Direct Labeling of Macroaggregated Albumin with Indium-111-Chloride Using Acetate Buffer

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Indium-111-labeled macroaggregated albumin (MAA) would be suitable for combined pulmonary perfusion and ventilation scan using a ^{99m}Tc ventilation agent. **Methods:** MAA suspended in 0.1 *M* sodium acetate buffer, pH 5.8, was incubated with ^{111}In-chloride for 30 min at room temperature. An in vitro study of the obtained ^{111}In-MAA was performed for labeling efficiency and stability in human normal serum. The ^{111}In-MAA was intravenously injected into normal mice, and the biodistribution was studied at 15 and 180 min postinjection. A gamma camera image was obtained at 15 min after injection. **Results:** MAA was directly and stably labeled with ^{111}In-chloride, and the labeling efficiency of the preparation was more than 96%. More than 90% of the administered ^{111}In-MAA was caught in the murine lung. The scintigraphy with ^{111}In-MAA showed a clearly visualized murine lung. **Conclusion:** Indium-111-MAA can be conveniently prepared by direct labeling at room temperature. It provides an alternative perfusion tracer for combined perfusion-ventilation imaging.

Key Words: indium-111-MAA; pulmonary perfusion agent

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It is desirable to perform combined pulmonary perfusion and ventilation imaging to assess and monitor pulmonary disease (*1*). When ^{81m}Kr-gas, as a pulmonary ventilation agent, is paired with ^{99m}Tc-macroaggregated albumin (MAA) as a perfusion agent, a simultaneously combined pulmonary perfusion and ventilation scan can be performed during a patient's single visit using a dual-isotope technique because the photo peaks of ^{81m}Kr (190 keV) and ^{99m}Tc (141 keV) are different. When a ^{99m}Tc-ventilation agent such as ^{99m}Tc-diethylenetriaminepentaacetic acid (DTPA) (*2*) or technegas (*3*) is used, there is no pulmonary perfusion agent suitable for a simultaneously combined pulmonary perfusion and ventilation scan using a dual-isotope technique. Indium-111-labeled (171 keV, 245 keV) MAA has been designed. Though MAA was first labeled with ^{111}In-chloride through a bifunctional chelate such as cyclic DTPA (*4*), it has been found that MAA suspended in a sodium acetate buffer may be directly and simply labeled with ^{111}In-chloride. We propose ^{111}In-MAA as a potential pulmonary perfusion agent.

MATERIALS AND METHODS

Direct Labeling of MAA with Indium-111-Chloride

One milliliter of original solution from a MACROKIT™ (Dainabot, Tokyo, Japan), which includes 2 mg MAA, 0.23 mg $SnCl_2$ and 9 mg benzylalcohol, was removed by centrifugation at 3000 rpm, 4° C for 1 min. The pellet was washed with phosphate-buffered saline (4.3 m*M* Na2HPO$_4$ [A0]H$_2$O, 1.4 m*M* KH$_2$PO$_4$, 137 m*M* NaCl, 2.7 m*M* KCl, pH 7.2) (PBS) three times and divided into two equal parts. One was suspended in 500 μl of 0.1 *M* sodium acetate, pH 5.8, and another was suspended in 500 μl of PBS as a control buffer. Each of these MAA suspensions was mixed with 500 μl of ^{111}In-chloride (74 MBq/ml: Nihon Medi-physics Co., Ltd., Nishinomiya, Japan) and incubated for 15, 30, 60 and 180 min at room temperature. Labeling efficiency of the obtained ^{111}In-MAA was estimated by a centrifugation method and by an electrophoresis method. The supernatant and the pellet were separated by centrifugation at 15,000 rpm at room temperature for 10 min. The radioactivity of the supernatant and the pellet was then measured with a well scintillation gamma counter (Aloka, Tokyo, Japan) after about 1 mo waiting for the decay of ^{111}In. Labeling efficiency was calculated as follows: {(counts per min of the pellet)/[(counts per min of the pellet)+(counts per min of the supernatant)]} × 100 (%). On the other hand, 0.9% agarose gel electrophoresis of ^{111}In-MAA and ^{111}In-chloride were performed. The gel sample was then placed in contact with the surface of the gamma camera (ZLC 7500, Siemens, Erlangen, Germany) with a medium-energy collimator. The data were entered into a digital computer (Scintipac 700, Schimadzu, Kyoto, Japan) with a 128 × 128 matrix size and the energy window ranging from 220–270 keV. With the ROIs being set, the amount of radioactivity found at the starting point or in the free ^{111}In-position was calculated as a percentage of the total activity and expressed as the labeling efficiency for the preparation. In vitro stabilities of ^{111}In-MAA were examined by the incubation of obtained ^{111}In-MAA with an equal volume of normal human serum or with an equal volume of PBS as a control for 15 and 180 min. The labeling efficiencies for the stability were estimated with both the centrifugation method and the agarose electrophoresis method as described above. In vitro studies were performed five times. The size of the ^{111}In-MAA particles in 0.1 *M* sodium acetate buffer, pH 5.8, was examined with an optical microscope and a hemocytometer using 40 X magnification compared with MAA in the original solution.

