Studies of Radiopharmaceutical-Enclosing Lipid-Protein Vesicles Formed from Native Plasma Components

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Vesicles 500–600 Å in diameter were formed by sonicating diluted samples of rat and mouse plasma. An average preparation of these vesicles formed from 1 ml of plasma contained 7.5 mg of plasma lipid and 7.3 mg of plasma protein. Plasma vesicles could be made to enclose such radiopharmaceuticals as $^{99m}TcO_4^-$, and the vesicles were found to be impermeable to this anion. We have studied the in vivo distribution patterns of ^{99m}Tc after intravenous injection into the rat or mouse of pertechnetate–plasma vesicles formed from rat or mouse plasma, and we find that the radioactivity remains primarily within the circulation even at 60 min after injection. In contrast, vesicles formed from artificial lipids are rapidly removed by the liver and spleen. Formation of vesicles from native plasma constituents offers a means of carrying drugs and radiopharmaceuticals in vivo in packages that have a low risk of being either toxic or antigenic.

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Artificial lipid vesicles can be used to carry drugs and radiopharmaceuticals in vivo, so that material enclosed and protected within a vesicle is distributed with it, rather than as a free drug or radiopharmaceutical (*1–3*). Other workers have also studied the use of lipid vesicles to carry various chemical compounds in in vitro and in vivo systems (*4–8*). After intravenous injection, artificial lipid vesicles are stable in blood, although substantial quantities are usually rapidly removed by the liver and spleen. Preliminary results in our laboratory indicate that removal of such vesicles by the liver can be reduced by precoating them with certain proteins (*3*). Since endogenous lipids are normally transported in blood in association with proteins (about 500 mg of lipid per 100 ml plasma) (*9*), it occurred to us that lipid–protein vesicles could be formed from these natural ingredients and that such vesicles might be less readily phagocytized than artificial lipid vesicles and hence have a longer residence time in the circulation. The present study indicates that this approach is feasible; it describes the formation of lipoprotein vesicles made from normal rat and mouse plasma components and presents evidence that such vesicles are able to carry enclosed radiopharmaceuticals in vivo with relatively low removal rates by liver and spleen.

MATERIALS AND METHODS

Blood was collected in heparinized tubes from female Sprague–Dawley rats (150 gm) or from C_3H mice (25 gm) fed ad libitum, and the plasma was collected after centrifuging the unrefrigerated blood at 1,000 g for 10 min. This plasma was used, without further treatment, to form the vesicles. Pertechnetate was obtained from a 500-mCi commercial ^{99}Mo–^{99m}Tc generator. To obtain pertechnetate-containing plasma vesicles, 1 ml of rat or mouse plasma was added to 1 ml of a salt buffer (composition given below) containing either 3 or 50 mCi of $^{99m}TcO_4^-$, and the mix was sonicated for 1 hr at 45°C with a 4-mm-diam probe.* The higher con-

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centration of pertechnetate was used to form the vesicles used in monitoring the in vivo distribution pattern by gamma imaging; the lower concentration served for counting of excised tissue samples in a well counter. The sonicated plasma samples were passed through a Sepharose 6B column (24 × 1.5 cm) to separate $^{99m}TcO_4^-$ trapped within the plasma vesicles from free $^{99m}TcO_4^-$. The eluting salt buffer had the following composition: 3.5 m*M* Na_2HPO_4, 1.5 m*M* NaH_2PO_4, 145 m*M* NaCl, 15 m*M* KCl, 1 m*M* $MgSO_4$, 1 m*M* $CaCl_2$, and 10 m*M* dextrose adjusted to pH 7.1. The first radioactive peak containing the plasma vesicles appeared immediately after the void volume of the column, whereas passage of free $^{99m}TcO_4^-$ was greatly retarded on the column. We estimate that approximately 0.1% of the sonicating solution (buffer and radiopharmaceutical) was enclosed within the vesicles. The ability of the plasma vesicles to contain the enclosed radiopharmaceutical was measured by dialysis (*1*). The protein content of the plasma vesicles and whole plasma was determined by the method of Lowry et al. (*10*). In some experiments phosphate-free Tris buffer, pH 7.2, was used in the formation of vesicles, and the phosphorus content of these vesicles was determined (*11*). The size of the plasma vesicles was determined by the light-scattering techniques described by Pecora and Aragon (*12*).

The pertechnetate–plasma vesicles formed from either rat or mouse plasma were injected intravenously into Sprague–Dawley (150 gm) rats or C_3H mice (25 gm). The in vivo distribution of the vesicles was studied in 5 rats and 30 mice. The in vivo distribution of ^{99m}Tc in rats was monitored with a scintillation camera, which permitted us to follow the distribution of radioactivity in one animal over a considerable period of time. The rats were placed prone on top of the low-energy collimator of the camera,† which was interfaced to a digital computer system.‡ Scintigraphic data were stored in list mode, and after completion of the study integration times for framing the data were selected and a series of images were mapped using a 64 × 64 matrix (4,096 pixels). With a light pen, regions of interest were chosen for the head, liver and right upper quadrant, spleen, bladder and lower abdomen, heart, and entire animal. Integral counts were recorded for each area at different intervals after injection.

