Blood-Pool Imaging Using Technetium-99m-Labeled Liposomes

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This study evaluated two ^{99m}Tc-liposome formulations as potential blood-pool agents in comparison with standard ^{99m}Tc-red cells and ^{99m}Tc-human serum albumin (HSA). **Methods:** Liposomes with no surface modification or coated with polyethylene glycol (PEG) were labeled with ^{99m}Tc using the lipophilic chelator, HMPAO. Autologous red cells were labeled with ^{99m}Tc using in vitro or in vivo techniques. Technetium-99m-HSA was supplied commercially. Rabbits were injected intravenously with ^{99m}Tc-liposomes, ^{99m}Tc-red cells or ^{99m}Tc-HSA. Static images were acquired and blood samples collected. **Results:** Technetium-99m-liposome images showed prominent blood-pool activity compared to lung and liver activities, which were similar to those acquired for ^{99m}Tc-red cells, but better than ^{99m}Tc-HSA. Heart-to-lung ratios were not significantly different between ^{99m}Tc-liposome formulations or for either formulation compared to ^{99m}Tc-red cells. The ratios were higher, however, than for ^{99m}Tc-HSA. Heart-to-liver ratios were higher for PEG ^{99m}Tc-liposomes than they were for neutral ^{99m}Tc-liposomes and ^{99m}Tc-HSA, but were not significantly different than ^{99m}Tc-red cells. Bladder activities for both ^{99m}Tc-liposome formulations were 3–6 times lower than for the other agents. PEG ^{99m}Tc-liposomes remained in circulation 1.6 times longer than any of the other agents. **Conclusions:** Technetium-99m-liposomes, independent of surface modification, had excellent circulation persistence and in vivo stability when compared to ^{99m}Tc-red cells and ^{99m}Tc-HSA. PEG ^{99m}Tc-liposomes performed better than neutral ^{99m}Tc-liposomes due to lower liver background activity. Advantages of PEG ^{99m}Tc-liposomes compared to ^{99m}Tc-red cells include: (a) only one venipuncture, (b) little exposure to patient's blood, (c) excellent in vitro and in vivo stability and (d) lack of drug interference.

Key Words: liposomes; technetium-99m-liposomes; blood-pool imaging; technetium-99m-HMPAO; ventriculography; polyethylene glycol

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The development of a safe, convenient and stable radiopharmaceutical for blood-pool imaging studies of cardiac function, venography and detection of gastrointestinal bleeding has so far remained an elusive goal (*1*). The most commonly used blood-pool agent has relied on labeling autologous red cells with ^{99m}Tc (*1*). Recently, human serum albumin (HSA) labeled with ^{99m}Tc has been substituted for ^{99m}Tc-red cells because of its simple preparation and reduced likelihood of transmission of potential blood borne pathogens (*1*). The use of ^{99m}Tc-HSA as a blood-pool agent, however, has been shown to be less than ideal due to its poor in vivo stability compared to ^{99m}Tc-red cells (*1*).

Several strategies have been explored to develop blood-pool agents that are more effective than ^{99m}Tc-HSA and that do not rely on the use of autologous ^{99m}Tc-red cells. First, HSA has been modified by conjugation with 2,3-dimercaptopropionyl before ^{99m}Tc labeling and shown to have greater in vivo stability than standard preparations of ^{99m}Tc-HSA in healthy volunteers (*2,3*). A second potential cell-free blood-pool agent currently in preclinical testing consists of polylysine polymer conjugation of diethylenetriacetic acid (DTPA) for chelation of ^{99m}Tc and containing a polyethylene glycol (PEG) for increased circulation times (PEG ^{99m}Tc-DTPA polylysine polymer) (*4*). Finally, a cell-free system comprised of polyethylene glycol (PEG) surface-modified liposomes has been studied as another blood-pool agent (*5,6*). In these early studies, PEG-coated liposomes were labeled with ^{99m}Tc using a DTPA phospholipid-based surface-labeling technique or with ^{67}Ga using an after-loading method (*5,6*).

In the present work, a newly developed after-loading technique was used to label liposomes with ^{99m}Tc using hexamethylpropyleneamine oxime (HMPAO). This lipophilic chelator is thought to carry ^{99m}Tc inside preformed liposomes, where it is trapped following conversion of the lipophilic HMPAO to its hydrophilic form in the presence of glutathione (*7*). This paper describes the results obtained for blood-pool imaging characteristics of two ^{99m}Tc-liposome formulations with both in vivo and in vitro labeled ^{99m}Tc-red cells and ^{99m}Tc-HSA in rabbits. The formulations consist of liposomes with no surface modification (neutral) or a PEG surface coating.

MATERIALS AND METHODS

Liposome Preparation

Two formulations were tested:

1. PEG liposomes comprised of distearoyl phosphatidylcholine (DSPC):cholesterol:distearoyl phosphoethanolamine-N-[Poly (ethylene glycol) 5000] (DSPE-PEG 5000):alpha-tocopherol (50:38:10:2 molar ratio).
2. neutral liposomes comprised of DSPC:cholesterol:alpha-tocopherol (66:32:2 molar ratio).