In Vivo Study and Imaging of Normal Mice with Indium-111-MAA

Six-week-old normal female Balb/c mice were injected intravenously with 70 μl ^{111}In-MAA suspensions (37 MBq/mg/ml). One

TABLE 1
Labeling Efficiency of Indium-111-MAA for Various Incubations at Room Temperature

Labeling buffer	Labeling efficiency (%)			
	15 min	30 min	60 min	180 min
0.1 M Acetate buffer pH 5.8	96.4 (0.18)	96.5 (0.45)	96.3 (0.18)	96.6 (0.29)
Phosphate-buffered saline pH 7.2	22.4 (2.75)	18.5 (2.29)	18.2 (2.40)	18.5 (2.36)

Each value represents the mean (1 s.d.) from five experiments.

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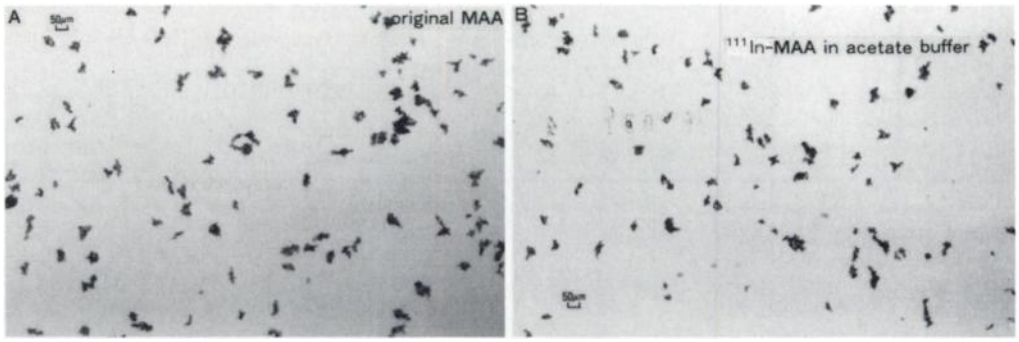

FIGURE 1. Microscopic view of MAA (X 40). (A) MAA particles in the original solution; (B) ^{111}In-MAA particles in 0.1-*M* sodium acetate, pH 5.8. Aggregates of MAA particles were not observed in (B) as in (A).

group of normal mice (n = 4) was killed 15 min after injection by heart puncture under general anesthesia. Another group (n = 4) was killed 180 min after injection. The organs were removed, weighed and counted using a well scintillation gamma counter. The results were expressed as percent injected dose/gram tissue (% ID/g) normalized to a 20-g mouse, percent injected dose/organ and organ-to-blood radioactivity concentration ratios. Whole-body images of normal mice also were obtained 15 min after injection of ^{111}In-MAA using a ZLC 7500 gamma camera equipped with a medium-energy collimator and collected on a 128 × 128 matrix with digital computer Scintipac 700. Animal studies were done in compliance with the relevant laws relating to the conduct of animal experiments. Statistical comparisons were made by the Mann-Whitney's U-test. Differences were considered statistically significant when $p < 0.05$.

Control Study

Technetium-99m-MAA (37 MBq/mg/ml) was used as a control. In vitro stabilities of ^{99m}Tc-MAA were examined by incubation with an equal volume of normal human serum or with an equal volume of PBS for 15 and 180 min. The stability of the labeled compound was estimated by the centrifugation method and the electrophoresis method. Six-week-old normal female Balb/c mice were injected intravenously with 70 μl ^{99m}Tc-MAA suspension, and biodistribution data were obtained in the same manner as described above.

