^{186}Re-Liposome Labeling Using ^{186}Re-SNS/S Complexes: In Vitro Stability, Imaging, and Biodistribution in Rats

Ande Bao, MS[1]; Beth Goins, PhD[1]; Robert Klipper, BA[1]; George Negrete, PhD[2]; and William T. Phillips, MD[1]

[1]*Department of Radiology, University of Texas Health Science Center at San Antonio, San Antonio, Texas; and* [2]*Department of Chemistry, University of Texas at San Antonio, San Antonio, Texas*

Liposomes are important carriers for controlling the spatial and temporal distribution of drug molecules or other bioactive molecules. Radiolabeled liposomes have potential applications in diagnostic imaging and radionuclide therapy. The purpose of this study was to develop a practical method for labeling liposomes with therapeutic rhenium radionuclides, using ^{186}Re as an example. **Methods:** An SNS pattern ligand, *N*,*N*-bis(2-mercaptoethyl)-*N*′,*N*′-diethylethylenediamine (BMEDA), and an S pattern ligand, benzene thiol (BT), were used to make 2 kinds of ^{186}Re-SNS/S complexes, ^{186}Re-BMEDA and ^{186}Re-BMEDA + BT. These ^{186}Re-SNS/S complexes were mixed with neutral liposomes encapsulating cysteine or $(NH_4)_2SO_4$ to prepare ^{186}Re-liposomes. The in vitro labeling stability of ^{186}Re-liposomes was investigated by incubation in 50% fetal bovine serum/50% phosphate-buffered saline, pH 7.4, at 37°C. Rat distribution studies of ^{186}Re-liposomes after intravenous injection were also performed. **Results:** The labeling efficiencies of ^{186}Re-liposomes were 52.9%–81.3% depending on the ^{186}Re-SNS/S complex chosen and whether cysteine- or $(NH_4)_2SO_4$-encapsulated liposomes were used. ^{186}Re-$(NH_4)_2SO_4$ liposomes labeled with ^{186}Re-BMEDA had the best in vitro labeling stability in serum with 89.8% ± 3.1% of the radioactivity associated with liposomes at 24 h and 76.2% ± 5.1% at 96 h. A specific activity of 1.85 GBq (50 mCi) of ^{186}Re per 50 mg of phospholipid could be achieved with good labeling stability. Biodistributions were followed for 72 h and showed good in vivo stability for ^{186}Re-liposomes that was characterized by a slow blood clearance and a gradually increasing spleen accumulation. ^{186}Re-BMEDA alone had fast blood clearance and no accumulation in spleen. **Conclusion:** A practical method for labeling liposomes with ^{186}Re using ^{186}Re-SNS/S complexes is described. The labeled ^{186}Re-liposomes were stable in serum and in vivo and could potentially be useful for radionuclide therapy.

Key Words: liposomes; ^{186}Re; SNS/S complexes; radiolabeling; biodistribution; radionuclide therapy

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Liposomes are double-membrane lipid vesicles. They have been widely studied as important carriers in controlling the spatial and temporal distribution of drug molecules or other bioactive molecules for targeted therapy. Liposomes have been investigated widely as universal carriers of tumor chemotherapeutic agents (*1*,*2*), as antigen carriers to stimulate immune response (*1*,*3*), as carriers of nucleic acid for gene therapy (*1*,*4*), and as carriers of antibiotics for infectious disease treatment (*1*).

Liposomes are promising carriers for radionuclide therapy for the following reasons:

- Biocompatibility: Lipids and cholesterol (Chol) used for liposome manufacture are common constitutes of cell membranes and therefore are easily metabolized.
- Varying uniform sizes: Liposomes with variable homogeneous particle size ranges can readily be produced by using the extrusion technique (*5*).
- Modification of surface properties: The surface of liposomes can be modified with different kinds of functional groups, such as antibodies, folic acid, peptides, and so forth (*6–9*), enabling radiolabeled liposomes to be used for molecular imaging and targeted radionuclide therapy.
- Controlled migration of liposomes and release of radioisotopes from liposomes: Use of different liposome components and different labeling methods to control the liposome migration and radioisotope release from liposomes may be helpful for delivering a uniform dose distribution in the tumor tissue (*10*).
- Use of physical modalities for targeting: Targeted hyperthermia and radiation to the targeted tissues (*11*,*12*) can significantly increase the accumulation of radiolabeled liposomes to targeted tissues.

There are many studies using ^{99m}Tc-, ^{67}Ga-, and ^{111}In-labeled liposomes for nuclear imaging (*13–16*), which have shown that radiolabeled liposomes have good accumulation characteristics in tumor, infection, and inflammation in vivo (*17*,*18*). Small unilaminar liposomes (*19*) composed of saturated lipids and Chol have been shown to have reduced

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For correspondence or reprints contact: William T. Phillips, MD, Department of Radiology, MSC 7800, UTHSCSA, 7703 Floyd Curl Dr., San Antonio, TX 78229-3900.

E-mail: phillips@uthscsa.edu

uptake by the reticuloendothelial system (RES) compared with liposomes with no Chol. Theoretic dose calculation studies by Emfietzoglou et al. (*20*) have suggested that if methods for labeling these small unilaminar liposomes with therapeutic radionuclides could be developed, these intravenously administered liposomes would deliver a high radiation dose to tumor tissues while sparing the red marrow and having acceptable doses to liver and spleen. These theoretic calculations were based on distribution studies of ^{67}Ga-labeled liposomes. Surface modification of liposomes using polyethylene glycol (PEG) can change the in vivo distribution of liposomes significantly after intravenous injection (*1*,*21*,*22*). In addition to intravenous delivery, other methods of delivering radiolabeled liposomes are also possible. Harrington et al. (*23*) have studied the biodistribution of ^{111}In-labeled pegylated liposomes via intratumoral or subcutaneous injection techniques. Their results showed that pegylated liposomes have potential as vehicles for intratumoral and subcutaneous drug delivery.

Until now, no promising method has been described for labeling liposomes with the ^{186}Re and ^{188}Re therapeutic radioisotopes. We have previously described a method for labeling liposomes using ^{99m}Tc-SNS/S complexes (*24*), which have been studied previously as general radiopharmaceuticals intended for use mainly as brain imaging agents (*25*). Studies using the crystal structures of stable Re-SNS/S complexes and ^{186}Re-labeled SNS/S complexes inferred that ^{99m}Tc-SNS/S complexes have a neutral core coordinate structure that is the same as the coordinate structure of Re-SNS/S complexes (*26*,*27*). The ^{186}Re-SNS/S complexes can also be achieved by using routine practical radiolabeling methods (*27*). In this article, we report a liposome labeling method using ^{186}Re-SNS/S pattern complexes (Fig. 1) and liposomes encapsulating cysteine or $(NH_4)_2SO_4$.

MATERIALS AND METHODS

Liposome Manufacture and Characterization

Two liposome formulations, cysteine-encapsulated liposomes and $(NH_4)_2SO_4$-encapsulated liposomes (transmembrane ammonium gradient liposomes), were studied for ^{186}Re labeling. All liposome formulations were comprised of the same lipid components, distearoyl phosphatidylcholine (DSPC) (Avanti Polar Lipids), Chol (Calbiochem), and α-tocopherol (Aldrich) at a molar percentage of 54:44:2. All lipids were used without further purification. Liposomes encapsulating cysteine were produced as previously outlined by Goins et al. (*21*). Liposomes encapsulating $(NH_4)_2SO_4$ were prepared from a modification of the method by Maurer-Spurej et al. (*28*).