These liposomes were prepared as previously described (*8,9*), except after rehydration with 100 m*M* reduced glutathione in Dulbecco's phosphate-buffered saline (PBS), pH 7.4, liposomes were extruded through a series of polycarbonate filters. The diameters of neutral and PEG liposomes were determined to be 138 nm ± 47 nm and 134 nm ± 37 nm, respectively, by particle-size analysis. Phospholipid concentration for the neutral and PEG liposomes was 63 m*M* and 60 m*M*, respectively (*10*). Intravesicular GSH concentration was estimated using a commercial assay kit to be 0.6 m*M* and 0.2 m*M* for the PEG and neutral liposomes, respectively.

Liposome Labeling Procedure

Liposomes (3 ml) were mixed with 1.5 ml of HMPAO preincubated with 10 mCi sodium pertechnetate in 5 ml 0.9% saline (*7*). Reconstituted kits were checked for contamination using a three-step, thin-layer chromatography system outlined in the HMPAO kit package insert. In all cases, the kits used for the liposome labeling studies contained >80% lipophilic HMPAO. After 30 min, the liposomes were separated from any free ^{99m}Tc by passage over a

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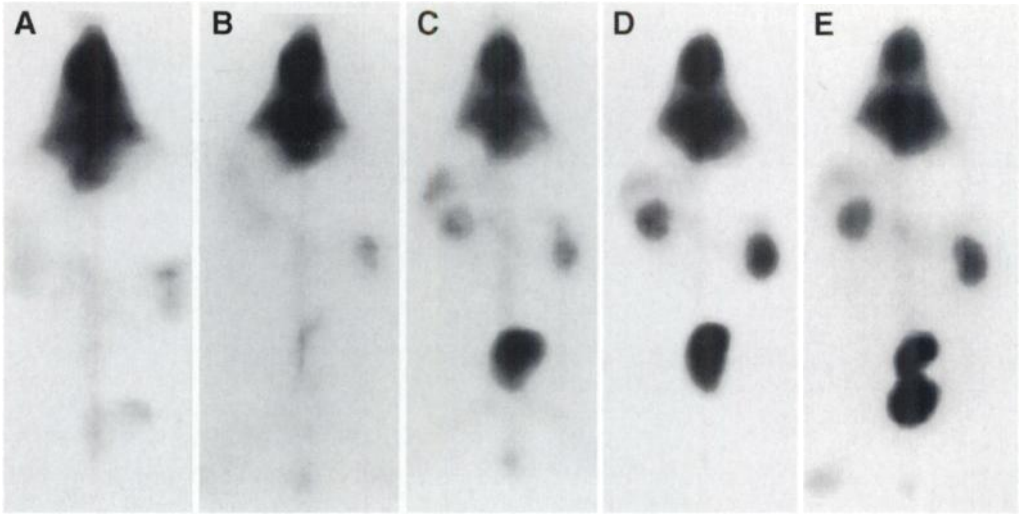

FIGURE 1. Whole-body images of rabbits acquired 45 min after intravenous injection of: (A) PEG ^{99m}Tc-liposomes, (B) neutral ^{99m}Tc-liposomes, (C) in vitro ^{99m}Tc-red cells, (D) in vivo ^{99m}Tc-red cells or (E) ^{99m}Tc-HSA.

Sephadex G-25 column. Labeling efficiencies were checked by determining the activity before and after column separation of ^{99m}Tc-liposomes using a dose calibrator. Mean labeling efficiencies of neutral ^{99m}Tc-liposomes and PEG ^{99m}Tc-liposomes were 52% and 66%, respectively. Post-column preparations of ^{99m}Tc-liposomes were used immediately for injection.

In Vitro Technetium-99m-Red Cell Labeling Procedure

Autologous blood (3 ml) was withdrawn via an ear artery into a heparinized syringe and labeled with ^{99m}Tc using an Ultratag kit (Mallinkrodt Medical, St. Louis, MO). After 20 min, labeled red cells were washed in normal saline and spun at 800 × g for 10 min. Supernatant and pellet fractions were checked for activity in a dose calibrator. Labeling efficiency was >97%.

In Vivo Technetium-99m-Red Cell Labeling Procedure

Red cells were labeled in vivo with ^{99m}Tc using a Pyrolite kit (DuPont Merck, N. Billerica, MA). Each rabbit was injected in an ear vein with 0.5 ml of Pyrolite rehydrated with 10 ml of normal saline, which represented 48–90 μg stannous chloride, 0.5 mg sodium pyrophosphate and 1.5 mg trimetaphosphate. After 20 min, 2.5 ml sodium pertechnetate (4 mCi) was injected via an ear vein.

