A Rapid Method for the Labeling of Albumin Microspheres with In-113 and In-111: Concise Communication

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A rapid and simple method for preparing microspheres labeled with In-113m or In-111 is described. The procedure requires 10 min and gives labeling yields approaching 100%. Biodistribution studies in rats, mice, and dogs show the product to be biologically stable, with approximately 90% of the injected dose localized in the lung at 1 hr postinjection. The convenient production of In-113m or In-111 albumin microspheres provides an alternate radiopharmaceutical to complement or substitute for Tc-99m microspheres in lung perfusion imaging and other circulation studies.

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Published methods for labeling human serum albumin (HSA) microspheres with In-113m are time-consuming and require multiple manipulations (*1–4*). This report describes a simple, rapid, and efficient method for labeling albumin microspheres with radioactive indium, which will provide an alternate radiopharmaceutical to complement or substitute for Tc-99m microspheres in lung perfusion imaging and other circulation studies.

MATERIALS AND METHODS

Microsphere labeling and quality control. The basic method used in labeling microspheres with indium was to add 1 cc of 5% sodium acetate, 4 cc of 0.05 N HCl, and 0.1 cc of In-111 (300 μCi) to a microsphere vial. The vial was briefly sonicated (30 sec) and then heated in a boiling-water bath for 5 min. The vial was sonicated again (30 sec) and allowed to cool to room temperature.

Initial studies indicated that sodium acetate could facilitate the complexing of indium by HSA microspheres. To study the effect of various quantities of sodium acetate on the labeling efficiency, 1-cc solutions containing 0, 10, 25, 50, 100, 200, and 300 mg of sodium acetate, respectively, were added to HSA lung microsphere kits followed by 6 cc of In-113m eluate (Sn-113/In-113m generator). The vials were sonicated for 30 sec and placed in a boiling-water bath for 5 min. The microsphere suspension was again sonicated for 30 sec and allowed to cool to room temperature.

Labeling yield was determined by thin-layer chromatography on ITLC-SG thin-layer strips with 0.05 N HCl as developing solvent. Under these conditions, free indium migrated with the solvent front and indium bound to the microspheres remained at the origin. Organ distribution studies of the various preparations were carried out in female Swiss Webster mice weighing approximately 20 g. Each animal was injected with 0.1 cc of the microsphere suspension and killed 5 min later by CO_2 asphyxiation. The organs were then removed, weighed, and counted.

The effect of heating on labeling efficiency was evaluated. Sodium acetate (1 cc of a 5% solution) and indium eluate (6 cc) were added to two microsphere vials. One vial was heated in a boiling-water bath, cooled to room temperature, and sonicated for 30 sec, whereas the other was sonicated for 15 min without heating. Each microsphere suspension

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was injected into five mice. The animals were sacrificed 5 min postinjection and the organs removed, weighed, and counted.

Human albumin microspheres (10–35 μm), not presoaked in tin, were also labeled with indium by the method previously described. Animal distribution studies in mice were carried out as above, and thin-layer chromatography studies determined labeling efficiency.

Biodistribution studies. In order to determine the rate of clearance of radioactive indium from the lung, microspheres were labeled with In-111 ($T_{1/2}$ = 2.8 d) and injected into rats. Organ distribution studies were carried out in 42 female Swiss Webster rats weighing 60–90 g. The animals were injected in the tail vein with 0.1 cc of the microsphere suspension. They were then placed in metabolic cages and given food and water. Six animals were killed by CO_2 asphyxiation at 5 min, and 1, 2, 4, 6, 24, and 48 hr postinjection. Urine and feces excreted by the animals at death were collected on blotter paper. Blood samples were taken by cardiac puncture, and the total activity in the blood was calculated based on the total weight of the animal (*8*). The organs were excised, washed briefly in water, and then dried. The remaining carcass was divided into three pieces for counting. Geometry corrections were made for the carcass and the samples of urine and feces.

The organ distribution of In-111 microspheres was also studied in dogs. The distribution of radioactivity was examined in two dogs (24 and 33 kg) after the simultaneous injection of In-111 microspheres, Tc-99m microspheres, and I-131 macroaggregated albumin into a femoral vein. The organ uptakes in the lungs, liver, spleen, and blood (carcass) were determined. Activity of each radionuclides in the organs was assayed using a Ge(Li) detector.

RESULTS

Microsphere labeling and quality control. At quantities of 50–300 mg of sodium acetate per vial, the pH ranged between 3 and 5.5 after addition of 3–6 cc of indium eluate. Using this formulation, labeling efficiency was greater than 95%. The effects of various amounts of sodium acetate on the lung uptake of labeled microspheres are summarized in Fig. 1. With greater than 50 mg of sodium acetate per vial, the animal data showed greater than 90% of the radioactivity in the lungs and minimal activity in the carcass and liver. When less than 50 mg of sodium acetate were used, the carcass accumulation of activity increased, suggesting the presence of free indium.