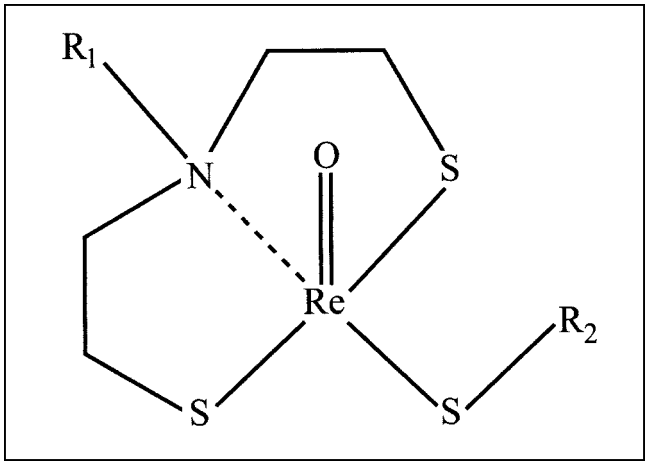

FIGURE 1. Structure of ^{186}Re/^{188}Re-SNS/S complexes.

Cysteine-Encapsulated Liposomes. DSPC, Chol, and α-tocopherol were mixed and dissolved in chloroform. Chloroform was then removed by rotary evaporation to form a lipid film. The lipid film was stored overnight in a vacuum desiccator to remove organic solvent. Samples were rehydrated with 300 mmol/L sucrose (Sigma) in sterile water for injection and warmed to 55°C for 15 min with periodic vortexing until the lipids were in suspension. The resultant multilamellar vesicles formed from rehydration were then frozen in liquid nitrogen and lyophilized. The resultant dry sugar-lipid preparations were then rehydrated with 200 mmol/L cysteine (Sigma) in Dulbecco's phosphate-buffered saline (PBS), pH 6.3, at a total lipid concentration of 120 mmol/L. The solutions were then diluted at a v/v ratio of 1 part lipid suspension to 2 parts PBS, pH 6.3, containing 150 mmol/L sucrose, and 100 mmol/L cysteine. The diluted lipid suspensions were then extruded through a series of polycarbonate filters (extruder, Lipex Extruder; filter, Whatman Nucleopore) at 55°C: 400-nm liposomes: 2 passes, 2 μm; then 5 passes, 400 nm; 100-nm liposomes: 2 passes, 2 μm; then 2 passes, 400 nm; then 2 passes, 200 nm; then 5 passes, 100 nm. The extruded lipid solution was then washed in PBS, pH 6.3, containing 75 mmol/L sucrose and centrifuged at 200,000 × g for 45 min to remove unencapsulated sucrose and cysteine and to concentrate the liposome sample. The washing step was repeated 3 times. The final liposome pellet was resuspended in PBS, pH 6.3, containing 300 mmol/L sucrose at a lipid concentration of 120 mmol/L and stored in the refrigerator at 4°C for up to 3 mo until needed for radiolabeling studies.

Liposomal size was monitored using 488-nm laser light scattering (Dynamic Light Scattering; Brookhaven Instruments). The measured sizes were 312.6 ± 27.7 nm for 400-nm cysteine liposomes and 115.8 ± 11.7 nm for 100-nm cysteine liposomes. Phospholipid concentrations determined using Stewart's method (*29*) were 44.4 mg/mL for 400- and 100-nm cysteine liposomes. Cysteine concentrations determined using a GSH-400 assay kit (OXIS International) were 5.5 mmol/L for 400-nm liposomes and 6.3 mmol/L for 100-nm liposomes. Bacterial and endotoxin tests were performed by Pathology Service Referral Laboratory, University Health System. No bacterial growth during 14 d of incubation at 37°C was observed for either 400- or 100-nm cysteine liposomes. A Gram-negative antigen screen test showed that the endotoxin level was >12.5 but <25 endotoxin units (EU)/mL for 100-nm cysteine liposomes, which were administered to rats.

$(NH_4)_2SO_4$-Encapsulated Liposomes. The lipid film samples prepared as described above were rehydrated with 300 mmol/L $(NH_4)_2SO_4$ (Sigma) in sterile water for injection (120 mmol/L total lipid) and warmed to 55°C for 15 min with periodic vortexing until the lipids were in suspension. The resultant multilamellar vesicles formed from rehydration were then frozen in liquid nitrogen and thawed at 55°C for 5 cycles. The solutions were then diluted at a v/v ratio of 1 part lipid suspension to 1 part 300 mmol/L $(NH_4)_2SO_4$ solution. The diluted lipid suspensions were then extruded through the series of polycarbonate filters as described

above. The extruded lipid solution was then stored in the refrigerator at 4°C for up to 6 mo until needed for radiolabeling studies.

Liposomal sizes were 443.1 ± 29.9 nm for 400-nm $(NH_4)_2SO_4$ liposomes and 147.7 ± 12.5 nm for 100-nm $(NH_4)_2SO_4$ liposomes. Phospholipid concentrations were 21.9 mg/mL for 400-nm $(NH_4)_2SO_4$ liposomes and 21.2 mg/mL for 100-nm $(NH_4)_2SO_4$ liposomes. No bacterial growth was observed for both 400- and 100-nm $(NH_4)_2SO_4$ liposomes. The endotoxin level was >5 but <12.5 EU/mL for the 100-nm $(NH_4)_2SO_4$ liposomes.

Preparation of ^{186}Re-SNS/S Complexes

An SNS pattern ligand, *N*,*N*-bis(2-mercaptoethyl)-*N*′,*N*′-diethylethylenediamine (BMEDA), and an S pattern ligand, benzene thiol (BT), were investigated. BMEDA was synthesized from a modification of the method by Corbin et al. (*30*), and the chemical structures were verified using ^{1}H/^{13}C NMR. BT was purchased from Aldrich. Two kinds of ^{186}Re-SNS/S complexes (Fig. 2), ^{186}Re-BMEDA and ^{186}Re-BMEDA + BT, were studied as intermediates of liposome labeling.

^{186}Re-aluminum perrhenate (^{186}Re-Al$(ReO_4)_3$) was purchased from Missouri University Research Reactor. Stannous chloride (Aldrich) was used as the reductant and glucoheptonate (GH) (Sigma) was used as an intermediate ligand to make ^{186}Re-SNS/S complexes. Either 2.0 μL (2.2 mg) of BMEDA or 1.0 μL (1.1 mg) of BMEDA and 0.5 μL (0.5 mg) of BT was pipetted into a new vial. Then, 1.0 mL of 0.17 mol/L GH-0.10 mol/L acetate solution, pH 5.0, was added, followed by the addition of 80 μL of stannous chloride (15 mg/mL). The pH of the solution was adjusted to 5.0 with 1.0 mol/L NaOH. After flushing the solution with N_2 gas, 44.4 MBq (1.2 mCi) or 504 MBq (13.6 mCi) of ^{186}Re-Al$(ReO_4)_3$ (0.4 μg Re per mCi ^{186}Re) was added. The vial was sealed and heated in an 80°C water bath for 1 h. The labeling efficiency of the ^{186}Re-BMEDA complex was checked by paper chromatography with either methanol (R_f values: $^{186}ReO_4^-$, 0.4–0.7; ^{186}Re-GH, 0.0–0.2; ^{186}Re-BMEDA or ^{186}Re-BMEDA + BT, 0.8–1.0) or saline (R_f values: $^{186}ReO_4^-$, 0.7–0.9; ^{186}Re-GH, 0.7–1.0; ^{186}Re-BMEDA or ^{186}Re-BMEDA + BT, 0.0–0.2) as the eluent.