Technetium-99m-HSA Labeling Procedure

Technetium-99m-HSA was purchased as a commercial kit and reconstituted.

Imaging Studies

Animal experiments were performed under the National Institutes of Health Animal Use and Care guidelines and were approved by the University of Texas Health Science Center at San Antonio Institutional Animal Care Committee. Male New Zealand white rabbits (3.5–4 kg) were anesthetized intramuscularly with ketamine:xylazine (50mg/10mg) and placed in the supine position. PEG ^{99m}Tc-liposomes (2.0 ml, 2 mCi, n = 6), neutral ^{99m}Tc-liposomes (2.0 ml, 1.3 mCi, n = 5), in vitro ^{99m}Tc-red cells (2.0 ml, 2.8 mCi, n = 4), in vivo ^{99m}Tc-red cells (2.0 ml, 4 mCi, n = 4) or ^{99m}Tc-HSA (2.0 ml, 3.0 mCi, n = 4) were injected through an ear vein. Phospholipid dose was approximately 17 mg phospholipid/kg body weight for both neutral and PEG ^{99m}Tc-liposomes. Whole-body and zoomed scintigrams (zoom of 2) magnifying the heart region were acquired using a gamma camera equipped with a high-resolution collimator. The camera was interfaced to a dedicated computer workstation and 1-min static images were acquired using a 64 × 64 matrix at 5, 22, 45 and 120 min postinjection. Five-minute static images were acquired for all radiopharmaceuticals at 20 hr due to loss of signal from isotope decay.

Image Analysis

Heart-to-liver and heart-to-lung ratios were determined from region of interest (ROI) analysis of zoomed static images. The count density of a 2 × 2-pixel box located over the heart, lung and liver in each image was recorded and these values were used to determine the heart-to-lung and heart-to-liver ratios. Bladder activity was determined by drawing a ROI around the bladder in each whole-body static image. A box was drawn around the entire body in the image to determine total body counts.

Blood Sampling

The circulation kinetics of the radiopharmaceuticals were determined from blood samples (100 μl) withdrawn via an ear artery. Samples were collected immediately following the infusion of the radiopharmaceutical (approximately 3 min), at 5 min, every 15 min for the first 2 hr, at 20 hr and at 44 hr. The activity of each sample was measured in a scintillation well counter. The activity measured in the sample at the 3 min time point for each animal was taken as the maximal value (100%) and the activities at the other time points were related to this value. A sample (100 μl) of each radiopharmaceutical was also counted as a standard reference.

Statistical Analysis

Values are reported as mean ± s.e.m. Statistical analysis was performed using Statworks software for the Macintosh computer. Student's unpaired t-test was used to compare the heart-to-lung ratios, heart-to-liver ratios and bladder activity for each agent at a given time. A p value <0.05 was considered statistically significant. Blood clearance curves were fitted to an exponential model to generate circulation half-lives using Scientist for Windows software with supplemental Pharmacokinetic Model Library (MicroMath, Salt Lake City, UT).

RESULTS

Whole-body gamma camera images of rabbits acquired 45 min postinjection of each blood-pool agent are shown in Figure 1. For cardiac function studies, the relationship of the distribution of the agent in the heart compared to the background activity associated with the lungs and liver is important, especially for accurate quantitation of ejection fraction. Comparison of these regions in the images showed that the activity associated with the blood pool was prominent for all agents except ^{99m}Tc-HSA. In these images, blood-pool activity was greater than lung activity for all agents tested. This observation was further substantiated by determining that the heart-to-lung ratios for each agent were ≥1, as outlined in Table 1. Comparison of the 45 min heart-to-lung ratios, which correspond to the

TABLE 1
Heart-to-Lung Ratios

Time (min)	PEG liposomes (n = 6)	Neutral liposomes (n = 5)	In vitro RBCs (n = 4)	In vivo RBCs (n = 4)	HSA (n = 4)
22	2.09 ± 0.21	2.09 ± 0.11*†	2.76 ± 0.16‡	1.92 ± 0.05†‡	1.66 ± 0.02
45	2.40 ± 0.25*	2.20 ± 0.09†‡	2.71 ± 0.11§	2.02 ± 0.12*†	1.58 ± 0.05
120	2.30 ± 0.21	2.31 ± 0.21*	2.75 ± 0.07§	2.09 ± 0.16†	1.70 ± 0.05

Values represent the mean ± s.e.m.
*$p < 0.05$ versus HSA.
†$p < 0.01$ versus in vitro RBCs.
‡$p < 0.01$ versus HSA.
§$p < 0.001$ versus HSA.