As the pH was increased from 3 to 6, using sonication only, the binding efficiency of indium to the microspheres also increased, but heating was necessary to maximize binding efficiency. For example, at pH 3, sonicated but unheated microspheres gave a labeling yield of less than 25% compared with 94% when the heating step was included (Table 1). Furthermore, the stannous ion was also essential to achievement of a high labeling efficiency and optimal biodistribution (Table 2).

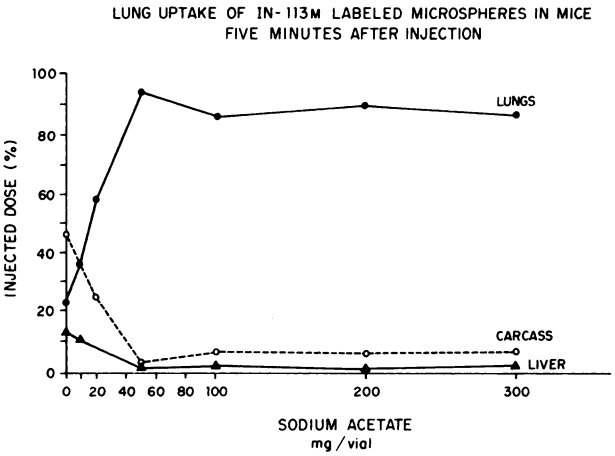

FIG. 1. Greater than 50 mg of sodium acetate in the reaction vial results in 90% of lung uptake of In-113m-labeled microspheres.

Biodistribution studies. The organ distributions for In-111-labeled microspheres showed very little clearance of indium from the lungs during the 48-hr period of observation, with 96.9% ± 1.0 of the injected dose recovered in the lungs at death 5 min postinjection, and 92.1% ± 1.2 recovered at 48 hr (Table 3).

TABLE 1. EFFECT OF HEATING AND SONIFICATION, VERSUS SONICATION ALONE, ON ORGAN DISTRIBUTION AND LABELING EFFICIENCY OF In-113m MICROSPHERES

	Labeling efficiency			
	Heating and sonication		Sonication only	
	94.1% bound	5.1% free % dose/ organ*	24.3% bound	75.7% free % dose/ organ*
Lungs		93		36.7
Liver		1.0		6.9
Spleen/pancreas		0.1		0.7
Kidneys		0.8		7.5
Stomach		0.2		1.3
Gut		0.4		6.9
Heart		0.2		0.8
Carcass		4.0		37.0
Urine and feces		0.3		2.2

* Each data point represents the mean of five mice.

TABLE 2. EFFECT OF TIN ON ORGAN DISTRIBUTION AND LABELING EFFICIENCY OF MICROSPHERES LABELED WITH In-113m

	Labeling efficiency			
	Tin present		Tin absent	
	89.3% bound	10.8% free % dose/ organ*	5.0% bound	95% free % dose/ organ*
Lungs		90.9		10.4
Liver		2.9		17.4
Spleen/pancreas		0.4		1.3
Kidneys		0.5		8.4
Stomach		0.9		1.2
Gut		0.4		7.3
Heart		1.2		1.5
Carcass		3.2		50.3
Urine and feces		0.7		2.3

* Each data point represents the mean of five mice.

Organ distribution studies in two dogs, following the simultaneous administration of In-111 microspheres, Tc-99m microspheres, and I-131 macroaggregates, are presented in Table 4. The results showed a very close correlation between dose percentage per organ for the three radiopharmaceuticals recovered in the lungs at 5 min after injection and sacrifice. Lung ratios of In-111 to Tc-99m were 1.13 and 0.98 in each dog, while indium-to-iodine ratios recovered in the lungs were 0.95 and 0.93.

DISCUSSION

Buchanan et al. (*1,2*) have described the labeling of microspheres with In-113m, and find as much as 94% of the injected dose localizing in the lungs of mice, but their several manipulations and the 1-hr preparation time are major disadvantages, especially when In-113m is used ($T_{1/2}$ = 1.73 hr). The method described in the present report requires approximately 10 min for preparation of the labeled material. The data presented for mice and rats showed that consistently greater than 90% of the In-111- and In-113m-labeled microspheres localized in the lungs. The dog data for In-111 microspheres showed 85–88% of the injected dose localized in the lungs; these results were equivalent to those obtained for Tc-99m microspheres and I-131 macroaggregated albumin in the same dogs.