^{186}Re-Liposome Labeling

For convenience, 400-nm liposomes were used for in vitro stability studies because a low-speed tabletop centrifuge could be used to quickly separate the liposome pellet from supernatant. Our previous studies using glutathione (GSH) liposomes labeled with ^{99m}Tc-SNS/S complexes suggested the good correlation between the in vitro labeling stability using 400-nm liposomes and the in vivo labeling stability using 100-nm liposomes. For rat distribution studies, 100-nm liposomes were used to permit better assessment of in vivo stability because it is known from the literature that liposomes with particle sizes of >100 nm are rapidly cleared from the blood (*1*).

400-nm Liposomes. Immediately before radiolabeling, 0.2 mL of 400-nm $(NH_4)_2SO_4$ liposomes containing 5 mg of DSPC (60 mmol/L total lipid) were prepared by dilution with 1.2 mL of PBS buffer, pH 7.4, and centrifugation at 11,000 × g for 10 min to remove the extraliposomal $(NH_4)_2SO_4$. The supernatant was discarded and 0.60 mL of PBS buffer, pH 7.4, was added to resuspend liposomes. Cysteine liposomes (400 nm) (120 mmol/L, 0.10 mL) and 0.50 mL of PBS buffer, pH 7.4, were moved to a new vial for cysteine liposome labeling.

Freshly washed 400-nm $(NH_4)_2SO_4$ liposomes were mixed with 22.2 MBq (0.6 mCi) or 252 MBq (6.8 mCi) of ^{186}Re-BMEDA or ^{186}Re-BMEDA + BT solution adjusted to pH 7.0 and incubated at 37°C for 1 h. The 400-nm cysteine liposomes prepared as described above were mixed with 22.2 MBq (0.6 mCi) of ^{186}Re-BMEDA or ^{186}Re-BMEDA + BT solution (pH 7.0) and incubated at 37°C for 1 h. The labeling efficiency was determined as the ratio of the activity associated with liposome pellet after centrifugation.

100-nm Liposomes. Immediately before radiolabeling, 2.0 mL of 100-nm $(NH_4)_2SO_4$ liposomes containing 50 mg of DSPC were diluted with 2.0 mL of PBS buffer, pH 7.4, and centrifuged at 47,000 × g for 45 min to remove the extraliposomal $(NH_4)_2SO_4$. The supernatant was discarded and 1.0 mL of PBS buffer, pH 7.4, was added to resuspend liposomes. Cysteine liposomes (100 nm) (120 mmol/L, 1.0 mL) were moved to a new vial for cysteine liposome labeling.

The preparation of ^{186}Re-BMEDA for the labeling process of 100-nm liposomes for biodistribution studies was similar to that described above. ^{186}Re-Perrhenate (3.7 GBq [100 mCi]) and 4.5 μL (5.0 mg) of BMEDA were used to make ^{186}Re-BMEDA. The liposomes encapsulating cysteine or $(NH_4)_2SO_4$ prepared as described above were mixed with 2.22 GBq (60 mCi) of the ^{186}Re-BMEDA solution and incubated at 37°C for 1 h. Sephadex G-25 column chromatography with PBS buffer, pH 7.4, was used to separate 100-nm radiolabeled liposomes from free ^{186}Re-BMEDA. The liposomes are eluted from the column first and can be conveniently collected and visualized due to their opacity. The labeling efficiency was determined from the ^{186}Re activity before and after separation using a Radix dose calibrator.

In Vitro Labeling Stability Study of ^{186}Re-Liposomes

The in vitro labeling stabilities of 400-nm ^{186}Re-cysteine liposomes and 400-nm ^{186}Re-$(NH_4)_2SO_4$ liposomes labeled using ^{186}Re-BMEDA or ^{186}Re-BMEDA + BT were studied comparably. After separation of 400-nm ^{186}Re-liposomes from free ^{186}Re-SNS/S complexes by centrifugation, evaluation of the in vitro

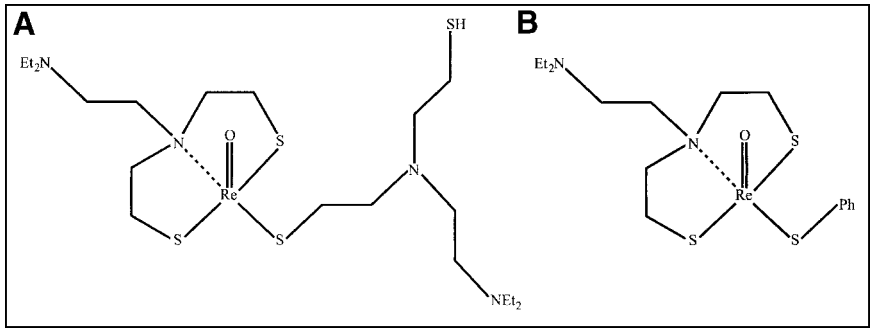

FIGURE 2. Structures of ^{186}Re-BMEDA (A) and ^{186}Re-BMEDA + BT (B).

TABLE 1
Labeling Efficiencies of 400-nm ^{186}Re-Liposomes Labeled with ^{186}Re-BMEDA and ^{186}Re-BMEDA + BT (n = 3)

Liposomes labeled with	Cysteine liposomes		$(NH_4)_2SO_4$ liposomes	
	^{186}Re-BMEDA	^{186}Re-BMEDA + BT	^{186}Re-BMEDA	^{186}Re-BMEDA + BT
Labeling efficiency (%)	52.9 ± 4.1*	68.7 ± 8.6	80.5 ± 7.8	81.3 ± 9.2

*$P < 0.05$ compared with $(NH_4)_2SO_4$ liposomes labeled with ^{186}Re-BMEDA or ^{186}Re-BMEDA + BT.

labeling stabilities of ^{186}Re-liposomes was performed by incubating ^{186}Re-liposomes (18.5 MBq [0.5 mCi] or 185 MBq [5 mCi] on average) in 1.6 mL of 50% fetal bovine serum (FBS) (GIBCO)-PBS buffer, pH 7.4, at 37°C. At certain times after serum incubation, 40 μL of ^{186}Re-liposome solution was removed with a micropipette to a test tube and counted for total radioactivity using a Minaxiγ A5550 γ-counter (Packard). Then, the incubation solution was centrifuged at 11,000 × g for 10 min and 40 μL of supernatant was removed to a fresh test tube and counted for radioactivity that did not associate with the liposome pellet. The liposome pellet and the remaining supernatant were resuspended and allowed to continue incubation until the next time point.

In Vivo Distribution of ^{186}Re-Liposomes in Normal Rats

The 100-nm ^{186}Re-liposomes were used for normal rat biodistribution studies. To ensure there was no free ^{186}Re-BMEDA in the liposome solutions, 100-nm ^{186}Re-liposome solutions were separated twice with Sephadex G-25 column chromatography before intravenous injection.