In mice the in vivo distribution of pertechnetate–plasma vesicles was determined by removing and weighing the liver, spleen, kidneys, stomach, heart, lung, thyroid, and samples of muscle, fat, and blood. These samples and a measured fraction of the injected dose of the vesicles were counted in a well scintillation spectrometer. The distribution of ^{99m}Tc in these samples was calculated and expressed either as percent of injected dose per gram of tissue or as percent of injected dose per organ.

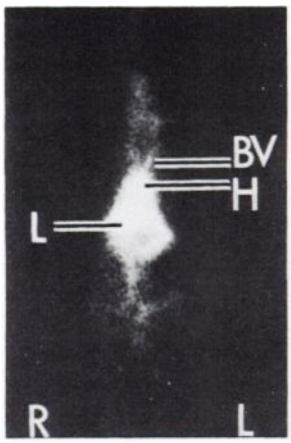

FIG. 1. Whole-body scintigram of rat 25 min after intravenous injection of $^{99m}TcO_4^-$–plasma vesicles. (H) Heart; (L) liver; (BV) blood vessels; (R) right side; (L) left side.

RESULTS

The size of the plasma vesicles, collected in four 0.5-ml fractions after passage through a Sepharose 6B column, ranged from 431 Å to 674 Å in diameter, with the largest units emerging in the first peak and the smallest in the last. Vesicle size was unchanged after storage at 4°C for 48 hr. These vesicles are nearly the same size as the artificial lipid vesicles made earlier in our laboratory (*1,3*). After dialysis of the pertechnetate–plasma vesicles against salt buffer for 1 hr, more than 95% of the ^{99m}Tc remained in the dialysis bag. Even after storage at 4°C for 24 hr, the vesicles retained more than 90% of the enclosed technetium. In contrast, free pertechnetate rapidly penetrated the dialysis sac and was recovered in the dialysis fluid (*1*). We believe that the properties of the plasma vesicles are similar to the artificial lipid vesicles previously described (*1–3*). Vesicles formed from 1.0 ml of plasma contained 7.3 mg of protein and 0.3 mg of phosphorus. Assuming that all the phosphorus is in lipids, the average lipid content can be estimated by multiplying the phosphorus weight by 25 (*11*), and this calculation gives 7.5 mg of lipid per milliliter of plasma. According to our measurement, rat plasma contains 82 mg of protein/ml; hence the vesicular protein constituted only about 9% of the original plasma proteins. This protein must be largely membrane-associated because the intravesicular volume is so small that only about 40 μg of plasma protein could be intravesicular. In contrast to the relatively small amount of plasma protein, the plasma vesicles contained almost all of the original plasma lipids. Variations in plasma lipid and protein content could occur if the

animals were in different nutritional states, and such variations would probably be reflected in differences in vesicular content and proportions of protein and lipid.

Pertechnetate–plasma vesicles were injected intravenously into five rats and the in vivo distribution of ^{99m}Tc was measured by gamma scintigraphy. A representative scintigram is shown in Fig. 1. Even 25 min after injection, large quantities of ^{99m}Tc remained within the blood stream since the heart and great vessels and some larger blood vessels in the neck, abdomen, and lower extremities are clearly visible in the scintigrams. The radioactivities in the heart and mediastinum, liver, brain, spleen, lower abdomen, and whole animal, as calculated by computer analysis, were compared within 2 min after injection and after 20 min after injection (Table 1). In Animal 1 at 0–10 sec, 30% of the total radioactivity remained in the area of the heart and mediastinum; at the later interval the activity of this area had been reduced only to 12%. These values for blood activity are considerably higher than those we found for artificial vesicles (*1–3*). With time ^{99m}Tc accumulated in other areas of the body, including liver, brain, and spleen (Table 1). In contrast to

TABLE 1. DISTRIBUTION OF RADIOACTIVITY IN RATS AFTER INTRAVENOUS INJECTION OF $^{99m}TcO_4^-$–PLASMA VESICLES AS COMPUTED BY ANALYZING SCINTIGRAPHIC AREAS OF INTEREST

Area of interest	No. of pixels in area	Percent of total radioactivity		Percent of total radioactivity/pixel	
Animal 1 (Fig. 1)		0–10 sec	1580–1590 sec	0–10 sec	1580–1590 sec
Heart and mediastinum	18	29.7	12.3	1.65	0.68
Liver	63	28.5	36.8	0.45	0.58
Brain	49	5.4	7.2	0.11	0.14
Spleen	11	1.6	4.8	0.14	0.43
Lower abdomen	49	10.6	9.6	0.22	0.19
Whole animal	765	100	100	0.13	0.13
Animal 2		60–120 sec	1470–1500 sec	60–120 sec	1470–1500 sec
Heart and mediastinum	20	14.1	9.7	0.70	0.49
Liver	34	22.4	38.5	0.66	1.13
Brain	38	7.1	2.6	0.19	0.07
Spleen	15	8.0	14.1	0.53	0.94
Lower abdomen	38	10.2	6.0	0.27	0.16
Whole animal	419	100	100	0.24	0.24

