jnm/ RADIOCHEMISTRY AND RADIOPHARMACEUTICALS

Evaluation of Liposome-Entrapped Radioactive Tracers as Scanning Agents. Part 1: Organ Distribution of Liposome [^{99m}Tc-DTPA] in Mice

Vicente J. Caride, William Taylor, John A. Cramer, and Alexander Gottschalk

Yale University School of Medicine, New Haven, Connecticut

We describe the use of liposomes as a delivery system for radiopharmaceutical localization. Liposomes [^{99m}Tc-DTPA] were injected intravenously in mice and showed preferential uptake in the liver and spleen. There was a steady decline of activity in all organs, suggestive of destruction of liposomes with subsequent release of ^{99m}Tc-DTPA into the circulation. Alteration of uptake from liver to spleen, lung, and bone marrow was achieved by prior loading of the circulation with nonradioactive liposomes. The same effect was produced in dogs and demonstrated with scintigraphy. We also showed scintigraphically in dogs how ^{99m}Tc-DTPA, when administered entrapped in liposomes, follows the pattern of distribution of liposomes. Liposomes seem to be suitable carriers for radiopharmaceuticals. Further studies should show the possibility of directing liposomes to specific targets.

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Under certain conditions globular micelles are formed in aqueous solutions of phospholipids and cholesterol (*1–3*). These structures have been referred to as liposomes (*4*). Liposomes have been used as carriers of antigens, antibodies, enzymes, drugs, and radiopharmaceuticals, and they are potentially able to transport several other compounds (*5–10*). It has also been postulated that by modifying the size of the liposomes and the properties of the surface of the liposomic membrane they can be directed toward certain target structures (*11*). We can imagine some of the possible combinations that could be obtained by introducing radioactive tracers into the lipid membrane of the liposome or into its aqueous content, or by attaching to the liposome certain molecules (i.e., antigens, antibodies, etc.) that could eventually act as a "membrane" receptor (Fig. 1).

We decided to study the diagnostic potentialities of liposome-carried radiopharmaceuticals. If modifications at the liposome membrane could be effectively achieved, these carriers should deliver the radiocompound to preselected regions in the organism. As a first step we have analyzed the simplest combination (Fig. 1B). We present the organ distribution of liposomes containing ^{99m}Tc-Sn-diethylenetriamine pentaacetic acid (DTPA) in mice. We have also obtained scintillation images of dogs injected with ^{99m}Tc-DTPA alone and with this agent carried in a liposome (coded liposome [^{99m}Tc-DTPA]) to emphasize the resulting change in organ distribution.

MATERIALS AND METHODS

Liposome preparation.* Liposomes are prepared from lecithin (phosphatidyl choline†) and cholesterol.‡ To 110 μl of a hexane solution containing

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For reprints contact: V. J. Caride, Nuclear Medicine Sect., Yale University School of Medicine, 333 Cedar St., New Haven, CT 06510.

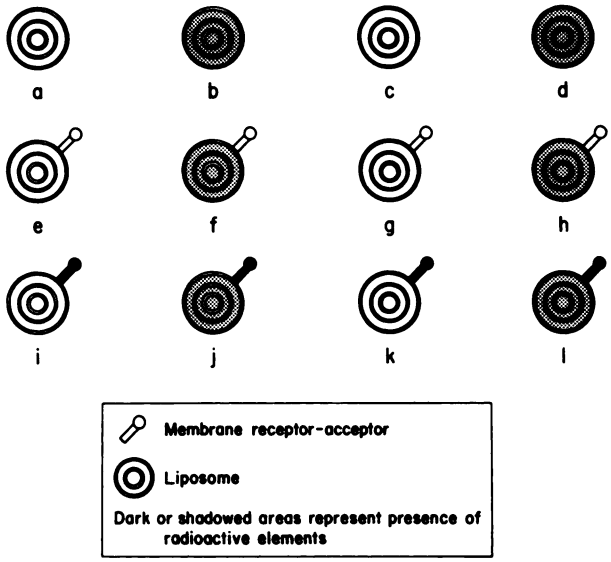

FIG. 1. Liposomes classified according to location of radioactive tracers and presence of membrane receptors. Circles are lipid bilayers. They are shown in concentric disposition to represent multiple lipid bilayers (liposomes) (a). Dark circles indicate the presence of radioactive tracer in lipid phase, i.e., ^{14}C-cholesterol. Separation between lipid bilayers are spaces filled with aqueous solution. This aqueous phase may contain water-soluble radioactive tracers (shadowed areas), i.e., ^{99m}Tc-DTPA (b). More complex molecules (e.g., antibodies) may be associated with both lipid and aqueous phase and are shown as small cylinders attached to liposome (e). They also may carry radioactive tracer (black cylinders) (i).