The animal experiments were performed according to the National Institutes of Health Animal Use and Guidelines and were approved by our Institutional Animal Care Committee. Rat distributions after intravenous injection of 100-nm ^{186}Re-cysteine liposomes or 100-nm ^{186}Re-$(NH_4)_2SO_4$ liposomes labeled with ^{186}Re-BMEDA were investigated in normal Sprague–Dawley male rats. For comparison, normal rat distributions of free ^{186}Re-BMEDA were also studied. After the rats were anesthetized by inhalation with isoflurane (3% in 100% oxygen), each rat (380 g on average) was injected with radiolabeled liposomes containing 114.7–133.2 MBq (3.1–3.6 mCi) of ^{186}Re and 4.2 mg of DSPC or 133.2 MBq (3.6 mCi) of free ^{186}Re-BMEDA. Planar images of the anesthetized rats in the prone position were collected at various times with a Picker Dyna 4 γ-camera interfaced to a Pinnacle computer workstation (MedaSys) (acquisition time: 1 min per image at baseline and at 1 and 4 h; 2 min per image at 24 and 72 h.). A low-energy, high-resolution collimator was used. The energy window was set at 137 keV ± 20%. The image size was set at 64 × 64.

After 72 h, anesthetized rats were euthanized by cervical dislocation and the biodistribution of ^{186}Re-liposomes or free ^{186}Re-BMEDA in various rat tissues was measured with the Minaxiγ A5550 γ-counter. Femur with bone marrow was taken as representative of bone and bone marrow. Bowel activity was determined by counting an aliquot of bowel plus contents after digestion in saturated NaOH. The 72-h total urine activity was determined by counting an aliquot of urine sample from each rat. Total blood, bone, muscle, and skin mass of rats were calculated as 5.4%, 10%, 40%, and 13% of total body weight, respectively (*31,32*).

Statistical Analysis

The MiniTab program (MiniTab Inc.) was used to perform statistical analysis. All average values are given as mean ± SD. The comparison of labeling efficiency and percentage injected dose (%ID) per organ between groups was determined using 1-way ANOVA. The acceptable probability for a significant difference was $P < 0.05$.

RESULTS

^{186}Re-Liposome Labeling Efficiency

The labeling efficiencies of 400-nm cysteine or 400-nm $(NH_4)_2SO_4$ liposomes labeled with 22.2 MBq (0.6 mCi) of ^{186}Re-BMEDA or ^{186}Re-BMEDA + BT are shown in Table 1. The labeling efficiency of ^{186}Re-cysteine liposomes labeled with ^{186}Re-BMEDA was significantly lower than that of ^{186}Re-$(NH_4)_2SO_4$ liposomes labeled with ^{186}Re-BMEDA or ^{186}Re-BMEDA + BT ($P < 0.05$). The labeling efficiency

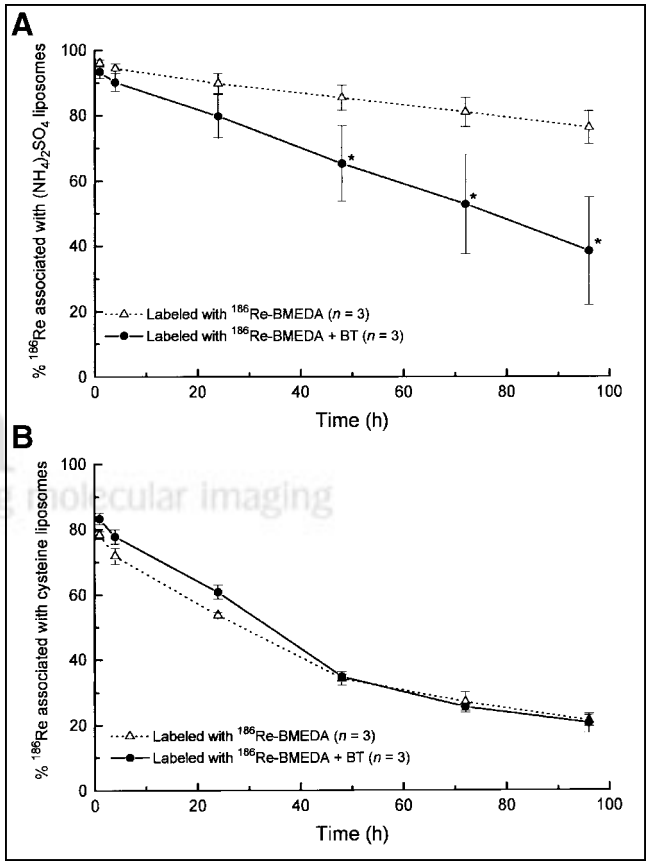

FIGURE 3. (A) In vitro labeling stability (n = 3) of 400-nm ^{186}Re-$(NH_4)_2SO_4$ liposomes labeled with ^{186}Re-BMEDA and with ^{186}Re-BMEDA + BT in 50% FBS-PBS buffer, pH 7.4, at 37°C. (B) In vitro labeling stability (n = 3) of 400-nm ^{186}Re-cysteine liposomes labeled with ^{186}Re-BMEDA and with ^{186}Re-BMEDA + BT in 50% FBS-PBS buffer at 37°C. ^{186}Re-$(NH_4)_2SO_4$ liposomes labeled with ^{186}Re-BMEDA had the best in vitro labeling stability of the liposomes tested. This stability could be retained for 96 h in 50% FBS-PBS buffer at 37°C (*$P < 0.05$).

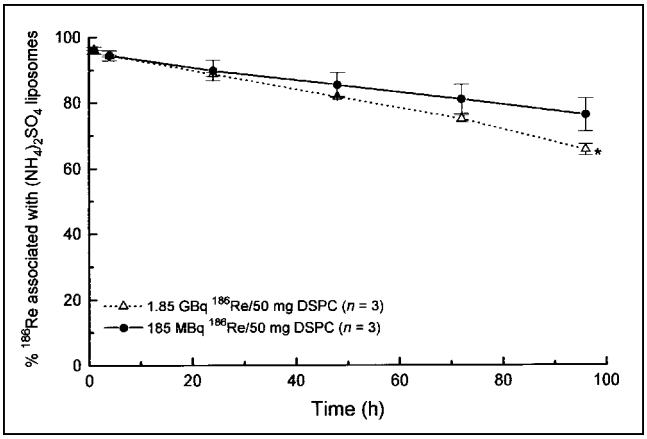

FIGURE 4. Effect of high specific activity on in vitro labeling stability of 400-nm ^{186}Re-$(NH_4)_2SO_4$ liposomes labeled with ^{186}Re-BMEDA. With $(NH_4)_2SO_4$ liposomes, specific activity of 1.85 GBq (50 mCi) of ^{186}Re per 50 mg of DSPC (1 mL 120 mmol/L liposomes) labeled to $(NH_4)_2SO_4$ liposomes also showed good in vitro labeling stability up to 96 h of incubation in 50% FBS-PBS buffer at 37°C (*$P < 0.05$).

of 400-nm ^{186}Re-$(NH_4)_2SO_4$ liposomes labeled with 252 MBq (6.8 mCi) of ^{186}Re-BMEDA was 74.3% ± 6.4% ($n = 3$).

