nm/PRELIMINARY NOTE

LUNG SCANNING USING ^{99m}Tc-LABELED MACROAGGREGATED FERROUS HYDROXIDE (Tc-MAFH) AS THE PERFUSION AGENT

R. E. Boyd and S. A. Ackerman

Australian Atomic Energy Commission, Sutherland, N.S.W., Australia

J. G. Morris and J. P. Huberty

Royal Prince Alfred Hospital and University of Sydney, Sydney, Australia

In 1955 Gerlitt (*1*) reported that technetium in the reduced form could be carried on ferric hydroxide, and the Tc-iron colloid described by Yeh *et al* (*2*) was based upon this reaction. We have studied this reaction further and have observed that preparations of Tc:Fe:ascorbic acid can also be made to yield a labeled precipitate of ferric hydroxide. From this we have developed what we believe to be a superior lung perfusion scanning agent with excellent radiochemical stability. The compound (technetium-macroaggregated ferrous hydroxide) has been administered intravenously to animals and humans where it localized predominantly in the lungs.

The Tc-MAFH possesses considerable advantages over other currently available lung perfusion preparations in that

1. It is easily and speedily prepared with the reducing agent and Tc-carrier combined in one chemical reagent,
2. It is prepared with a high labeling efficiency,
3. It is amenable to terminal autoclaving,
4. It has an adequate shelf life and
5. It facilitates most effective exploitation of the superior physical properties of the radionuclide ^{99m}Tc.

MATERIALS AND METHODS

The stages in the production of ^{99m}Tc-macroaggregated ferrous hydroxide were:

1. Reduction of pertechnetate with ferrous sulphate and stannous chloride,
2. Precipitation of the mixed hydroxides by sodium hydroxide,
3. Selection of the larger particles by slow speed centrifugation,
4. Resuspension of the precipitate in a gelatin vehicle,
5. Terminal autoclaving of the final Tc-MAFH preparation.

The ^{99m}Tc-pertechnetate in 5 ml isotonic saline solution was placed in a sterile centrifuge tube and 1 ml ferrous sulphate solution (1 mg Fe^{++} per ml) and 1 ml stannous chloride solution (0.6 mg Sn^{++} per ml) were added. The pH of the solution was raised to 6–7 by the rapid addition of 0.1 *N* sodium hydroxide (0.25 ml). The solution turned a green color immediately, but after a few seconds a precipitate of the mixed hydroxides of iron (II) and tin (II) separated out. (If the sodium hydroxide is added slowly, complete oxidation occurs in which ferrous is converted to ferric hydroxide; this results in a reduced tagging of the precipitate with the ^{99m}Tc and gives rise to smaller particles).

The solution was then centrifuged at 600 rpm for 1 min and the supernate drawn off and discarded. The residue was resuspended in 5 ml of sterile isotonic saline and centrifuged again at 600 rpm for a further minute; the supernate was drawn off and discarded. The residue was transferred in 1 ml saline to a multidose vial containing 5 ml saline solution. To this mixture was added 1 ml gelatin solution (10 wt%), 1 ml stannous chloride solution (0.6 mg Sn^{++} per ml). The final pH was adjusted to 6–7 by the addition of 0.3 ml of 0.1 *N* sodium hydroxide. The vial was sealed and then autoclaved at 121°C for 33 min.

RESULTS

Radiochemistry. Between 60 and 70% of the available ^{99m}Tc was tagged to the large ferrous hydroxide particles. The final suspension contained insoluble iron (II) and tin (II) each at a concentration of 60–100 μg/ml. Electrophoretic examination revealed that less than 1% of the ^{99m}Tc was present as ionic pertechnetate and this radiochemical

Received May 23, 1969; revision accepted Aug. 12, 1969.

For reprints contact: R. E. Boyd, Isotope Production Manager, A.A.E.C. Research Establishment, Private Mail Bag, Sutherland, 2232 N.S.W., Australia.