RESULTS

The labeling efficiency of ^{111}In-MAA obtained in 0.1 *M* sodium acetate, pH 5.8, was more than 96%, whereas that obtained in PBS ranged from 18%–22% (Table 1). Aggregates of ^{111}In-MAA particles were not found microscopically (Fig. 1). In 0.9% agarose gel, most of the ^{111}In-MAA did not migrate during electrophoresis because of the large particle size and therefore remained in the well (at the starting point). Indium-111-chloride migrated to the anode. Labeling efficiency calculated by the centrifugation method generally corresponded well with that obtained by the electrophoresis method. For in vitro stability examinations, ^{111}In-MAA and ^{99m}Tc-MAA were incubated with an equal volume of human normal serum or with PBS for 15 and 180 min. Indium-111 dissociated from ^{111}In-MAA was 0% and about 2% when incubated with human normal serum for 15 and 180 min, whereas free ^{99m}Tc from ^{99m}Tc-MAA was 0% and from 1% to 2%, respectively. No free ^{111}In or ^{99m}Tc was found from ^{111}In-MAA or ^{99m}Tc-MAA when incubated with PBS. The biodistribution of normal mice with ^{111}In-MAA showed very high lung activities (90.3% ID/organ and 83.3% ID/organ at 15 and 180 min, respectively) (Table 2). The organs except for the lung showed very low activity 15 min after injection. However, slightly increased radioactivities of the liver, kidney and spleen were found at 180 min after injection compared to those obtained at 15 min. Compared with the biodistribution of ^{111}In-MAA with ^{99m}Tc-MAA, there was no significant difference in the biodistribution except for the stomach between ^{111}In-MAA and ^{99m}Tc-MAA (Tables 2, 3). Murine lung was clearly visualized after injection of ^{111}In-MAA and showed homogeneous radioactivity in an imaging study (Fig. 2). However, other organs such as the liver, spleen, bone marrow or kidney were not demonstrated on the imaging.

DISCUSSION

A simple method for the direct labeling of MAA with ^{111}In-chloride has been described. A high labeling efficiency of more than 96% was obtained within 30 min incubation of MAA

TABLE 2
Biodistribution of Indium-111-MAA in Normal Mice at 15 and 180 min After Intravenous Administration

	At 15 min (n = 4)			At 180 min (n = 4)		
Organ	% ID/g	% ID/organ	Organ/blood radioactivity concentration ratio	% ID/g	% ID/organ	Organ/blood radioactivity concentration ratio
Lung	653.1 (55.7)	90.3 (0.58)	728.8 (24.0)	645.5 (102.3)	83.3 (2.89)	511.1 (183.9)
Liver	0.37 (0.10)‡	0.54 (0.11)	0.41 (0.10)	1.05 (0.26)‡	1.27 (0.48)	0.78 (0.25)
Kidney	3.04 (0.92)‡	0.71 (0.20)	3.44 (1.21)	10.1 (2.46)‡	1.97 (0.50)	7.60 (1.31)
Intestine	0.16 (0.05)	0.47 (0.09)	0.19 (0.05)	0.23 (0.06)	0.63 (0.22)	0.18 (0.04)
Stomach	0.10 (0.30)	0.06 (0.02)	0.11 (0.03)	0.12 (0.03)	0.06 (0.02)	0.09 (0.03)
Spleen	0.20 (0.04)‡	0.02 (0.01)	0.23 (0.05)	0.82 (0.08)‡	0.08 (0.05)	0.64 (0.17)
Muscle*	0.15 (0.01)	0.02 (0.01)	0.17 (0.02)	0.16 (0.05)	0.02 (0.01)	0.12 (0.03)
Bone†	0.24 (0.06)	0.02 (0.01)	0.27 (0.09)	0.31 (0.15)	0.02 (0.01)	0.23 (0.08)
Blood	0.90 (0.08)	—	1.00	1.34 (0.32)	—	1.00

*Left femoral quadriceps muscle.
†Left femur.
‡$p < 0.05$.
Each value represents the mean ± 1 s.d. from four mice.