images, showed that the ratio for PEG ^{99m}Tc-liposomes was greater but not significantly different than the neutral ^{99m}Tc-liposome ratio. Also the ratio for PEG ^{99m}Tc-liposomes was not significantly lower than the value determined for in vitro ^{99m}Tc-red cells, unlike the value determined for neutral ^{99m}Tc-liposomes, which was significantly less ($p < 0.01$). Ratios determined for both liposome-based agents were slightly greater than the in vivo ^{99m}Tc-red cell value, but were not statistically different. Both liposome formulations, however, were significantly greater than the ratio for ^{99m}Tc-HSA ($p < 0.05$). Like the liposome-based ratios, heart-to-lung ratios for both ^{99m}Tc-red cell preparations were significantly greater than for ^{99m}Tc-HSA ($p < 0.001$ for in vitro ^{99m}Tc-red cells; $p < 0.05$ for in vivo ^{99m}Tc-red cells). During the initial 120 min, there were no statistically significant differences in the heart-to-lung ratios as a function of time for any of the agents. Although the ratios for both ^{99m}Tc-liposome-based agents did increase during this period, these increases were not significant.

The images also showed that blood-pool activity was greater than liver activity for all agents except ^{99m}Tc-HSA, which was the only agent with heart-to-liver ratios <1, as outlined in Table 2. Blood-pool activity was similar for both ^{99m}Tc-liposome formulations, but there was less liver activity associated with PEG ^{99m}Tc-liposomes than neutral ^{99m}Tc-liposomes, which lead to a higher heart-to-liver ratio for PEG ^{99m}Tc-liposomes compared to neutral ^{99m}Tc-liposomes. This ratio for PEG ^{99m}Tc-liposomes was not significantly different from the in vitro ^{99m}Tc-red cell ratio, whereas the ratio for neutral ^{99m}Tc-liposomes was significantly lower. Heart-to-liver ratios for both liposome formulations were significantly greater than ^{99m}Tc-HSA at all time points studied. Likewise, the ratios for both in vitro and in vivo ^{99m}Tc-red cells were significantly greater than the ratios for ^{99m}Tc-HSA at all time points. A comparison between ^{99m}Tc-red cell labeling methods showed that the ratio for in vitro ^{99m}Tc-red cells was greater than the ratio for in vivo ^{99m}Tc-red cells ($p < 0.05$) at 45 min. As with the heart-to-lung ratios, there were no significant effects of time on the heart-to-liver ratios for the blood-pool agents, with the exception of the ratio for ^{99m}Tc-HSA, which decreased significantly.

At 45 min postinjection, there were major differences in the organ distribution of the blood-pool agents in the abdominal region where blood-pool agents are used to detect sites of gastrointestinal bleeding (Fig. 1). There was very little spleen activity seen with either in vitro or in vivo ^{99m}Tc-red cells, indicating that the red cells were not damaged during the labeling process. The ^{99m}Tc-liposome formulations showed greater activity in the spleen than either ^{99m}Tc-red cells or ^{99m}Tc-HSA. This result is not surprising, since liposomes are known to be cleared from the circulation by the spleen (*11*). Yet, spleen activity reported in the present study for both liposome formulations is lower than previously reported by our laboratory for liposome formulations comprised of negative and neutral surface charges (*8,9*). Lack of bowel activity in the images for each agent tested was also observed. The most noticeable difference in the abdominal region is the low activity associated with the kidneys and bladder for both ^{99m}Tc-liposome formulations compared to both in vitro and in vivo ^{99m}Tc-red cells and ^{99m}Tc-HSA.

Figure 2 shows images of the same rabbits acquired at 20 hr postinjection. At this time, there is still sufficient activity

TABLE 2
Heart-to-Liver Ratios

Time (Mins)	PEG liposomes (n = 6)	Neutral liposomes (n = 5)	In vitro RBCs (n = 4)	In vivo RBCs (n = 4)	HSA (n = 4)
22	1.71 ± 0.09§	1.40 ± 0.07†‡	2.02 ± 0.11§	1.50 ± 0.14¶	1.09 ± 0.05*
45	1.79 ± 0.15††	1.43 ± 0.05**	1.81 ± 0.10§	1.56 ± 0.11††	0.97 ± 0.06
120	1.53 ± 0.07§‡‡	1.31 ± 0.08**	1.89 ± 0.13§	1.62 ± 0.12††	0.91 ± 0.05

*$p < 0.05$ versus 120 minutes.
†$p < 0.05$ versus PEG liposomes, HSA.
‡$p < 0.01$ versus in vitro RBCs.
§$p < 0.001$ versus HSA.
¶$p < 0.05$ versus in vitro RBCs, HSA.
**$p < 0.01$ versus in vitro RBCs, HSA.
††$p < 0.01$ versus HSA.
‡‡$p < 0.05$ versus in vitro RBCs.
Values represent mean ± s.e.m.