In Vitro Labeling Stability of ^{186}Re-Liposomes

The in vitro labeling stabilities of ^{186}Re-cysteine liposomes and ^{186}Re-$(NH_4)_2SO_4$ liposomes labeled with 22.2 MBq (0.6 mCi) of ^{186}Re-BMEDA or ^{186}Re-BMEDA + BT are shown in Figure 3. ^{186}Re-$(NH_4)_2SO_4$ liposomes labeled with ^{186}Re-BMEDA had the best in vitro labeling stability after incubation in 50% FBS-PBS, pH 7.4, at 37°C for 96 h (Fig. 3A). There was 89.8% ± 3.1% ($n = 3$) radioactivity associated with $(NH_4)_2SO_4$ liposomes at 24 h and 76.2% ± 5.1% ($n = 3$) radioactivity associated with liposomes at 96 h. In contrast, ^{186}Re-$(NH_4)_2SO_4$ liposomes labeled with ^{186}Re-BMEDA + BT had lower in vitro stability compared with ^{186}Re-$(NH_4)_2SO_4$ liposomes labeled with ^{186}Re-BMEDA ($P < 0.05$ at 48, 72, and 96 h) (Fig. 3A). ^{186}Re-$(NH_4)_2SO_4$ liposomes labeled with ^{186}Re-BMEDA and with ^{186}Re-BMEDA + BT behaved in a similar fashion with a linear ^{186}Re release from the liposomes ($R^2 = 0.995$ and 0.999, respectively; $P < 0.0001$), although ^{186}Re-$(NH_4)_2SO_4$ liposomes labeled with ^{186}Re-BMEDA + BT showed faster ^{186}Re release.

^{186}Re-Cysteine liposomes showed less in vitro labeling stability compared with ^{186}Re-$(NH_4)_2SO_4$ liposomes. A similar in vitro labeling stability for ^{186}Re-cysteine liposomes labeled with ^{186}Re-BMEDA and with ^{186}Re-BMEDA + BT was observed (Fig. 3B).

The effect of high specific activity (10 times more ^{186}Re activity per mg of lipid) on the in vitro stability of ^{186}Re-$(NH_4)_2SO_4$ liposomes labeled with ^{186}Re-BMEDA was investigated. A labeling efficiency of 74.3% ± 6.4% was achieved using 2.52 GBq (68 mCi) of ^{186}Re per 50 mg of DSPC. A specific activity of 1.87 ± 0.16 GBq (50.5 ± 4.4 mCi) of ^{186}Re per 50 mg of DSPC labeled to $(NH_4)_2SO_4$ liposomes also showed good in vitro labeling stability up to 96 h of incubation in 50% FBS-PBS buffer at 37°C (Fig. 4). There were 89.1% ± 0.6% ($n = 3$) radioactivity associated with liposomes at 24 h and 66.7% ± 1.5% ($n = 3$) radioactivity associated with liposomes at 96 h ($P < 0.05$ at 48, 72, and 96 h).

Normal Rat Distribution of ^{186}Re-Liposomes

The labeling efficiency for 100-nm ^{186}Re-cysteine liposomes was 70.0% and for 100-nm ^{186}Re-$(NH_4)_2SO_4$ liposomes was 82.6%. The γ-camera images of rats acquired at different times after intravenous injection of 100-nm ^{186}Re-$(NH_4)_2SO_4$ liposomes labeled with ^{186}Re-BMEDA, 100-nm ^{186}Re-cysteine liposomes labeled with ^{186}Re-BMEDA, or ^{186}Re-BMEDA alone are shown in Figure 5. Both 100-nm ^{186}Re-liposome formulations tested showed slow blood clearances and spleen accumulations. The spleen accumulation is stable even at 72 h after intravenous injection. This spleen uptake is a common feature of liposome distribution with the liposome composition used in this study after intravenous injection in rats (*1,17*). Other liposome formulations that have surface modification with PEG have greatly reduced spleen accumulation (*21,22*). The ^{186}Re-

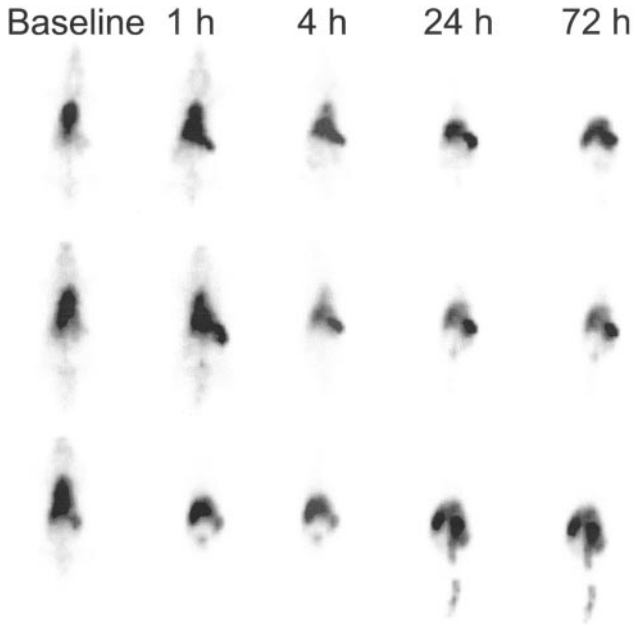

FIGURE 5. γ-Camera images of normal rats via intravenous injection method. (Top row) Images of rat at baseline and at 1, 4, 24, and 72 h after intravenous injection of 100-nm ^{186}Re-$(NH_4)_2SO_4$ liposomes labeled with ^{186}Re-BMEDA. (Middle row) Images of rat at corresponding times after intravenous injection of 100-nm ^{186}Re-cysteine liposomes labeled with ^{186}Re-BMEDA. (Bottom row) Images of rat at corresponding times after intravenous injection of ^{186}Re-BMEDA alone. ^{186}Re-$(NH_4)_2SO_4$ liposomes and ^{186}Re-cysteine liposomes labeled with ^{186}Re-BMEDA showed slow blood clearances and spleen accumulations, which are common features of liposome distribution after intravenous injection in rats. ^{186}Re-BMEDA alone showed fast blood clearance, fast excretion from bowel and urine, and no spleen accumulation.

liposomes also have delayed excretion from the hepatobiliary system compared with ^{186}Re-BMEDA alone. ^{186}Re-BMEDA alone had a faster blood clearance and did not show any spleen accumulation (Fig. 5). ^{186}Re-BMEDA alone also showed significant excretion from the hepatobiliary system and high activity in kidneys.

Normal rat distributions of ^{186}Re-cysteine liposomes and ^{186}Re-$(NH_4)_2SO_4$ liposomes labeled with ^{186}Re-BMEDA at 72 h are listed in Table 2. For comparison, biodistributions of the free ^{186}Re-BMEDA used for liposome labeling were also obtained. The 100-nm ^{186}Re-$(NH_4)_2SO_4$ liposomes and 100-nm ^{186}Re-cysteine liposomes labeled with ^{186}Re-BMEDA showed significant spleen accumulation at 72 h after intravenous injection ($P < 0.001$). This shows the common feature of liposome distribution after intravenous injection in rats (*1,17*). ^{186}Re-BMEDA alone did not have any spleen accumulation.

