^{99m}Tc-HUMAN ALBUMIN MICROSPHERES (HAM) FOR LUNG IMAGING

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Human albumin microspheres (HAM) labeled with ^{99m}Tc constitute the first short-lived radiopharmaceutical for pulmonary perfusion imaging which has been developed into a commercial kit (The 3M Co., Nuclear Products Div., St. Paul, Minn.). This new agent provides the combined advantages of easy laboratory preparation and optimal imaging characteristics for both the rectilinear scanner and the scintillation camera. We have previously reported negative animal toxicity studies in which HAM was injected into the brain, celiac axis, kidneys, and lungs of dogs in doses up to 30 times an estimated human equivalent (*1*). This report summarizes an investigation of the pulmonary clearance, clinical toxicology, immunology, and effectiveness of HAM when used in humans for lung imaging.

MATERIALS AND METHODS

The kit for labeling HAM with ^{99m}Tc consists of a reaction vessel containing HAM, thiosulfate, and a cationic exchange resin; and a diluent solution composed of isotonic saline, a dispersing agent and a preservative (Fig. 1). The labeling process is initiated by adding pertechnetate to the reaction vessel through the exchange resin. The vial is immediately placed in a sonicated water bath (Branson Model 220, 50–55 kHz, 100 watts at room temperature) and shaken for 1–2 min to dissolve the reagent tablet and evenly distribute the microspheres, technetium, and sulfur colloid for maximum surface interaction without clumping or precipitation. After agitating the vial for 6–10 min in a boiling water bath, the supernatant is withdrawn through the fritted filter, and the microspheres are washed and resuspended in diluent. Sonication should be performed immediately before withdrawing a dose since the microspheres tend to aggregate (Fig. 2). Washing with diluent should be repeated if more than 1 hr elapses between preparation and injection to insure a minimum of unbound technetium.

PULMONARY CLEARANCE

The rate of biologic clearance of intrapulmonary HAM was studied in rats histologically and with whole-organ radioactivity analysis, and in humans by external radiation monitoring. In the histologic study, labeled HAM were stained with the fluorescent dye lissamine rhodamine (RB-200) and mixed with nonbiodegradable carbonized polystyrene microspheres (CM) of the same diameter. After thorough

Received Sept. 16, 1970; revision accepted Dec. 6, 1970.

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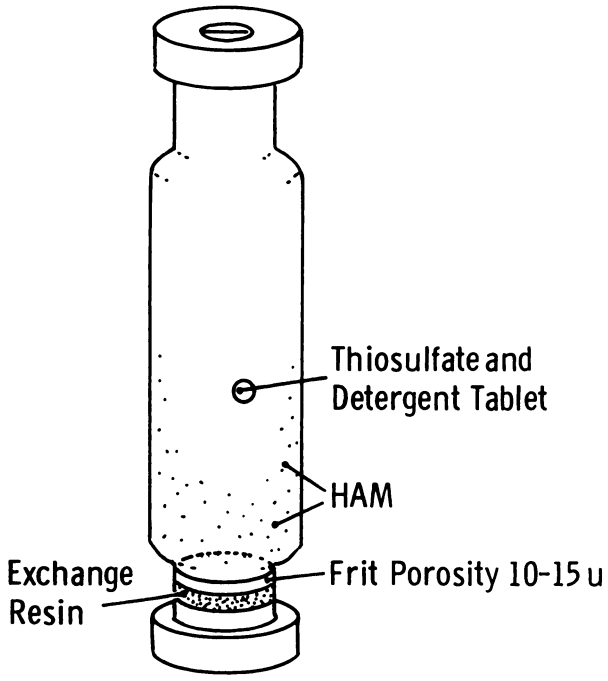

FIG. 1. Reaction vessel—sterile evacuated glass vial containing 5 mg HAM, and reagents for labeling. As pertechnetate is introduced through cationic exchange resin, HCl is produced which reduces thiosulfate to form sulfur colloid.