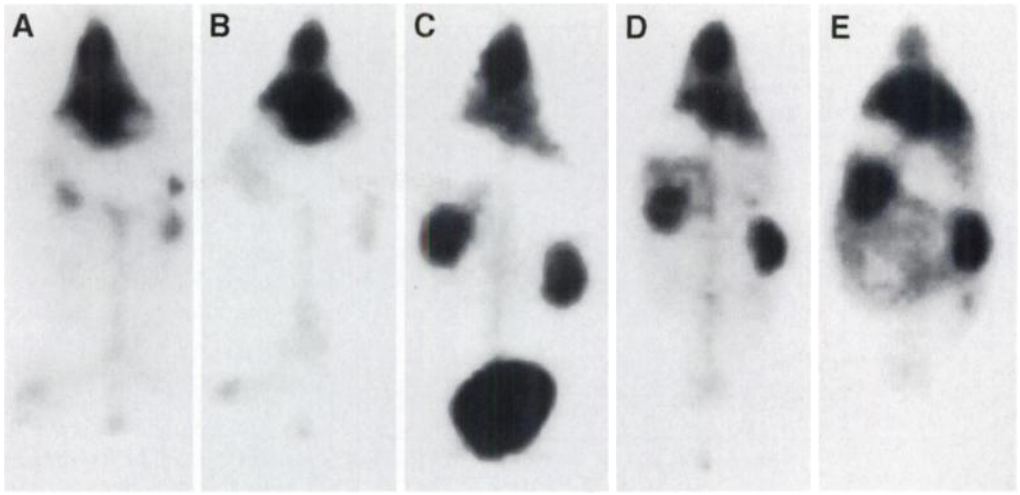

FIGURE 2. Whole-body images of rabbits acquired 20 hr after intravenous injection of: (A) PEG ^{99m}Tc-liposomes, (B) neutral ^{99m}Tc-liposomes, (C) in vitro ^{99m}Tc-red cells, (D) in vivo ^{99m}Tc-red cells or (E) ^{99m}Tc-HSA.

associated with the blood pool for both PEG ^{99m}Tc-liposomes, neutral ^{99m}Tc-liposomes, in vitro ^{99m}Tc-red cells and in vivo ^{99m}Tc-red cells compared to ^{99m}Tc-HSA. There is also more activity associated with the blood pool than with the lungs for each agent. In these delayed images, liver activity definitely increased for ^{99m}Tc-HSA, increased slightly for both PEG ^{99m}Tc-liposomes and neutral ^{99m}Tc-liposomes, but remained constant for both in vitro and in vivo ^{99m}Tc-red cells. The slight increase in liver activity for the liposomes, which was greater for neutral ^{99m}Tc-liposomes than for PEG ^{99m}Tc-liposomes, is most likely the result of the natural removal process of the liposomes by liver phagocytic cells (*11*). At 20 hr, PEG ^{99m}Tc-liposomes and neutral ^{99m}Tc-liposomes had more spleen activity than both in vitro and in vivo ^{99m}Tc-red cells and ^{99m}Tc-HSA. Also, there was little kidney or bladder activity following injection of either PEG ^{99m}Tc-liposomes or neutral ^{99m}Tc-liposomes compared to both in vitro and in vivo ^{99m}Tc-red cells and ^{99m}Tc-HSA. In addition, rabbits injected with ^{99m}Tc-HSA showed significant bowel activity compared to those receiving either PEG ^{99m}Tc-liposomes, neutral ^{99m}Tc-liposomes, in vitro ^{99m}Tc-red cells or in vivo ^{99m}Tc-red cells. Finally, we note that rabbits receiving an injection of PEG ^{99m}Tc-liposomes, neutral ^{99m}Tc-liposomes or ^{99m}Tc-HSA, but not ^{99m}Tc-red cells, showed activity in the thigh which became inflamed after receiving multiple intramuscular injections of anesthesia.

Figure 3 shows the clearance kinetics for the blood-pool agents over the first 120 min (inset) as well as at later time points. At 45 min, in vivo stability for both ^{99m}Tc-liposome formulations was similar to in vivo ^{99m}Tc-red cells and significantly greater than in vitro ^{99m}Tc-red cells and ^{99m}Tc-HSA. By 120 min, both liposome formulations continued to remain in circulation at higher levels than the other agents including in vivo ^{99m}Tc-red cells. Blood clearance curves were fitted to a bolus one compartment model using pharmacokinetic curve fitting software. PEG ^{99m}Tc-liposomes (half-life = 36.3 hr) remained in circulation longer than neutral ^{99m}Tc-liposomes (half-life = 23.2 hr), in vivo ^{99m}Tc-red cells (half-life = 14.6 hr), in vitro ^{99m}Tc-red cells (half-life = 8.8 hr) and ^{99m}Tc-HSA (half-life = 2.1 hr). This longer circulation time of PEG ^{99m}Tc-liposomes agrees with those noted by other researchers and is thought to be due to the decreased recognition of PEG-coated liposomes by liver and spleen macrophages (*6*).