100 mg of lecithin/ml, 7.5 mg of cholesterol dissolved in chloroform is added and the mixture evaporated under nitrogen gas at room temperature. A thin layer of a lecithin–cholesterol solid mixture is deposited on the walls of the vial.

Two to three milliliters of sodium pertechnetate ($Na^{99m}TcO_4$), eluted from a molybdenum–technetium generator with 0.9% saline, is added to a "^{99m}Tc-DTPA kit"||: a vial containing 5 mg of diethylenetriamine pentaacetic acid (DTPA) and 0.25 mg of stannous chloride.

Two different procedures were utilized to add ^{99m}Tc-Sn-DTPA to the lipid mixture. (A) In the initial procedure, 1–2 ml of the ^{99m}Tc-Sn-DTPA solution, plus 2–3 ml of normal saline, constitute the "swelling solution" that is added to the lecithin–cholesterol mixture. After the addition of ^{99m}Tc-Sn-DTPA in saline, a good dispersion of the mixture is obtained by magnetic stirring at 37°C for 10 min. (B) In a later procedure, we add to the lecithin–cholesterol mixture 0.1 ml of ^{99m}Tc-Sn-DTPA and 0.3 cm^3 of methylethyl ketone¶ and the result is evaporated under nitrogen to obtain a thin layer of ^{99m}Tc-Sn-DTPA at the top of the lipid layer. Physiologic saline (2 ml) is then added and good dispersion obtained by magnetic stirring at 30°C for 30 min. The final product is milky in appearance and free of particulate material.

Separation techniques. In order to remove all the radioactivity not attached to the liposomes we used one of the following procedures:

Centrifugation. The liposome [^{99m}Tc-Sn-DTPA] preparation is centrifuged for 5 min at about 1,350 g. The supernatant is then removed and the pellet resuspended in 2–5 ml of normal saline and carefully mixed by magnetic stirring or vortex mixing. This procedure is repeated two more times.

Sephadex G-200 filtration. With this technique a better separation is obtained. We used a 9 × 13-cm Pharmacia column packed with 40–120 μM G-200 Sephadex. Fractions of 1 cm^3 each are collected until two radioactive peaks are visualized.

Size uniformity. For the purpose of obtaining liposomes of uniform size we used sonication and/or Millipore filtrations.

Sonication. Performed in a water bath sonicator,** this step required at least 30 min. Temperature is kept at 15°C or less by adding ice to the ultrasound bath.

Millipore filtration. Using a 1.2-μm filter, this procedure is simply performed by forcing the preparations through the membrane. The filtrate is then checked for uniformity and size on a hemocytometer slide under a microscope with a magnification of 400×.

Determination of liposome concentration. Liposome concentration was determined by absorbance using a Beckman DUR spectrophotometer at 410 nm and expressed as mg/ml of lecithin–cholesterol mixture (*5*). Calculations of final yield were performed in a well counter and expressed as percentage of the initially added activity found in the final preparation after centrifugation or Sephadex G-200 filtration. Liposome [^{99m}Tc-Sn-DTPA] preparations are maintained at 4°C and under positive N_2 pressure until used. Dried lecithin–cholesterol mixtures were stored for several weeks at temperatures below 0°C and under positive N_2 pressure, and were prepared for use by simply adding the saline solution of ^{99m}Tc-Sn-DTPA.

Animal experiments. In vivo experiments were performed on Swiss mice 8–12 weeks old. Two-tenths milliliters of a liposome [^{99m}Tc-Sn-DTPA] preparation (0.12 mg/ml) was administered intravenously in a tail vein and the total activity injected calculated by counting the syringe before and after injection, or by counting the whole animal at a reproducible geometry. In experiments designed to show the effects of liposome concentration on organ distribution, the animals received 0.2 ml of a 0.01-mg/ml liposome preparation or 0.2 ml of a 1.2-mg/ml liposome preparation in a tail vein. The procedure noted above was followed to determine the