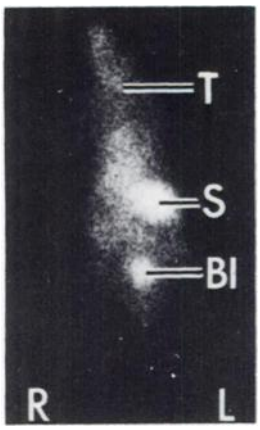

FIG. 2. Whole-body scintigram of rat 17 min after intravenous injection of free $^{99m}TcO_4^-$. (S) Stomach; (B) bladder; (R) right side; (L) left side.

TABLE 2. DISTRIBUTION OF ^{99m}Tc IN C_3H MICE 5 MIN AFTER INTRAVENOUS INJECTION OF $^{99m}TcO_4^-$–PLASMA VESICLES, CALCULATED FROM REMOVED TISSUE SAMPLES (MEANS AND RANGES FOR THREE MICE)

Tissue	Percent injected dose per gram tissue		Percent injected dose per organ	
	Range	Mean	Mean	Range
Liver	10.6	9.8–11.8	16.6	14.9 –18.3
Spleen	16.2	12.6–19.3	1.9	1.7 – 2.2
Kidney (L)	4.2	2.7– 6.4	0.7	0.4 – 1.1
Kidney (R)	4.2	3.0– 6.0	0.8	0.6 – 1.1
Stomach	1.2	0.9– 1.3	0.2	0.18– 0.28
Heart	7.9	5.0–10.2	1.0	0.6 – 1.3
Lung	10.0	8.4–12.2	1.8	1.4 – 2.3
Muscle	1.7	1.4– 2.1	—	—
Fat	0.6	0.4– 0.9	—	—
Blood	29.7	26.7–33.6	60*	—

* Estimated.

the scintigraphic findings observed with pertechnetate–plasma vesicles (Fig. 1), scintigraphy at 17 min after the intravenous injection of free $^{99m}TcO_4^-$ showed that most of the ^{99m}Tc activity had accumulated in the stomach, bladder, and thyroid (Fig. 2). Previous studies have shown that very soon following intravenous injection of artificial lipid vesicles containing ^{99m}Tc, most of the activity was localized in liver and spleen, and blood-to-liver ratios were less than unity (*2*).

Pertechnetate vesicles formed from mouse plasma were injected into three C_3H mice and the distribution of the radioactivity calculated (Table 2). The blood-to-liver ratio 5 min after injection was also relatively high at 2.8. We estimate that about 60% of the radioactivity was still circulating at the time of death and only about 17% was present in the

liver. In contrast, the corresponding liver and spleen uptake of the artificial vesicles (*3*) was higher, with a blood-to-liver ratio of 0.5 at 5 min after injection. In various experiments a total of 35 animals has been injected with pertechnetate–plasma vesicles, and in all cases the technetium was distributed with the vesicles rather than as the free pertechnetate.

DISCUSSION

These experiments show that technetium-carrying vesicles can be formed in vitro in rat plasma, and upon reinjection they escape being trapped in the liver and spleen much longer than do the previously studied artificial lipid vesicles. Thus, the radiopharmaceutical can be carried effectively to all parts of the body. Excised-tissue experiments in mice show similarly delayed trapping. The sluggishness of RES trapping may be due to the protecting presence of native protein in the vesicle walls; phagocytosis of the artificial lipid vesicles was similarly delayed when they were coated with protein (*3*).

The vesicles formed from plasma had many of the same physical characteristics as artificial lipid vesicles formed from diphosphatidylcholine, gangliosides, and cholesterol (*1–3*). Both are small polydisperse bodies about 500 Å in diameter, which are not sedimented by centrifugation at 1,000 g and are not precipitated in the alcohol–ether mixture. The plasma vesicles can also be made to contain radiopharmaceuticals, such as $^{99m}TcO_4^-$. Like artificial vesicles, plasma vesicles are highly impermeable to the enclosed anion.

Since the plasma vesicles are made from native substances, the risks of toxicity or antigenicity should be very low, and the method offers a means of retaining enclosed drugs, radiopharmaceuticals, and possibly contrast agents in inert form within the circulatory system.

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FOOTNOTES

* Biosonik, Bronwill Scientific, Rochester, N.Y.
† Ohio-Nuclear Series 100 (Solon, Ohio).
‡ Hewlett–Packard Model 5407A (Waltham, Mass.).

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