The acetate ion in this reaction mixture rapidly complexes with the indium (*10,11*), thereby preventing indium from precipitating from solution as indium hydroxide. The acetate also functions as a buffering agent, raising the pH of the acid indium chloride solution to the range of 3–5.5 where the labeling yield is optimal. Our data show that stannous ion is a prerequisite for the labeling of microspheres by indium; the mechanism of labeling may involve a complex relationship between indium, stannous ion, and coordination sites on albumin. Sodium acetate has been used as parenteral therapy for acidosis and in solutions for hemo- and peritoneal dialysis; the quantity used in the indium microsphere formulation is much less than the gram quantities used clinically (*12*).

Based on the results of this study, the best procedure for the optimal labeling of albumin microspheres with radioindium chloride requires addition of 1 ml of a 10% sodium acetate solution and 3–6 ml of In-113m or In-111 in 0.05 N HCl to a microsphere-labeling vial. The vial is immersed in a water or oil bath at 100°C for 5 min and then cooled in an operating ultrasonic bath for 1 min. Final pH ranges from 3.0 to 5.5. This procedure results in a labeling yield of 90% or greater and a biologically stable product suitable for lung imaging and other circulation studies.

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TABLE 3. BIOLOGIC DISTRIBUTION OF In-111-LABELED ALBUMIN MICROSPHERES IN RATS

	Percentage of injected dose remaining*						
Organ†	5 min	1 hr	2 hr	4 hr	6 hr	24 hr	48 hr
Lungs	96.9 ± 1.0	96.6 ± 2.4	92.5 ± 3.1	95.8 ± 2.5	95.2 ± 1.9	93.5 ± 4.6	92.1 ± 1.2
Liver	0.2 ± 0.1	0.5 ± 0.5	0.6 ± 0.6	1.2 ± 1.9	0.6 ± 0.2	0.2 ± 0.1	1.5 ± 0.8
Kidneys	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
Stomach	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.0	0.1 ± 0.1	0.0	0.1 ± 0.0
Gut	0.1 ± 0.1	0.3 ± 0.3	1.2 ± 0.7	0.6 ± 0.7	0.9 ± 0.4	0.4 ± 0.2	0.4 ± 0.2
Heart	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
Carcass	1.6 ± 0.8	1.3 ± 0.6	2.8 ± 1.5	1.2 ± 0.3	1.3 ± 0.3	1.7 ± 0.5	2.9 ± 0.8
Urine and feces	0.6 ± 0.4	0.6 ±	2.3 ± 1.0	0.8 ± 0.8	1.5 ± 1.2	3.2 ± 3.8	2.5 ± 1.4
Blood (total)	0.7 ± 0.5	0.2 ± 0.1	0.6 ± 0.3	0.4 ± 0.1	0.4 ± 0.2	0.3 ± 0.1	0.2 ± 0.1

* Mean ± one standard deviation.
† At all time periods, the percentage injected dose in the spleen, pancreas, eyes, and ovaries was less than 0.1%.

TABLE 4. COMPARISON OF ORGAN DISTRIBUTION OF In-111 MICROSPHERES, Tc-99m MICROSPHERES, AND I-131 ALBUMIN MACROAGGREGATES 5 MIN AFTER SIMULTANEOUS INJECTION IN DOGS

	Dog 1		Dog 2	
	% dose/g	% dose/ organ	% dose/g	% dose/ organ
Lung				
In-111 MS	0.77	88	0.72	85
Tc-99m MS	0.68	78	0.74	87
I-131 MAA	0.82	93	0.78	92
Liver				
In-111 MS	0.0018	0.60	0.0005	0.26
Tc-99m MS	0.0017	0.58	0.0004	0.19
I-131 MAA	0.0034	1.2	0.0009	0.45
Heart				
In-111 MS	0.0016	0.26	0.0061	1.1
Tc-99m MS	0.0029	0.45	0.0046	0.81
I-131 MAA	0.0021	0.33	0.0024	0.43
Carcass 1/blood 2				
In-111 MS		4.6	0.0108	11
Tc-99m MS		6.4	0.22	23
I-131 MAA		4.3	0.0074	7.8
Lung ratios (% dose/organ)				
I-111/Tc-99m	1.13		0.98	
In-111/I-131	0.95		0.93	
Blood = 7% body weight				

large work loads may consider having both a Tc-99m and an In-113m generator available for use in labeling microspheres intended for regional pulmonary perfusion studies; in particular, those institutions using Tc-99m-labeled aerosols (*5–7*) would be able to perform both ventilation and perfusion studies at the same time. Research aimed at studying acute change in small-vessel perfusion as a consequence of experimental intervention may be facilitated by the complementary use of microspheres labeled separately with In-113m, In-111, and Tc-99m (*8,9*). Finally, nuclear medicine facilities in regions without reliable access to Tc-99m would be able to offer lung scanning with In-113m microspheres without delay or compromise in patient care.

ACKNOWLEDGMENT

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