Figure 4 shows the corresponding bladder activity for each blood-pool agent during the first 120 min postinjection. By 45 min, bladder activity was 4.0% ± 1.1% and 3.1% ± 0.6% for PEG ^{99m}Tc-liposomes and neutral ^{99m}Tc-liposomes, respectively. These values were significantly less than the value for in vitro ^{99m}Tc-red cells, in vivo ^{99m}Tc-red cells and ^{99m}Tc-HSA. Increased bladder activity is due to leakage of the ^{99m}Tc label from the red cells and dissociation of the ^{99m}Tc label from the HSA. In both instances, free ^{99m}Tc label is excreted through the kidneys and bladder. Both in vitro and in vivo ^{99m}Tc-red cells had significantly less bladder activity than ^{99m}Tc-HSA. Also, there was more bladder activity associated with the in vivo red cell labeling method compared to the in vitro method. A similar trend in the bladder activity was seen at 120 min, which showed a continual increase in the bladder activity for ^{99m}Tc-red cells and ^{99m}Tc-HSA, while the bladder activity for both ^{99m}Tc-liposome formulations remained constant throughout the experimental period. These results show the excellent in vivo stability of the ^{99m}Tc label associated with the liposomes compared to other agents currently in clinical use.

DISCUSSION

Technetium-99m-labeled liposomes were evaluated in this study in order to determine if they meet the criteria of being a safe, convenient and stable radiopharmaceutical for blood-pool imaging studies. Both ^{99m}Tc-liposome formulations were compared with ^{99m}Tc-red cells labeled using standard in vivo and in vitro methods. The labeling efficiencies, blood circulation times and bladder activities for both red cell labeling methods were similar to those seen in other studies comparing the in vivo and in vitro methods (*1*). Also, as demonstrated by other researchers

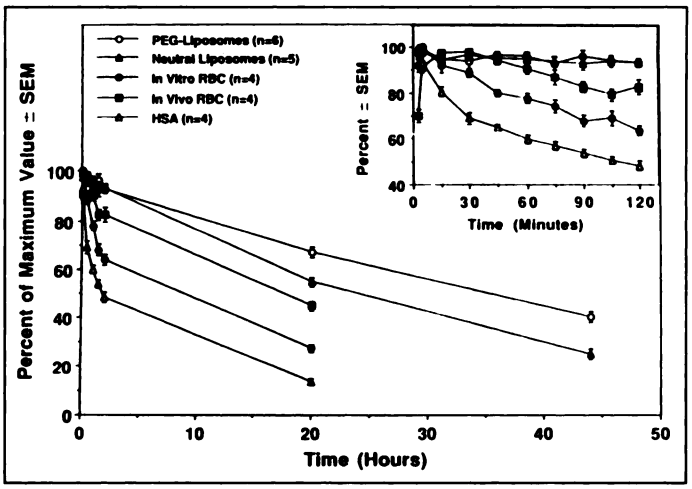

FIGURE 3. Clearance of blood-pool imaging agents from the bloodstream after intravenous injection in rabbits. Inset: Clearance pattern during the first 120 min postinjection.

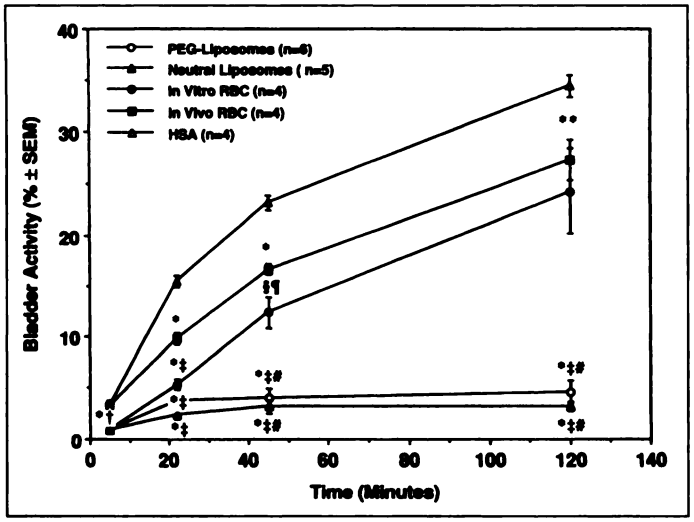

FIGURE 4. Bladder activity during the first 120 min after intravenous injection of blood-pool agents in rabbits. Differences considered to be statistically significant are indicated as follows: *$p < 0.001$ versus ^{99m}Tc-HSA, †$p < 0.01$ versus in vivo ^{99m}Tc-red cells, ‡$p < 0.001$ versus in vivo ^{99m}Tc-red cells, §$p < 0.05$ versus in vivo ^{99m}Tc-red cells, ¶$p < 0.01$ versus ^{99m}Tc-HSA, #$p < 0.01$ versus in vitro ^{99m}Tc-red cells, **$p < 0.05$ versus ^{99m}Tc-HSA.

and confirmed in the present study, heart-to-lung and heart-to-liver ratios were better for the in vitro ^{99m}Tc-red cells than in vivo ^{99m}Tc-red cells due to lower background ^{99m}Tc activity in the lungs and liver for in vitro ^{99m}Tc-red cells (*1*).