TABLE 1. DISTRIBUTION (PER WHOLE ORGAN) OF LIPOSOME [^{99m}Tc-DTPA] AFTER INTRAVENOUS INJECTION*

	Time after injection						
Organ	5 min	15 min	30 min	45 min	90 min	120 min	21 hr
Liver	47.32 ±8.09	44.61 ±6.79	44.42 ±5.91	42.86 ±5.57	41.42 ±10.26	43.33 ±4.31	11.42 ±0.59
Spleen	3.07 ±1.18	2.73 ±1.40	4.19 ±1.41	3.06 ±0.78	5.62 ±0.92	2.27 ±1.22	0.467 ±0.28
Kidney	7.69 ±4.43	3.26 ±2.42	1.14 ±0.25	3.03 ±2.46	2.89 ±2.75	1.48 ±0.76	0.49 ±0.113
Lung	2.45 ±1.52	1.37 ±0.96	0.57 ±0.20	0.98 ±0.53	1.08 ±0.93	0.64 ±0.54	0.131 ±0.05
Stomach	0.63 ±0	3.29 ±1.85	1.317 ±0.42	1.48 ±0.014	1.19 ±0.24	1.43 ±0.73	0.16 ±0.07

* Values are mean percent of injected radioactivity ± the s.e. of the mean. Each group contains four mice, except those at 15 min (eight mice), 30 min (nine mice), and 120 min (six mice). Data at 5 min for stomach are based on two mice.

TABLE 2. DISTRIBUTION (% PER GRAM OF TISSUE) OF LIPOSOME [^{99m}Tc-DTPA] AFTER INTRAVENOUS INJECTION*

	Time after injection					
Organ	5 min	15 min	30 min	45 min	120 min	21 hr
Liver	22.97 ±6.9	19.31 ±4.7	21.66 ±3.31	18.10 ±2.78	16.86 ±1.78	4.58 ±0.79
Spleen	35.79 ±14.11	31.16 ±18.43	30.31 ±5.67	34.66 ±13.96	16.85 ±11.88	5.48 ±2.61
Kidney	13.30 ±10.81	5.27 ±0.19	1.98 ±0.39	5.16 ±4.55	1.88 ±0.22	0.956 ±0.26
Lung	10.22 ±7.78	5.52 ±4.15	2.34 ±0.98	4.34 ±3.08	1.17 ±0.23	0.64 ±0.23
Stomach	2.43 ±0.27	12.13 ±8.28	6.01 ±2.36	4.32 ±0.12	4.9 ±1.5	0.45 ±0.24
Blood	5.9 ±0.53	4.28 ±2.43	5.1 ±0.9	3.12 ±0.29	2.79 ±0.02	0.33 ±0.01

* Values are mean percent of injected radioactivity ± the s.e. of the mean. Each group contains four mice, except 15 min (eight mice). Data for stomach at 5, 30, and 45 min are based on two mice.

total amount of radioactivity injected. Mice were then killed at different times and organs weighed and counted, the resultant activities being corrected for the physical decay of ^{99m}Tc. Finally, another group of mice received intravenously 0.2 ml of a 37-mg/ml preparation of nonradioactive liposome followed by 0.2 ml of liposome [^{99m}Tc-Sn-DTPA]. Mice were killed 30 min after the injections. An unpretreated (control) group was injected intravenously only with a similar amount of liposome [^{99m}Tc-Sn-DTPA] and killed at 30 min. The final results were expressed as a percentage of the dose per organ as well as percentage of the dose per gram of tissue.

Whole-body scans were performed in dogs at several intervals after the intravenous injection of liposome [^{99m}Tc-Sn-DTPA], with and without previous administration of 5 ml of nonradioactive liposome (37 mg/ml).

RESULTS

Liposome preparation. When the initial procedure was followed, 1–3% of the initial activity of the added ^{99m}Tc-Sn-DTPA was found to accompany the liposome pellet after centrifugation. With the modified procedure the yield was higher: 13.75% ± 4.12. The distribution of liposome [^{99m}Tc-Sn-DTPA] in mice was the same for both procedures.

Organ distribution of liposome [^{99m}Tc-Sn-DTPA]. The results presented in Table 1 correspond to studies performed with a liposome concentration of 0.12 mg/ml. The results are expressed as percentage of the injected dose per organ at several postdose times up to a maximum of 21 hr. In Table 2 the data are presented as percentages of the injected dose per gram of target tissue. Each measurement averages the results of at least four animals (n = 4): at 15 min n = 8; at 30 min n = 9; and at 120 min n = 6.