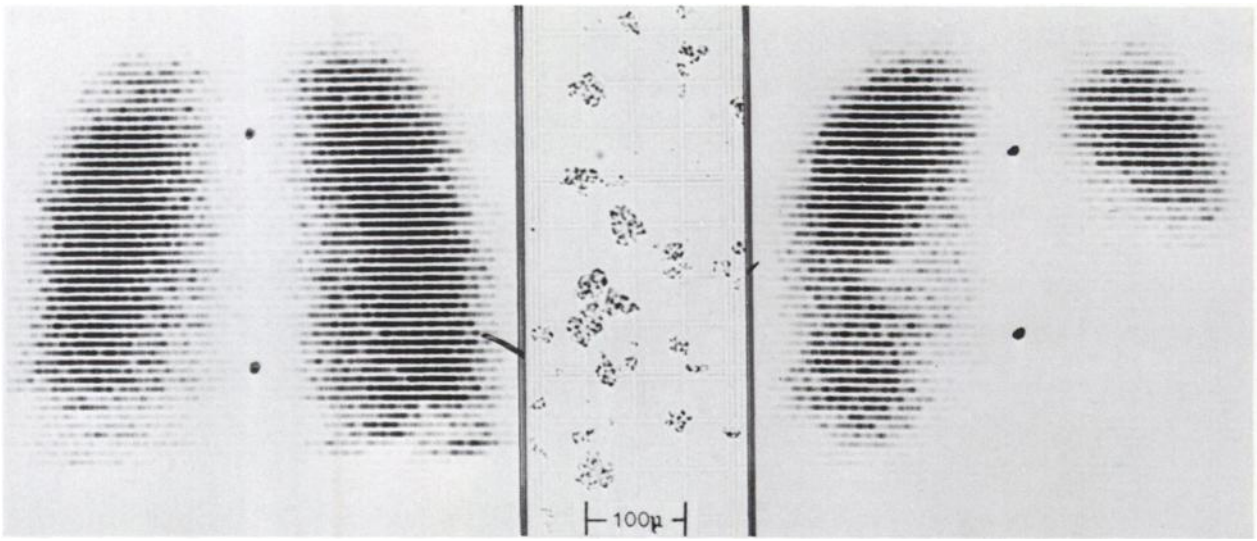

FIG. 1. At left is normal posterior lung scan. In middle are Tc-MAFH particles in hemocytometer. At right is lung scan made with Tc-MAFH showing multiple perfusion defects.

purity persisted for at least 24 hr. The osmolality of the preparation was slightly hypotonic (220–250 milliosmoles).

Particle-size distribution. Examination by optical microscopy indicated that the Tc-MAFH particles were between 10 and 50 microns in dia and that 70% of the particles were in the optimal 20–50-micron range. This 70% contained 98.5% of the total radioactivity. No significant alteration in particle sizes was observed over a period of 24 hr. The number of particles per milliliter as measured in a hemocytometer was approximately 3×10^6.

Organ distribution studies. Organ distribution of radioactivity in 25 rats was studied at varying intervals after intravenous injection of Tc-MAFH. Each rat was anesthetized intraperitoneally with 6 mg sodium Nembutal and then given 50 μCi of Tc-MAFH through the tail vein. Groups of rats were killed at intervals ranging from 5 min to 24 hr. The animals were dissected, and the radioactivity in the lungs, liver, kidneys, intestine, spleen, stomach, heart, blood and carcass was measured in a large-volume well scintillation counter.

The distribution of ^{99m}Tc-MAFH in the organs at varying intervals after injection is given in Table 1.

Five minutes after injection the distribution of Tc-MAFH radioactivity was approximately 98% in the lungs, 1% in the liver and 1% throughout the remainder of the animal. The rate of clearance of the ^{99m}Tc from the lungs was exponential with a biological half-time of approximately 24 hr. In the liver and to a smaller degree in the kidneys, spleen and carcass, the radioactivity increased with time.

The shelf-life of Tc-MAFH was determined by studying a series of rats which were injected with preparations of ages varying from 3 to 24 hr. The rats were sacrificed 5 min after injection, and the organ radioactivity distribution was measured as described. Preparations up to 8 hr old retained their lung specificity of greater than 95%, and with a preparation which was 24 hr old, approximately 90% of the activity localized in the lungs and 3% in the liver.

TABLE 1. ^{99m}Tc DISTRIBUTION (% INJECTED DOSE) IN TISSUES OF RATS AFTER INTRAVENOUS INJECTION OF ^{99m}Tc-MACROAGGREGATED FERROUS HYDROXIDE

	Time after injection				
Organs	5 min	2 hr	4 hr	6 hr	24 hr
Lung	97.5	94.9	89.8	75.1	49.8
Liver	1.2	2.7	5.8	13.7	26.6
Kidney	0.4	1.1	1.7	3.7	7.2
Intestine	<0.1	0.2	0.4	1.1	2.2
Spleen	0.1	0.6	1.0	4.1	7.8
Stomach	<0.1	<0.1	<0.1	0.2	0.2
Blood	0.3	<0.1	<0.1	0.2	0.7
Heart	<0.1	<0.1	<0.1	<0.1	0.1
Carcass	0.3	0.4	0.8	1.7	5.5