Comparisons between ^{186}Re-cysteine liposomes and ^{186}Re-$(NH_4)_2SO_4$ liposomes indicate that ^{186}Re-$(NH_4)_2SO_4$ liposomes have significantly higher radioactivity in spleen, blood, liver, kidney, muscle, and bone with bone marrow at 72 h ($P < 0.001$ for spleen, liver, kidney, and bone with bone marrow; $P < 0.05$ for blood and muscle); ^{186}Re-$(NH_4)_2SO_4$ liposomes have significantly higher radioactivity in the total activity of bowel and feces ($P < 0.001$) and significantly lower excretion from urine at 72 h ($P < 0.01$).

DISCUSSION

There are 3 types of liposome labeling methods (*33*). An ideal labeling method consists of trapping the radioisotopes within the inner space of premanufactured liposomes with high labeling efficiency (*34*). To perform this labeling method, a radiolabeled chemical with a certain lipophilicity is required so that it can go across the lipophilic double membrane of the liposome. After crossing the lipophilic double membrane, this radiolabeled chemical can transfer to a hydrophilic chemical within the inner space of the liposome where it could potentially be trapped in a stable fashion.

Phillips et al. (*13*) developed an effective ^{99m}Tc-liposome labeling method with ^{99m}Tc-hexamethylpropyleneamine oxime (HMPAO) and GSH-encapsulated liposomes. By using this labeling method, the labeling can be conveniently performed after liposomes are manufactured, resulting in very stable ^{99m}Tc-liposomes. ^{99m}Tc-Liposomes have been widely investigated as diagnostic imaging agents. These studies have demonstrated that ^{99m}Tc-liposomes can be effective agents for infection and inflammation imaging, tumor imaging, lymphoscintigraphy, and blood-pool measurement (*9,17,18,21*).

It is hypothesized that the lipophilic ^{99m}Tc-HMPAO crosses the double membrane of liposomes, after which it reacts with GSH and transforms into a hydrophilic composite in the inner space of liposomes. The ^{99m}Tc is thus trapped in the inner space of liposomes.

^{99m}Tc-HMPAO was introduced as a brain imaging agent. Since ^{99m}Tc-HMPAO is a ^{99m}Tc-N_4 pattern complex, it has low stability for remaining in the original neutral coordinate structure (*35*). Although rhenium and technetium share similar chemical characteristics, there have been no reports of radiolabeled Re-HMPAO. The most likely reason, which

TABLE 2
Normal Rat Distributions of 2 Kinds of 100-nm ^{186}Re-Liposomes Labeled with ^{186}Re-BMEDA and ^{186}Re-BMEDA Alone at 72 Hours ($n = 4$)

	%ID per organ (mean ± SD)			%ID per gram (mean ± SD)		
Organ	^{186}Re-Cysteine liposomes	^{186}Re-$(NH_4)_2SO_4$ liposomes	^{186}Re-BMEDA alone	^{186}Re-Cysteine liposomes	^{186}Re-$(NH_4)_2SO_4$ liposomes	^{186}Re-BMEDA alone
Spleen	3.88 ± 0.44*	9.80 ± 0.93*	0.21 ± 0.05	12.03 ± 1.51	32.50 ± 6.17	0.38 ± 0.14
Blood	0.32 ± 0.05†	0.52 ± 0.11	0.49 ± 0.04	0.013 ± 0.001	0.020 ± 0.004	0.022 ± 0.001
Liver	8.55 ± 0.61*	22.75 ± 0.43*	5.97 ± 0.35	0.71 ± 0.08	1.82 ± 0.26	0.54 ± 0.05
Kidney	5.22 ± 0.48*	8.83 ± 0.09‡	10.42 ± 0.93	2.02 ± 0.26	3.43 ± 0.44	4.49 ± 0.37
Lung	0.12 ± 0.02	0.16 ± 0.02	0.37 ± 0.06	0.060 ± 0.021	0.087 ± 0.018	0.23 ± 0.03
Heart	0.027 ± 0.004	0.035 ± 0.002	0.076 ± 0.005	0.022 ± 0.004	0.028 ± 0.004	0.069 ± 0.007
Brain	0.0026 ± 0.0006	0.0037 ± 0.0008	0.0052 ± 0.0007	0.0016 ± 0.0003	0.0024 ± 0.0003	0.0035 ± 0.0007
Muscle	0.57 ± 0.14	1.01 ± 0.30‡	0.79 ± 0.07	0.0032 ± 0.0009	0.0054 ± 0.0019	0.0048 ± 0.0005
Bone and marrow	1.28 ± 0.20*	6.14 ± 1.00*	0.58 ± 0.06	0.028 ± 0.004	0.13 ± 0.03	0.014 ± 0.000
Skin	0.84 ± 0.22	1.44 ± 0.28	1.48 ± 0.51	0.014 ± 0.004	0.023 ± 0.005	0.029 ± 0.012
Testis	0.044 ± 0.014	0.082 ± 0.011	0.058 ± 0.004	0.012 ± 0.005	0.022 ± 0.004	0.015 ± 0.003
Feces	6.08 ± 0.57	16.26 ± 6.36	20.54 ± 4.35	1.83 ± 0.65	3.63 ± 0.49	4.82 ± 1.53
Bowel	5.38 ± 1.44	12.37 ± 3.58	6.67 ± 3.31	0.24 ± 0.05	0.52 ± 0.14	0.34 ± 0.19
Urine	50.09 ± 15.30‡	11.33 ± 0.50*	26.93 ± 4.23	1.06 ± 0.47	0.39 ± 0.09	0.79 ± 0.08

*$P < 0.001$ compared with ^{186}Re-BMEDA alone.
†$P < 0.01$ compared with ^{186}Re-BMEDA alone.
‡$P < 0.05$ compared with ^{186}Re-BMEDA alone.
^{186}Re-Cysteine liposomes have significantly lower total feces and bowel radioactivity level compared with ^{186}Re-BMEDA alone ($P < 0.001$).

can be derived from studies of technetium and rhenium chemistry, is that rhenium probably does not form a stable neutral coordinate structure with HMPAO.

Hafeli et al. (*33*) reported a method for labeling liposomes with ^{186}Re/^{188}Re. These liposomes were labeled by incorporating a ^{186}Re/^{188}Re complex into liposomes during liposome manufacture, making this method impractical in a clinical setting. The labeling efficiency with this method also was low (method I, 0%–20.9%; method II, 45.5% ± 5.3%), and a biodistribution study of $^{186/188}$Re-labeled liposomes was not reported.

Another report of labeling liposomes with a therapeutic radionuclide has been described. Utkhede et al. reported a ^{90}Y-liposome labeling method using a cation ionophore and diethylenetriaminepentaacetic acid–encapsulated liposomes (*36*). The ^{90}Y uptake by liposomes was >95%. The limitation of this method is that the labeling must be performed at a temperature of >41°C, which makes the labeling impractical for use with temperature-sensitive liposomes. In addition, ^{90}Y may not be an ideal radionuclide for liposome labeling because of the following inherent physical and biochemical characteristics: (a) $^{90}Y^{3+}$ is a bone-seeking chemical (*37*), so that the dissociated $^{90}Y^{3+}$ accumulates in bone, potentially resulting in a high radiation dose to bone marrow; and (b) ^{90}Y is a pure β-emitter (*38*), which makes it difficult to trace the in vivo distribution of ^{90}Y-labeled agents directly.