TABLE 3. ORGAN DISTRIBUTION AT 30 MIN OF LIPOSOME [^{99m}Tc-DTPA] IN ANIMALS PREVIOUSLY INJECTED WITH NONRADIOACTIVE LIPOSOMES*

Organ	Mean % uptake per organ		Mean % uptake per gram of tissue	
	L[^{99m}Tc-DTPA] + Nonradioactive L	L[^{99m}Tc-DTPA] Control	L[^{99m}Tc-DTPA] + Nonradioactive L	L[^{99m}Tc-DTPA] Control
Liver	31.71 ±6.6	44.42 ±5.91	16.56 ±2.07	21.66 ±3.39
Spleen	8.35 ±1.99	4.19 ±1.41	65.13 ±7.87	30.31 ±5.67
Kidney	1.99 ±0.55	1.14 ±0.25	3.62 ±0.92	1.98 ±0.39
Lung	2.16 ±1.14	0.57 ±0.20	10.18 ±5.46	2.34 ±0.98
Bone	0.20 ±0.11	0.116 ±0.048	2.05 ±0.88	1.19 ±0.21

* Values are mean percent of injected activity ± s.e. of the mean. The "nonradioactive" group contains seven mice, the control group four. Data for bone are based on three mice (nonradioactive group) and two mice (control group). Control mice did not receive nonradioactive liposomes before administration of liposome [^{99m}Tc-DTPA].

These values served as controls for the rest of the experiments. The uptake by the liver remained unchanged up to 120 min (43.99% ± 1.99), but at 21 hr it was 11.42% ± 0.59. The same decrease is observed when data are expressed per gram of tissue: from 22.07% ± 2.51 to 4.58% ± 0.79. A similar situation is observed in the spleen. The mean value from 5 to 120 min is 4.45% ± 2.95 for the whole organ and 29.81% ± 7.3 per gram of tissue. At 21 hr these values decreased to 4.67% ± 0.286 and 5.48% ± 2.61, respectively. The removal of liposomes from the circulation is rapid. Five minutes after the injection only 10% of dose is still circulating (assuming a blood volume of 2 cm^3). This value dropped to less than 1% in 24 hr.

Results obtained using a diluted liposome preparation (0.01 mg/ml of the lecithin–cholesterol mixture, or a total amount of 0.002 mg) resulted in an hepatic uptake of 39.26% ± 1.62 and a splenic uptake of 2.81% ± 0.62 at 30 min. With a more concentrated preparation (1.2 mg/ml of the lecithin–cholesterol mixture, a total amount of 0.24 mg) results were 44.2% ± 7.6 for the liver and 2% ± 0.92 for spleen at 30 min. In both situations the hepatic uptake was not significantly different from our control value for 30 min.

The organ distribution of ^{99m}Tc-Sn-DTPA liposomes, administered 30 min after injection of nonradioactive liposomes, is shown in Table 3, together with the nonpretreated control values. The pretreatment slightly decreases liver uptake of the radioactive liposomes but significantly increases that in the spleen. There is also increase in the kidneys, lungs, and bones.

Liposome [^{99m}Tc-DTPA] scintigraphy. Whole-body scans were performed in dogs after intravenous

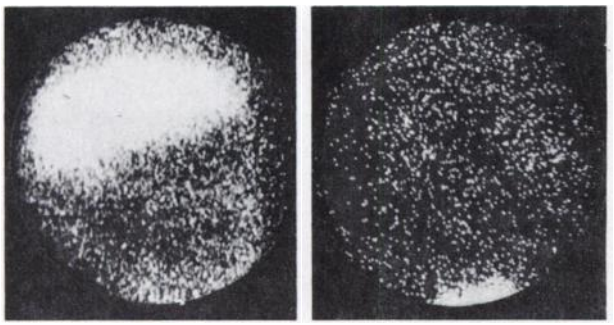

FIG. 2. (A) Scan of dog done 100 min after administration of 200 μCi of liposome [^{99m}Tc-DTPA]. (B) Scan performed 100 min after administration of 200 μCi of ^{99m}Tc-DTPA. Note that radioactive tracer, when incorporated into liposome, follows organ distribution of its carrier.

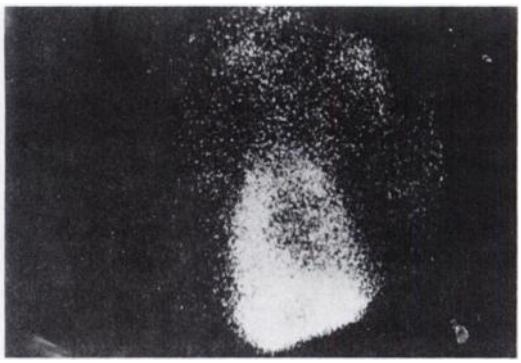

FIG. 3. Scintigram done 60 min after intravenous injection of nonradioactive liposome followed by 200 μCi of liposome [^{99m}Tc-DTPA] in dog. Lung uptake is markedly increased.

administration of 200 μCi of liposome [^{99m}Tc-Sn-DTPA]. The liver and spleen were clearly delineated. The kidney and bladder were also visualized. Variable amounts of radioactivity were observed in the lungs. A scan of a dog after administration of ^{99m}Tc-Sn-DTPA is shown (Fig. 2) to stress the difference in organ distribution.