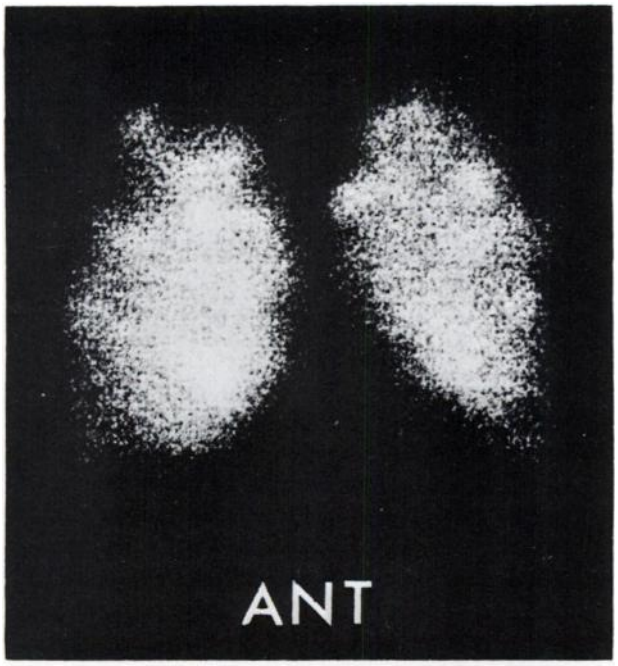

FIG. 2. "Hot spots" due to aggregates of ^{99m}Tc-HAM, observed when sonication was omitted from labeling process (two patients), and when injection was made via indwelling venous catheter (one patient).

shaking and sonication of the mixture, aliquots containing 40,000–50,000 total particles were immediately injected into twenty-seven 300-gm rats through an external jugular vein. The rats were sacrificed at intervals from 0 to 72 hr postinjection. The ratio of HAM to CM was determined in each rat by counting the number of microspheres in five representative 50-micron lung sections under ultraviolet microscopy. The decrease in this ratio with time was used as a measure of the rate of HAM clearance.

Approximately 80% of the microspheres remained in the lung at 3 hr, but less than 3% were present at 12 hr. The mean clearance $T_{50\%}$ was 5 hr. In a separate group of 27 rats sacrificed at identical intervals for quantitation of total-lung radioactivity, the initial rate of clearance resulted in a 5-hr $T_{50\%}$, but the residual radioactivity at 12 hr was greater than anticipated from the histologic study (Fig. 3A). Similar results were found in the human radioactivity clearance studies (Fig. 3B).

HAM trapped in the pulmonary capillary bed showed progressive morphologic changes with phagocytes present at their periphery (Fig. 4). The CM were unchanged.

TABLE 1. CARDIOPULMONARY FUNCTION AFTER INJECTION OF ^{99m}Tc-HAM (11 PATIENTS)

Measurements	Preinjection (mean)	Postinjection (mean)	p*
Pressures (mm Hg)			
Systemic	107 ± 31	106 ± 30	>0.4
Pulmonary artery	22 ± 9	22 ± 9	>0.2
Pulmonary capillary	13 ± 8	13 ± 8	>0.4
Heart rate	95 ± 24	93 ± 23	>0.1
Pa_{O_2}	73 ± 13	70 ± 13	>0.4
Pa_{CO_2}	36 ± 4	36 ± 4	>0.4
pH	7.44 ± 0.03	7.44 ± 0.03	>0.4

* Probability that the pre- and postinjection means are from the same population. $p > 0.05$ indicates no significant change.

CLINICAL TOXICOLOGY

One hundred fifteen consecutive patients who had a clinical indication for lung scanning were selected for this phase of the investigation. Their final clinical diagnoses were: pneumonia, 17; congestive heart failure, 18; chronic obstructive pulmonary disease,