^{99m}Tc-SNS/S pattern complexes (*25*) were initially studied for potential use as brain imaging agents. Because no stable nuclides exist for technetium, rhenium was used as a technetium analog to analyze the structure of these SNS/S pattern complexes. It was proven (*26*,*27*) that Re-SNS/S complexes have a chemistry similar to that of ^{99m}Tc-SNS/S complexes and have a core coordinate structure of the complex that is neutral so that lipophilic complexes can be made with this kind of coordinate system. The lipophilic characteristic of ^{99m}Tc-SNS/S, ^{186}Re-SNS/S, or ^{188}Re-SNS/S complexes suggests that these complexes can cross the lipophilic double membrane of a liposome.

Nock et al. (*39*) suggested that a ligand exchange behavior occurred between ^{99m}Tc-SNS/S complexes and other molecules containing a thiol group, such as GSH and cysteine. This suggests that ^{99m}Tc-SNS/S and ^{186}Re/^{188}Re-SNS/S complexes may change into hydrophilic composites after they enter into the inner space of liposomes and react with preencapsulated GSH, cysteine, or other hydrophilic chemicals containing a thiol group and be trapped.

Liposomes encapsulating citrate or other weak acids with lower inner space pH (pH gradient liposomes) or encapsulating $(NH_4)_2SO_4$ (ammonium gradient liposomes) have been used in the entrapment of chemotherapeutic agents for drug delivery (*1*,*2*,*28*). The mechanism of the drug entrapment in these kinds of liposomes was studied, and it has been shown that the chemotherapeutic agents containing an amine group can be entrapped in the lower pH environment of liposome inner space. The ^{186}Re complexes we have studied also contain amine groups that make the entrapment of ^{186}Re-SNS/S complexes by the ammonium gradient mechanism possible. In the ammonium gradient situation, after radiolabeled complexes enter into the liposome's inner space, the amine group of the complex is protonized and the complex becomes hydrophilic and trapped. It would also be possible to use both the ligand exchange mechanism with GSH/cysteine and the ammonium or pH gradient mechanism described above to potentially further improve the labeling of liposomes with ^{186}Re/^{188}Re.

In this study, there was similar in vitro labeling stability of ^{186}Re-cysteine liposomes labeled with ^{186}Re-BMEDA compared with that of ^{186}Re-cysteine liposomes labeled with ^{186}Re-BMEDA + BT. But ^{186}Re-$(NH_4)_2SO_4$ liposomes labeled with ^{186}Re-BMEDA + BT showed lower in vitro labeling stability compared with that of ^{186}Re-$(NH_4)_2SO_4$ liposomes labeled with ^{186}Re-BMEDA ($P < 0.05$ at 48, 72, and 96 h). The reason for this difference is likely related to the fact that ^{186}Re-BMEDA has more amine groups that can be protonized. ^{186}Re-BMEDA + BT has less amine groups available and is more lipophilic compared with ^{186}Re-BMEDA. In comparison, ^{186}Re-cysteine liposomes have similar hydrophilic structures after the ligand exchange reaction between cysteine and ^{186}Re-BMEDA or ^{186}Re-BMEDA + BT.

Typical distributions of standard liposome formulations in the 100- to 200-nm size range that are administered via intravenous injection have the slow blood-pool clearance and spleen accumulation (*1*,*17*). Our experiments showed the stability of ^{186}Re-liposomes labeled with ^{186}Re-BMEDA. A significantly higher spleen accumulation was observed even at 72 h after intravenous injection of ^{186}Re-liposomes compared with ^{186}Re-BMEDA alone ($P < 0.001$). ^{186}Re-BMEDA alone showed fast blood clearance, fast excretion from bowel and urine, and no spleen accumulation (Fig. 5).

The labeling method reported in this article may be applied to different kinds of liposomes with different lipid compositions. To show the feasibility of the labeling method, we used nonspecifically targeted liposomes made from DSPC and Chol. These liposomes used in the normal distribution studies ranged in size from 115 to 150 nm. The liposome dose was only 4.2 mg of DSPC per rat. These liposomes were used to assess in vivo stability by monitoring the normal distribution in the spleen. Although high spleen uptake provides assurance of in vivo liposome stability, this high spleen uptake may not be desirable for intravenous liposome radiotherapy. Changes in liposome size and surface characteristics can greatly affect liposome biodistribution. Small liposomes of <100 nm with a high Chol content were found to have relatively low spleen uptake and excellent calculated dose distribution to tumors (*19*). In addition, liposome surface modifications using PEG (*1*,*21*,*22*) or functional groups (*6*–*9*)—such as antibodies and the biotin–avidin system—and physical modalities (*11*,*12*)—such as hyperthermia and radiation—can change the behavior of radiolabeled liposomes significantly, potentially resulting in much lower levels in liver and spleen and much higher accumulation in tumors.

The amount of liposomes injected can also change the in vivo behavior of liposomes. Lower spleen and liver radioactivity may also be achieved by injecting unlabeled liposomes beforehand, thus saturating the RES (*1*). In addition, some reports have suggested that radiolabeled liposomes can be used to treat tumors using intratumoral injection techniques (*23*,*40*). This flexibility in the preparation of different liposome structures and the use of various administration techniques indicates the great potential of using radiolabeled liposomes in radionuclide therapy.

By using the above methods, liposomes can be radiolabeled with high specific activity to meet the requirements of clinical treatment. Based on our experiments, a specific activity of 1.85 GBq (50 mCi) of ^{186}Re per 50 mg of DSPC can be readily achieved. Considering the presence of carrier in ^{186}Re-perrhenate and the half-life difference between ^{186}Re and ^{188}Re, 1.85 GBq (50 mCi) of ^{186}Re corresponds to >22.2 GBq (>600 mCi) of carrier-free ^{188}Re on the same 50-mg lipid dose.

CONCLUSION

We have introduced a method of labeling liposomes with ^{186}Re by using ^{186}Re-SNS/S complexes and liposomes encapsulating hydrophilic chemicals containing thiol groups or an ammonium gradient. This labeling method is convenient and permits achievement of high specific activities. ^{186}Re/^{188}Re-Liposomes have potential for clinical radionuclide therapy applications.

