be noticed that the recovery of the 25-μm spheres was also very low. In the complete absence of radioactive counts in any of the other organs and with no evidence for relocation or elimination, underdosing due to faulty redispersion or other problems in drawing up the dose appears to be the only reasonable explanation. This conclusion is supported by the discrepancies observed in the scanning times.

Quantitative Recovery of Radioactive Spheres from Tissue Samples—The radioactive spheres that remained in the organs were quantitated. Blank tissue homogenates were used for dilution when count rates of undiluted homogenates were too high. Sample tubes were filled to about the same weight. Under these conditions, the efficiency of the counter was constant over the count rates observed.

From the qualitative distribution observed by means of the scanning, it appeared evident that there was no major variation between dogs in the same group. It was felt that sacrificing only one dog per group would be sufficient to obtain the results for the recovery and distribution of radioactive spheres in the 8-, 15-, and 25-μm size ranges. In the 3-μm range, both of the dogs were sacrificed to obtain an estimate of the variations in the distribution between these two animals. Although small samples of tissue were removed from one dog for a histological evaluation, the results presented in Table III are in good agreement, thus adding confidence to the results obtained from the single determination of the other size ranges.

CONCLUSION

The results of both qualitative and quantitative evaluations of the distribution indicate that most particles above 8 μm are filtered out by

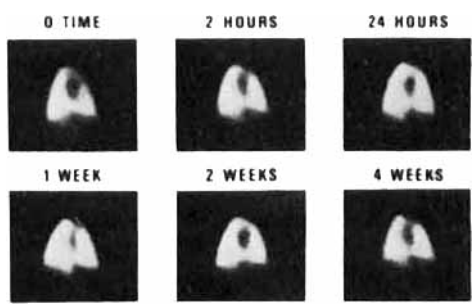

Figure 5—*Distribution of 25-μm spheres up to 4 weeks after bolus intravenous administration in Dog 837.*

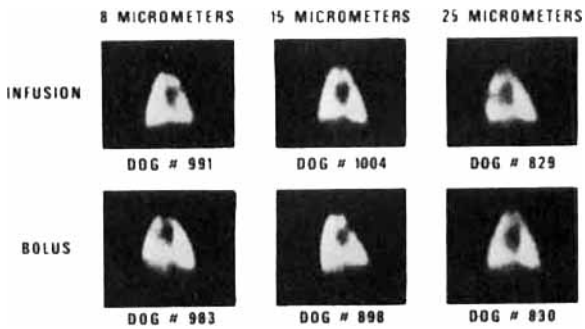

Figure 6—*Comparison of distribution after infusion and bolus administrations of radioactive microspheres (initial scan).*

Table III—Quantitative Recovery of Radioactivity in Organs Expressed as Percent of Dose

Organ	3 μm, Dog 885	3 μm, Dog 995	8 μm, Dog 997	15 μm, Dog 897	25 μm, Dog 937
Lung	3.2	2.7	91.2	94.8	62.72
Heart	—[a]	—[a]	0.1	—[a]	—[a]
Liver	76.2	78.7	0.6	—[a]	—[a]
Spleen	18.3	16.4	0.1	—[a]	—[a]
Kidneys	0.4	0.1	—[a]	—[a]	—[a]
Brain	—[a]	—	—	—	—
Gallbladder	—[a]	—	—[a]	—[a]	—[a]
Lymph node	—[a]	—	—[a]	—[a]	—[a]
Blood	—[a]	—	—[a]	—[a]	—[a]
Total recovery	98%	98%	92%	95%	63%

[a] Less than 0.05% of initial dose.

the lung and appear to localize there indefinitely.

The information obtained using the 3-μm spheres indicates an initial, presumably mechanical retention by the lungs with subsequent relocation by phagocytosis in the liver and the spleen for indefinite periods (33). More detailed studies of this phenomenon are warranted; by using spheres of higher specific activity or more sensitive scanning equipment, it would be possible to obtain more precise data on the relocation characteristics observed for these smaller spheres.

Although the administration of large doses of microspheres can be regarded as a major factor contributing to the death of several experimental animals, other possibilities must be taken into account. The relatively high maintenance doses of barbiturates needed to maintain the dogs absolutely still during scanning may have been responsible in part. Moreover, since the suspensions used were prepared elsewhere, there is no guarantee as to the purity of the vehicle. These points are raised since acute effect experiments at much higher levels of spheres did not result in death to any experimental animal (33).

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