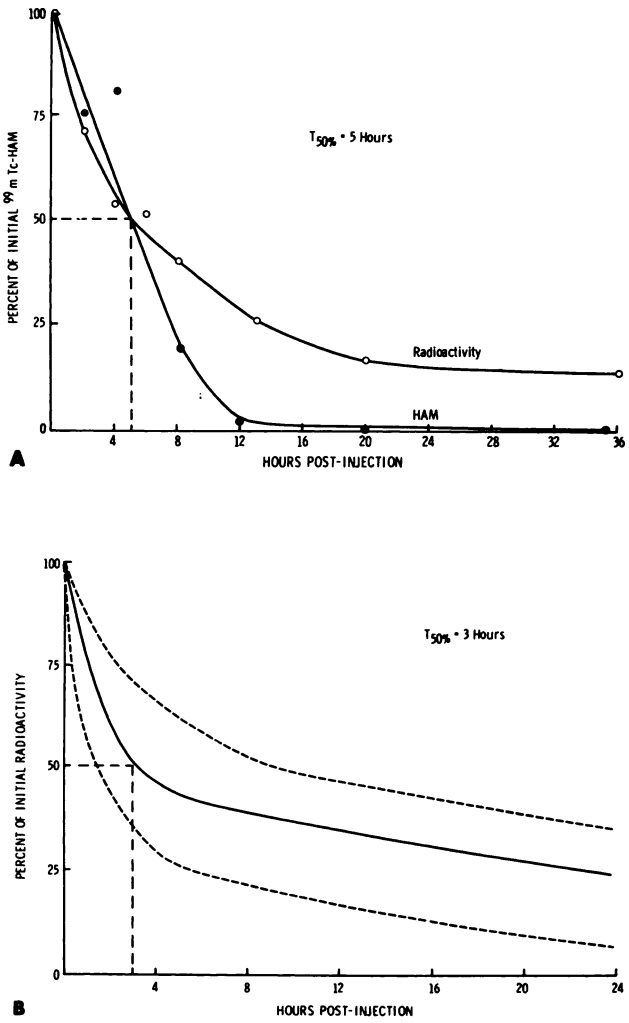

FIG. 3. Mean pulmonary clearance of ^{99m}Tc-HAM. A shows clearance in rats, determined by change in HAM/CM ratio, and by whole-organ radioactivity analysis. Each point represents three animals. B shows decrease in humans (40 patients), determined by external radiation monitoring. Range limits are indicated.

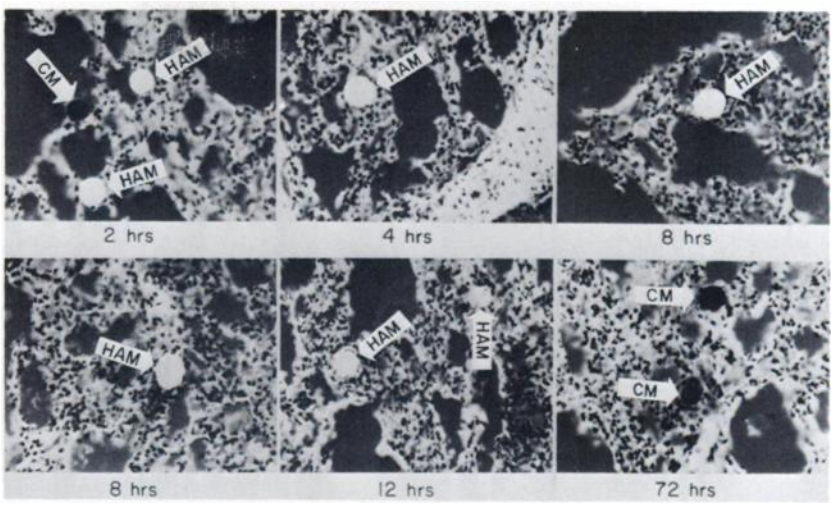

FIG. 4. Morphologic changes of intrapulmonary HAM (fluorescent stain). Progressive loss of spherical shape and surface regularity. No change in CM (arrows).

23; pulmonary thromboembolus, 41; normal, 4; and other, 12 (granuloma, abscess, fibrosis, tuberculosis, tumor). After injection of 1–4 mCi of ^{99m}Tc-HAM (0.3–1.8 mg of protein = 45,000–270,000 microspheres), the patients were continuously monitored for 6 hr with frequent recording of vital signs. In 25 of these patients, 1-hr preinjection and 24–48-hr postinjection CBC, BUN, SGOT, and urinalysis were performed. In order to determine the effect of HAM on cardiopulmonary function, an additional 11 patients were injected with a lung imaging dose directly into the pulmonary artery while undergoing cardiac catheterization (Table 1). No significant changes occurred in any of the parameters measured.

IMMUNOLOGY

Antibody response to ^{99m}Tc-HAM was assessed by the bentonite particle flocculation test and a fluorescent antibody technique. Blood samples were collected from 20 patients immediately before and 15 days after a single injection of HAM for the flocculation testing. The antigen coating for the particles was human serum albumin denatured by heating at 65°C for 20 min at pH 6.5. HAM was incubated in six of these patients' sera and tested using fluorescein-labeled antihuman IgM and IgG antibodies. No reactive antibody was demonstrated by either test.

CLINICAL EFFECTIVENESS

For pulmonary perfusion imaging, an average adult dose of 2 mCi of ^{99m}Tc was used, with a specific activity of 4 mCi/mg of microspheres. Counting rates of 200,000–400,000 cpm over the lungs permitted completion of a single scan view in less than 10 min with a high-speed scanner, thus minimizing respiratory motion artifact. With the scintillation camera, a lung image could be obtained with respiration suspended using several consecutive periods of breath-holding (Fig. 5). The definition of pulmonary morphology including vascular indentations, fissures, and peripheral pulmonary margins was excellent. Consequently, we believe it was easier to recognize perfusion abnormalities and exclude artifacts in comparison with our experience using ^{131}I-MAA. Because of the rapid pulmonary clearance of HAM and simultaneous increase in liver-spleen radioactivity, we found it advantageous to complete imaging during the first half-hour after injection.