As can be seen from the images (Fig. 1), heart-to-lung ratios and heart-to-liver ratios, ^{99m}Tc-liposomes could potentially be substituted for ^{99m}Tc-red cells as blood-pool agents. At 45 min, heart-to-lung and heart-to-liver ratios for PEG ^{99m}Tc-liposomes were not significantly different from ^{99m}Tc-red cell ratios, regardless of the labeling method employed. On the other hand, heart-to-lung and heart-to-liver ratios for neutral ^{99m}Tc-liposomes did not significantly differ for in vivo ^{99m}Tc-red cells, but were significantly less than in vitro ^{99m}Tc-red cells. Therefore, PEG ^{99m}Tc-liposomes would make a better blood-pool agent than neutral ^{99m}Tc-liposomes.

Although heart-to-lung and heart-to-liver ratios for the ^{99m}Tc-liposomes were lower than in vitro ^{99m}Tc-red cells in this rabbit model, both ^{99m}Tc-liposomal agents remained in the vasculature longer than ^{99m}Tc-red cells. The half-life of 8.8 hr for in vitro ^{99m}Tc-red cells determined in rabbits in this study is less than the biological half-life of 29 hr reported in humans, but agree with other studies in rabbits by the manufacturer (Ultratag package insert, *personal communication*, Dr. Robert Wolfangel, Mallinckrodt Medical). Also, during the first 120 min, bladder activities for both ^{99m}Tc-liposome formulations were significantly lower than both ^{99m}Tc-red cell preparations. This increased blood retention and low bladder activity for both ^{99m}Tc-liposome formulations show the excellent in vivo stability of ^{99m}Tc label associated with the liposomes compared to ^{99m}Tc-red cells labeled by either method. There are several other advantages for using ^{99m}Tc-liposomes, and for using PEG ^{99m}Tc-liposomes over ^{99m}Tc-red cells in blood-pool imaging studies in particular. These advantages include increased safety, because the technologist does not have to unnecessarily handle potentially contaminated blood during the red cell labeling procedure, and less chance of accidentally injecting in vitro labeled ^{99m}Tc-red cells from one patient to another. Technetium-99m-liposomes are also more convenient to use than ^{99m}Tc-red cells because they require only one venipuncture and can be labeled before the patient arrives. In addition, ^{99m}Tc-liposomes have been found to be very stable following reconstitution and labeling with little decomposition of the ^{99m}Tc label (>6 hr) in previous studies (*7*). In contrast, it is recommended that in vitro ^{99m}Tc-red cells be reinjected into the patient within 30 min (Ultratag package insert). Therefore, unlike both in vivo and in vitro ^{99m}Tc-red cells, ^{99m}Tc-liposomes could be prepared ahead of time for emergency situations. Technetium-99m-liposomes would be more reliable than ^{99m}Tc-red cells for gastrointestinal bleeding studies because of their excellent in vivo stability, as shown by the lack of significant bladder activity. Furthermore, ^{99m}Tc-liposomes are unlikely to be affected by medications prescribed for the patient that can interfere with the labeling and stability of ^{99m}Tc-red cells (*1,12*).

Both ^{99m}Tc-liposomes and ^{99m}Tc-HSA are convenient radiopharmaceuticals because they require few steps, use cheap and widely available sodium pertechnetate and can be prepared prior to the patient's arrival. A number of differences in their properties should be noted, however. Technetium-99m-liposomes would be safer to produce and administer than ^{99m}Tc-HSA, because HSA is derived from human blood, which is a source for the transmission of bloodborne pathogens between patients. Only recently has this safety issue been overcome by producing HSA using recombinant DNA technology (*13*). Another difference between the two agents is the improved in vivo stability of ^{99m}Tc-liposomes over ^{99m}Tc-HSA. The ^{99m}Tc-HSA is cleared rapidly from the blood pool compared to both ^{99m}Tc-liposome formulations. By 120 min, there was 8 times (34.4% for ^{99m}Tc-HSA versus 4.5% for PEG ^{99m}Tc-liposomes) more renal excretion of the ^{99m}Tc label. This lack of in vivo stability produces heart-to-lung and heart-to-liver ratios for ^{99m}Tc-HSA that are significantly less than the ratios for both ^{99m}Tc-liposome formulations. Recently, Verbeke et al. (*2,3*) developed a dimercaptopropionyl-modified HSA and showed that it had superior in vivo stability when compared to a conventional kit of ^{99m}Tc-HSA in human volunteers. This in vivo stability was similar to that shown for ^{99m}Tc-liposomes in that there was good retention of the modified HSA in the bloodstream and low bladder activity. Heart-to-lung and heart-to-liver ratios for the modified HSA were also shown to be comparable to ratios determined for in vitro ^{99m}Tc-red cells. Despite these positive features, a major disadvantage of the modified HSA agent is that it still uses HSA as a starting material. Also, long-term storage of modified HSA may not be as reliable as ^{99m}Tc-liposomes due to oxidation of the sulfhydryl groups (*2–4*).