Autoclaving. The facility of being able to autoclave Tc-MAFH represented a major advantage this material had over Tc-labeled albumin macroaggregates. It was observed, however, that the volume of air inside the bottle above the suspension was a critical parameter. The contents of bottles less than half full underwent thermal decomposition when autoclaved whereas those nearly full were unaffected. To prevent this, a procedure has been adopted in which just prior to insertion in the autoclave the bottle caps are punctured with sterile needles and the air inside the bottle displaced with oxygen-free

nitrogen. Under these conditions decomposition resulting in the release of free pertechnetate is maintained at less than 1%.

Clinical application. Scans of 250 patients were performed using a commercial rectilinear scanner (Picker Magnascanner 3 × 2-in. crystal) with a medium and a low energy collimator (2107, 2102B, respectively). A dose of 750 μCi Tc-MAFH was used, and multiple views were done. Examples of photoscans and a photomicrograph of the Tc-MAFH particles in a hemocytometer are shown in Fig. 1.

The photoscans obtained using ^{99m}Tc-MAFH were superior to those obtained with ^{131}I-labeled macroaggregated albumin because of the greatly improved counting statistics and excellent collimation possible. The scanning time was greatly reduced, posterior and both lateral views being done in the time usually required for one posterior view.

Careful clinical observations of the patients were carried out during and after the administration of the preparation; particular attention was paid to the pulse rate and temperature. No abnormalities were recorded. Serial studies were performed on many of the patients and again no adverse signs or symptoms were detected.

Toxicity. The bulk of the toxicological information on iron compounds is related to long-term exposures by inhalation; however, the soluble salts, particularly ferric salts, are known to have toxic effects when administered directly into the blood stream. Trow *et al* (*3*) in their search for a less toxic carrier for ^{113m}In than ferric hydroxide proposed the use of aluminum hydroxide. In Tc-MAFH we have used the reported greater tolerance for the ferrous ion as compared to the ferric ion. The toxicity of ferrous or ferric hydroxides is not well documented but the quoted lethal doses for their soluble salts is $FeCl_3 \cdot 6H_2O$ 7.2 mg/kg and $FeSO_4 \cdot 7H_2O$ 99 mg/kg, respectively, in rabbits. Hence the substitution of ferrous for ferric hydroxide would suggest a reduction in chemical toxicity by a factor of 12.

The addition of stannous chloride, later converted to the insoluble hydrated oxide, to Tc-MAFH does increase the toxicity risk. However, the quantity administered even if it is assumed to be in its most toxic (soluble) form represents only 7 μg/kg.

DISCUSSION

These results indicate that ^{99m}Tc-macroaggregated ferrous hydroxide is a superior agent for lung perfusion scanning. It is well localized in the lungs, and liver and spleen uptake is at an acceptable minimum. The change in the activity in the lungs over a period of 2 hr after injection is insignificant, permitting multiple views to be done with either a rectilinear scanner or gamma camera.

The use of a combined reducing agent and carrier ($FeSO_4$) in this agent greatly simplifies its preparation. The addition of stannous chloride protects the compound from atmospheric and/or radiolytic oxidation over extended periods. The Tc-MAFH can be prepared under aseptic conditions in 10 min by trained technical staff and then terminally autoclaved at 121°C for 33 min without any risk of decomposition.

SUMMARY

A method for preparing ^{99m}Tc-macroaggregated ferrous hydroxide (Tc-MAFH) has been described. Tc-MAFH is easy to prepare and possesses adequate radiochemical stability. The majority of the labeled macroaggregates lie in the optimal range of 20–50 microns.

This radiopharmaceutical enables the superior physical properties of ^{99m}Tc to be more effectively used in lung perfusion studies.

REFERENCES

1. GERLITT, J. B.: *Proc. Intern. Conf. Peaceful uses of Atomic Energy*, Vol. 7, United Nations, New York, 1956, p. 145.

2. YEH, S. H., OHLSEN, J., KOUNTZ, S. L. AND KRISS, J. P.: Distribution and scintiphotography studies of ^{99m}Tc-iron colloid in the rabbit. *J. Nucl. Med.* **10**:56, 1969.

3. TROW, R. S., BROWN, D. W., AHRENS, C. A., CLEAVELAND, J. D. AND LEE, J. I.: ^{113m}In aluminum hydroxide particles for lung scanning. *J. Nucl. Med.* **10**:377, 1969.