DISCUSSION

Radionuclide imaging of the lungs with ^{131}I-MAA has achieved widespread acceptance as a reliable

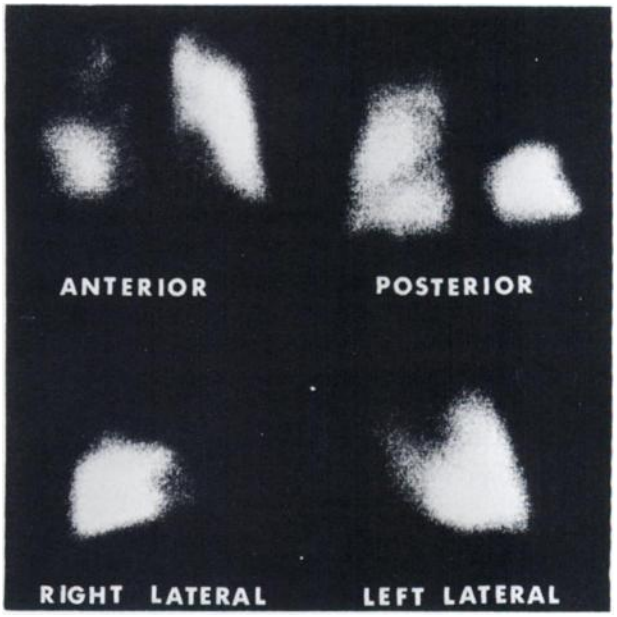

FIG. 5. ^{99m}Tc-HAM scintillation camera image obtained with respiration suspended during consecutive periods of breath-holding. (Metastatic carcinoma of lung.)

means of demonstrating macroscopic pulmonary perfusion defects. In spite of significant contributions to clinical research as well as patient care, the information provided by the test has been limited by several characteristics of the radiopharmaceutical. These undesirable features include a high cost compounded by a short shelf-life, a potentially high patient radiation dose due primarily to the ^{131}I-beta spectrum, and inefficient imaging of ^{131}I with the Anger scintillation camera. The development of ^{99m}Tc-HAM is a response to the need for an improved radiopharmaceutical. Other short-lived perfusion imaging agents such as ^{113m}In-ferric hydroxide particles and ^{99m}Tc-MAA are difficult to prepare and have considerable variation in the final product. By contrast, the preparation of ^{99m}Tc-HAM is simple, rapid, and consistently results in a tagging efficiency greater than 70% with the commercial kit. Particle size is controlled within a remarkably narrow range (15–30 microns) by the manufacturer utilizing standard sieving techniques after formulation.

Because of the possibility of injury to the lungs produced by microembolization, the toxicology of any new perfusion imaging agent must be defined, particularly the characteristics of its biologic clearance. Ceramic and carbonized microspheres, for example, were found to be cleared very slowly if at all from the pulmonary circulation and might therefore produce permanent damage such as pulmonary fibrosis. The clearance data presented here demonstrates without question that HAM disappears from the lung with a biologic life comparable to that of MAA, although the mode of biodegradation may be different. While the clearance of MAA has been attributed to hemodynamic factors (*2*), our observations suggest that cellular activity plays an important role in the removal of HAM from the lungs since phagocytes were consistently noted at their periphery in the histologic sections. Enzymatic activity, if present, would be relatively specific since no changes in HAM morphology were noted during incubation in gastric juice, rat liver extract, and trypsin (*3*).

We have previously reported negative animal toxicity studies in which HAM was injected not only into the venous circulation, but directly into the brain, celiac axis, and kidneys of dogs. In the current investigation, the clinical and laboratory testing of humans including cardiopulmonary function studies demonstrated no HAM toxicity following intravenous injection. Although no antibody formation was detected following a single injection of HAM, its antigenicity should be evaluated in patients who have received multiple injections. With an estimated 50,000 lung scans performed using MAA through 1968, only two adverse reactions have been reported in the literature to date, both of which resulted in death (*4,5*). The patients involved had severe preexisting pulmonary hypertension, and the reactions were not attributed to an immunologic response. In such patients ^{99m}Tc-HAM may be more safe since an imaging dose can contain less than 10% of the particles in a 300 μCi dose of ^{131}I-MAA.

SUMMARY

Technetium-99m-HAM is simple and rapid to prepare using a commercial kit. The pulmonary clearance $T_{50\%}$ is comparable to that of MAA. We found no evidence of clinical toxicity, and initial immunologic studies were negative. Because of the optimal imaging characteristics of ^{99m}Tc for the Anger scintillation camera as well as the rectilinear scanner, this radiopharmaceutical may prove to be the agent of choice for pulmonary perfusion imaging.

ACKNOWLEDGMENTS

We express our appreciation to The 3M Company, Nuclear Products Division for supplying the HAM kits and the carbonized microspheres, and to David Marrack for his contributions in performing the immunologic tests.

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