TABLE 3
Biodistribution of Technetium-99m-MAA in Normal Mice at 15 and 180 min After Intravenous Administration

Organ	At 15 min (n = 4)			At 180 min (n = 4)		
	% ID/g	% ID/organ	Organ/blood radioactivity concentration ratio	% ID/g	% ID/organ	Organ/blood radioactivity concentration ratio
Lung	632.7 (45.5)	93.0 (6.24)	956.4 (136.2)	608.4 (64.3)	86.8 (5.38)	685.2 (182.9)
Liver	0.48 (0.08)	0.67 (0.19)	0.68 (0.12)	0.81 (0.28)	1.78 (0.35)	1.77 (0.54)
Kidney	4.39 (1.65)	1.23 (0.38)	6.59 (2.35)	6.46 (1.81)	1.50 (0.39)	7.32 (2.72)
Intestine	0.45 (0.07)	0.71 (0.13)	0.70 (0.22)	0.47 (0.10)	0.52 (0.16)	0.44 (0.14)
Stomach	4.40 (3.31)	1.15 (0.48)	6.53 (4.67)	6.34 (1.11)	1.69 (0.48)	6.95 (2.55)
Spleen	0.34 (0.04)	0.04 (0.01)	0.51 (0.13)	0.34 (1.60)	0.06 (0.01)	0.77 (0.31)
Muscle*	0.31 (0.03)	0.05 (0.01)	0.46 (0.03)	0.28 (0.10)	0.02 (0.01)	0.15 (0.09)
Bone†	0.43 (0.08)	0.03 (0.02)	0.76 (0.13)	0.72 (0.09)	0.02 (0.01)	0.40 (0.12)
Blood	0.67 (0.10)	—	1.00	0.92 (0.15)	—	1.00

*Left femoral quadriceps muscle.
†Left femur.
Each value represents the mean (1 s.d.) from four mice.

suspended in 0.1 *M* sodium acetate, pH 5.8, with ^{111}In-chloride at room temperature. The in vitro and in vivo studies showed high stability of the ^{111}In-MAA at least up to 180 min, suggesting that this would be enough time for pulmonary perfusion studies. Slight increased tracer activities in the liver, kidney and spleen were found at 180 min after injection compared with those obtained at 15 min. These findings appeared to be due to slight elution of ^{111}In from ^{111}In-MAA and/or slight degradation of ^{111}In-MAA. The biodistribution of ^{111}In-MAA was similar to that of ^{99m}Tc-MAA except for lack of stomach activity. The increased stomach activity of ^{99m}Tc-MAA may be due to free ^{99m}Tc eluted from ^{99m}Tc-MAA. Methods of direct labeling of microspheres with positron emitters such as ^{68}Ga have been demonstrated (*5–8*). In our previous study (*4*), ^{111}In-DTPA-MAA was obtained by an incubation of MAA conjugated with DTPA, with the labeling efficiency more than 96%. Indium-111-DTPA-MAA administered intravenously into normal mice also gave clearly visualized murine lung scintigraphy. Both ^{111}In-MAA and ^{111}In-DTPA-MAA are promising pulmonary perfusion agents.

CONCLUSION

A convenient method to label MAA with ^{111}In presents new opportunities for reliable, simultaneous imaging of pulmonary perfusion using ^{111}In-MAA and ventilation using ^{99m}Tc aerosol.

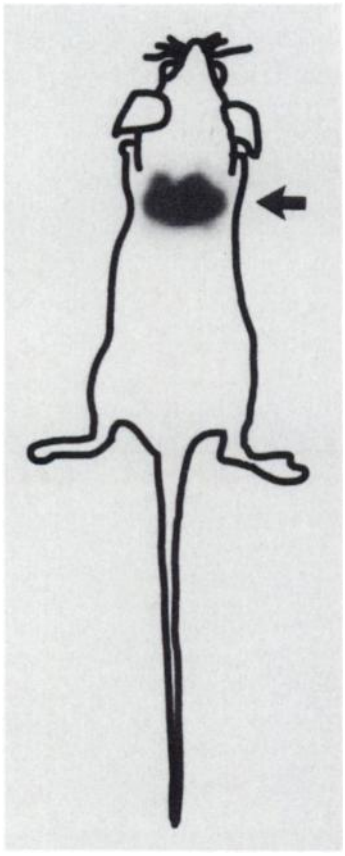

FIGURE 2. Dorsal scintigraphy of normal mouse after intravenous injection of ^{111}In-MAA. Murine lung was clearly and homogeneously demonstrated. The arrow shows murine lung.

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