On two occasions before the injection of the radioactive liposomes, a large amount (185 mg) of cold liposome was administered. The images obtained showed increasing amounts of radioactivity in the lungs and bone marrow with preferential liver–spleen uptake (Fig. 3).

DISCUSSION

Five minutes after the injection of liposome [^{99m}Tc-Sn-DTPA], almost 50% of the injected radioactivity was present in the liver (47%) and spleen (3%). There was a continuous decline of this value with time, but the rate of decline was slower for the liver than for the spleen. If the reticuloendothelial system is exclusively responsible for the uptake and destruction of liposomes, similar rates of disappearance would be expected. It seems that liposomes, unlike colloids, are only partly removed by the reticuloendothelial system. In fact, the presence of liposomes inside hepatocytes has been shown by electron microscopy (*6,12*). Our data are consistent with the concept that the metabolism of liposomes in the liver occurs both in reticuloendothelial cells and polygonal cells.

After the circulation has been loaded with nonradioactive liposomes, the decrease in hepatic uptake and the increase in uptake by spleen, lung, and bone marrow suggest a competitive process with displacement of the liposome [^{99m}Tc-Sn-DTPA] to other organs. This indicates that the mechanism of liposome uptake can be saturated to a certain extent.

Single-compartment vesicles were used as vehicles for radiopharmaceuticals (*13*). Vesicles are obtained by prolonged sonication of liposomes (hours) until the clearing of the suspension indicates the change from multiple-bilayer to single-bilayer structures (*14*). Both liposomes and vesicles are produced from the same materials. The difference resides in the spatial arrangement: vesicles have only one lipid membrane enclosing the aqueous phase while liposomes have multiple concentric lipid membranes containing the aqueous phase between them. For this reason, vesicles have a smaller water space within them and consequently trap fewer ions (*4*). For a meaningful comparison of in vivo distribution of liposomes and vesicles, it would be necessary to know the variation in organ distribution of lipid vesicles with time.

The carrier function of liposomes is clearly demonstrated in Fig. 2, where the liposomes carried ^{99m}Tc-Sn-DTPA, which is usually excreted through the kidneys, into the liver and spleen. In the same way, it may be possible to introduce other tracers or more complex molecules into cells or organs in which they usually cannot be localized by conventional techniques.

CONCLUSIONS

Our studies clearly show that liposomes can act as carriers for radiopharmaceuticals. Liposome-entrapped ^{99m}Tc-Sn-DTPA has a different distribution from ^{99m}Tc-Sn-DTPA.

The uptake of the liposome carrier system can be at least partially saturated; this causes a distinct alteration of the liposome distribution.

ACKNOWLEDGMENTS

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FOOTNOTES

* We will express the composition of liposomes using the notation proposed by Weissman: substances entrapped within the liposomes are written in square brackets following the word *liposome*, e.g., liposome [^{99m}Tc-DTPA] or L[^{99m}Tc-DTPA] (see Ref. *5* for complete details).

† Sigma Chemical Co., St. Louis, Mo.

‡ Fisher Scientific Co., Fair Lawn, N.J.

|| Diagnostic Isotopes, Upper Saddle River, N.J.

¶ J. T. Baker Chemical Co.

** Bransonic 50-55KH2.

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SNM TECHNOLOGIST SECTION
24th Annual Meeting

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The Technologist Section has set aside time for a nuclear medicine technologists program at the 24th Annual Meeting in Chicago, June 20–23, 1977.

The Scientific Program Committee welcomes the submission of abstracts for 12-min papers from technologists for the meeting. Abstracts must be submitted on an official abstract form. The format of the abstracts must follow the requirements set down on the abstract form. These abstract forms are available only from the Technologist Section, Society of Nuclear Medicine, 475 Park Ave. South, New York, NY 10016.

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DEADLINE: February 10, 1977

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For additional information contact Paul E. Christian, Division of Nuclear Medicine, University of Utah Medical Center, 50 North Medical Drive, Salt Lake City, UT 84132; tel. (801) 581-2121.

DEADLINE: April 15, 1977