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REFERENCES

1. Lasic DD, Papahadjopoulos D, eds. *Medical Applications of Liposomes*. Amsterdam, The Netherlands: Elsevier; 1998:15–449.
2. Drummond DC, Meyer O, Hong K, Kirpotin DB, Papahadjoupolos D. Optimizing liposomes for delivery of chemotherapeutic agents to solid tumors. *Pharmacol Rev.* 1999;51:691–743.
3. Pietrobon PJ. Liposome design and vaccine development. *Pharm Biotechnol.* 1995;6:347–361.
4. Lasic DD. *Liposomes in Gene Therapy.* Boca Raton, FL: CRC Press; 1997:67–197.
5. Hope MJ, Bally MB, Webb G, Cullis PR. Production of large unilamellar vesicles by a rapid extrusion procedure: characterization of size distribution, trapped volume and ability to maintain a membrane potential. *Biochim Biophys Acta.* 1985;812:55–65.
6. Oku N. Anticancer therapy using glucuronate modified long-circulating liposomes. *Adv Drug Deliv Rev.* 1999;40:63–73.
7. Wang S, Low PS. Folate-mediated targeting of antineoplastic drugs, imaging agents, and nucleic acids to cancer cells. *J Control Release.* 1998;53:39–48.
8. Willis MC, Collins B, Zhang T, et al. Liposome-anchored vascular endothelial growth factor aptamers. *Bioconjug Chem.* 1998;9:573–582.
9. Phillips WT, Klipper R, Goins B. Novel method of greatly enhanced delivery of liposomes to lymph nodes. *J Pharmacol Exp Ther.* 2000;295:309–313.
10. Thierens HM, Monsieurs MA, Brans B, et al. Dosimetry from organ to cellular dimensions. *Comput Med Imaging Graph.* 2001;25:187–193.
11. Matteucci ML, Anyaramdhatla G, Rosner G, et al. Hyperthermia increases accumulation of technetium-99m-labeled liposomes in feline sarcomas. *Clin Cancer Res.* 2000;6:3748–3755.
12. Koukourakis MI, Koukouraki S, Giatromanolaki A, et al. High intratumoral accumulation of stealth liposomal doxorubicin in sarcomas: rationale for combination with radiotherapy. *Acta Oncol.* 2000;39:207–211.
13. Phillips WT, Rudolph AS, Goins B, Timmons JH, Klipper R, Blumhardt R. A simple method for producing a technetium-99m-labeled liposome, which is stable in vivo. *Nucl Med Biol.* 1992;19:539–547.
14. Woodle MC. ^{67}Gallium-labeled liposomes with prolonged circulation: preparation and potential as nuclear imaging agents. *Nucl Med Biol.* 1993;20:149–155.
15. Essien H, Hwang KJ. Preparation of liposomes entrapping a high specific activity of $^{111}In^{3+}$-bound inulin. *Biochim Biophys Acta.* 1988;944:329–336.
16. Oku N. Delivery of contrast agents for positron emission tomography imaging by liposomes. *Adv Drug Deliv Rev.* 1999;37:53–61.
17. Goins B, Klipper R, Rudolph AS, Cliff RO, Blumhardt R, Phillips WT. Biodistribution and imaging studies of technetium-99m-labeled liposomes in rats with focal infection. *J Nucl Med.* 1993;34:2160–2168.
18. Goins B, Klipper R, Rudolph AS, Phillips WT. Use of technetium-99m-liposomes in tumor imaging. *J Nucl Med.* 1994;35:1491–1498.
19. Semple SC, Chonn A, Cullis PR. Influence of cholesterol on the association of plasma proteins with liposomes. *Biochemistry.* 1986;35:2521–2525.
20. Emfietzoglou D, Kostarelos K, Sgouros G. An analytic dosimetry study for the use of radionuclide-liposome conjugates in internal radiotherapy. *J Nucl Med.* 2001;42:499–504.
21. Goins B, Phillips WT, Klipper R. Blood-pool imaging using technetium-99m-labeled liposomes. *J Nucl Med.* 1996;37:1374–1379.
22. Boerman OC, Oyen WJ, van Bloois L, et al. Optimization of technetium-99m-labeled PEG liposomes to image focal infection: effects of particle size and circulation time. *J Nucl Med.* 1997;38:489–493.
23. Harrington KJ, Rowlinson-Busza G, Syrigos N, Uster PS, Vile RG, Stewart JSW. Pegylated liposomes have potential as vehicles for intratumoral and subcutaneous drug delivery. *Clin Cancer Res.* 2000;6:2528–2537.
24. Bao A, Phillips W, Negrete G, Klipper R, Goins B. ^{99m}Tc/^{186}Re/^{188}Re-Liposome radiolabeling for nuclear imaging and targeted radionuclide therapy. In: Nicolini M, Mazzi U, eds. *Technetium, Rhenium and Other Metals in Chemistry and Nuclear Medicine: 6.* Padova, Italy: SGE; 2002:381–386.
25. Mastrostamatis SG, Papadopoulos MS, Pirmettis IC, et al. Tridentate ligands containing the SNS donor atom set as a novel backbone or the development of technetium brain-imaging agents. *J Med Chem.* 1994;37:3212–3218.
26. Pirmettis IC, Papadopoulos MS, Mastrostamatis CP, Raptopoulou CP, Terzis A, Chiotellis E. Synthesis and characterization of oxotechnetium(V) mixed-ligand complexes containing a tridentate N-substituted bis(2-mercaptoethyl) amine and a monodentate thiol. *Inorg Chem.* 1996;35:1685–1691.
27. Pelecanou M, Pirmettis IC, Papadopoulos MS, Terzis A, Chiotellis E, Stassinopoulou CI. Structural studies of ReO(V) mixed ligand [SNS][Cl] and [SNS][S] complexes. *Inorg Chim Acta.* 1999;287:142–151.
28. Maurer-Spurej E, Wong KF, Maurer N, Fenske DB, Cullis PR. Factors influencing uptake and retention of amino-containing drugs in large unilamellar vesicles exhibiting transmembrane pH gradients. *Biochim Biophys Acta.* 1999;1416:1–10.
29. Stewart JCM. Colorimetric determination of phospholipids with ammonium ferrothiocyanate. *Anal Biochem.* 1980;104:10–14.
30. Corbin JL, Miller KF, Pariyadath N, Wherland S, Bruce AE. Preparation and properties of tripodal and linear tetradentate N, S-donor ligands and their complexes containing the MoO_2^{2+} core. *Inorg Chim Acta.* 1984;90:41–51.
31. Petty C. *Research Techniques in the Rat.* Springfield, IL: Charles C. Thomas; 1982:66–70.
32. Frank DW. Physiological data of laboratory animals. In: Melby EC Jr, Altman NH, eds. *Handbook of Laboratory Animal Science*. Vol. III. Boca Raton, FL: CRC Press; 1976:23–64.
33. Hafeli U, Tiefenauer LX, Schubiger PA, Weder HG. A lipophilic complex with ^{186}Re/^{188}Re incorporated in liposomes suitable for radiotherapy. *Nucl Med Biol.* 1991;18:449–454.
34. Tilcock C. Delivery of contrast agents for magnetic resonance imaging, computed tomography, nuclear medicine and ultrasound. *Adv Drug Deliv Rev.* 1999;37:33–51.
35. Hung JC, Corlija M, Volkert WA, Holmes RA. Kinetic analysis of technetium-99m d,l-HMPAO decomposition in aqueous media. *J Nucl Med.* 1988;29:1568–1576.
36. Utkhede D, Yeh V, Szucs M, Tilcock C. Uptake of yttrium-90 into lipid vesicles. *J Liposome Res.* 1994;4:1049–1061.
37. Kutzner J, Becker M, Grimm W. Bone-seeking behavior of rhenium and yttrium complexes [in German]. *Nuklearmedizin.* 1983;22:162–165.
38. Weber DA, Eckerman KF, Dillman LT, Ryman JC. *MIRD: Radionuclide Data and Decay Schemes.* Reston, VA: Society of Nuclear Medicine; 1989:159.
39. Nock BA, Maina T, Yannoukakos D, Pirmettis IC, Papadopoulos MS, Chiotellis E. Glutathione-mediated metabolism of technetium-99m SNS/S mixed ligand complexes: a proposed mechanism of brain retention. *J Med Chem.* 1999;42:1066–1075.
40. Nomura T, Koreeda N, Yamashita F, Takakura Y, Hashida M. Effect of particle size and charge on the disposition of lipid carriers after intratumoral injection into tissue-isolated tumors. *Pharm Res.* 1998;151:128–132.