A direct comparison study between ^{99m}Tc-liposomes and other synthetically derived agents was not feasible, although several points can be discussed (*4*). First, PEG ^{99m}Tc-DTPA polylysine copolymer and ^{99m}Tc-liposomes would both be safe because the agents are derived independent of potentially infectious blood products and are produced from compounds known to be biocompatible. These agents are also convenient in that they can be packaged in kit form and easily prepared as needed. The main differences in the ^{99m}Tc-liposomes and polymer concern the stability of the agent following injection into the body. Although both the polymer and the ^{99m}Tc-liposomes showed similar circulation half-lives in rabbits (31.5 and 35 hr, respectively), the polymer demonstrated a lack of in vivo stability when compared to the ^{99m}Tc-liposomes. Approximately 25% of the ^{99m}Tc label was excreted by the animals receiving polymer at 24 hr postinjection, which was thought to be due to the low affinity of the DTPA chelator for ^{99m}Tc (*4*). The added in vivo stability of ^{99m}Tc-liposomes compared to the polymer may also be due to the fact that any free ^{99m}Tc label was removed when the liposomes were passed through a

column before injection. The distribution of the polymer in the liver was greater than in vitro ^{99m}Tc-red cells, thus producing a lower heart-to-liver ratio which may be related to the size of the polymer. We also observed lower heart-to-liver ratios for both ^{99m}Tc-liposome preparations compared to in vitro ^{99m}Tc-red cells, with the PEG ^{99m}Tc-liposomes having higher ratios than neutral ^{99m}Tc-liposomes.

Initially, the use of liposomes as blood-pool agents was limited due to their rapid removal from circulation (*14,15*). In recent years, advances in formulation by the addition of PEG-phospholipids and processing technology using extrusion have lead to a liposome product that is less toxic and has increased shelf stability. Moreover, the development of a method to label liposomes with ^{99m}Tc that resulted in both chemical and biological stability following intravenous injection has been instrumental in the diagnostic use of liposomes. The glutathione-HMPAO method used in the present study is superior to an earlier liposome surface labeling technique because of its excellent in vivo stability (*16,17*). The lack of dissociation of the ^{99m}Tc label from liposomes labeled by the glutathione-HMPAO method results in better counting statistics for cardiac studies and increases the potential for definitive detection of gastrointestinal bleeding sites over interfering bowel and bladder activity.

A column purification step was used before injecting ^{99m}Tc-liposomes because the labeling efficiencies determined for these particular liposome preparations was lower than those measured previously for other formulations (*7*). This additional step increased both preparation time and contamination risk. Recent studies by our group indicate that these low labeling efficiencies occur because of the presence of glutathione on the outside of the liposome which immediately converts HMPAO to its hydrophilic form. Experiments are underway to remove any extravesicular glutathione by washing the liposomes during manufacturing. Preliminary results from these washing studies indicate that labeling efficiencies >95% can be achieved and that these higher labeling efficiencies will make column purification unnecessary. Syringes packed with common gel filtration media are also being tested in order to develop a closed kit system for producing a sterile ^{99m}Tc-liposome product.

The use of HMPAO for ^{99m}Tc-liposome labeling has its advantages and disadvantages. The main advantage is that HMPAO is an approved radiopharmaceutical for brain imaging and white blood cell labeling. Prior clinical approval of this agent potentially could make the approval process for ^{99m}Tc-liposomes easier. Disadvantages of HMPAO include cost, the need for recently eluted pertechnetate and chemical instability once reconstituted with pertechnetate. The recently added white cell labeling indication for HMPAO may increase demand and in turn lower cost. Also, the need for freshly eluted pertechnetate may not be as important in ^{99m}Tc-liposome labeling as it is in brain imaging. The chemical instability of HMPAO, previously requiring injection within 30 min of reconstitution, has recently been extended to 4 hr with the addition of methylene blue to the HMPAO kit. For both white cell labeling and liposome labeling, however, the instability of the HMPAO kit is of little importance, since the liposomes can be prepared before reconstitution of the HMPAO kit. Once labeled the liposomes also remain stable for more than 6 hr.

CONCLUSION

This study demonstrates the feasibility of using ^{99m}Tc-liposomes labeled with the glutathione-HMPAO method as blood-pool agents. Technetium-99m-liposomes composed of two different lipid formulations had sufficient circulation persistence and excellent in vivo stability when compared to ^{99m}Tc-red cells and ^{99m}Tc-HSA. For imaging applications, ^{99m}Tc-liposomes with a PEG surface modification would be a better agent than neutral ^{99m}Tc-liposomes due to lower liver background activity. Future directions for the use of PEG ^{99m}Tc-liposomes include developing a long-term storage strategy, scaling up current processing techniques to meet good manufacturing practices and conducting toxicity and efficacy testing in various animal models.

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