IN VIVO DISTRIBUTION OF VESICLES LOADED WITH RADIOPHARMACEUTICALS: A STUDY OF DIFFERENT ROUTES OF ADMINISTRATION

I. Ross McDougall*, June K. Dunnick, Michael L. Goris, and Joseph P. Kriss

Stanford University Medical Center, Stanford, California

The in vivo distribution of vesicles containing radiopharmaceuticals in their cavities has been studied using three routes of administration: intravenous, subcutaneous, and intraperitoneal. The in vivo distribution in mice was determined by dissection of the animals and calculation of radioactivity in the organs. In rats the in vivo distribution was assessed by scintigraphy using a scintillation camera-digital computer unit. After intravenous injection of vesicles, radioactivity is concentrated to some extent in the liver and spleen but the pattern of distribution is different from that of the corresponding free radiopharmaceutical or radiocolloid made of the corresponding radionuclide. The permeability of the vesicular membrane to contained radiopharmaceutical has been shown to vary according to the chemical composition of the vesicles. Vesicles can be used to introduce materials in vivo and the potential exists for their specific targeting by coupling other molecules to their surfaces.

Investigations in our laboratory have indicated that $^{99m}TcO_4^-$ (and other radiopharmaceuticals) can be enclosed within vesicles, single compartmental spherules composed of a bilayer of polar lipids (*1*). After intravenous injection of such vesicles into mice, the distribution of radioactivity in sampled tissues differed from that of either free pertechnetate or technetium-sulfur colloid (*1*). This communication presents autopsy and scintigraphic evidence of the in vivo distribution of vesicle-enclosed radiopharmaceuticals in mice and rats after intravenous, subcutaneous, or intraperitoneal injection. These studies were designed to determine whether vesicles might be useful carriers for diagnostic or therapeutic agents.

MATERIALS AND METHODS

Vesicles were prepared in the following manner: 44 μM phosphatidyl-choline [prepared by the method of Singleton, et al (*2*)] in ethanol and 4 μM gangliosides (Supelco, Bellefonte, Pa., Catalog No. 04-0632) in chloroform/methanol (1/1) were evaporated together to dryness with a Buchler flash evaporator. One milliliter of toluene was added to the dry lipids and the mixture was evaporated to dryness for a second time; 1.4 ml of salt buffer (Table 1) containing 1–3 mCi $^{99m}TcO_4^-$ in a volume of 0.1 ml eluted from a ^{99}Mo-molybdenum generator (New England Nuclear Corp., Boston, Mass.) was added to the dried lipids, and using a Vortex mixer, the lipids were suspended in the buffer to produce a cloudy whitish solution. At this stage, the solution contains liposomes, multilaminated structures each layer of which is a continuous lipid bilayer. The liposomal solution was transferred to a round-bottomed glass test tube, fixed in an ice bath to maintain a constant cold temperature, and subjected to ultrasonic irradiation with a Biosonik probe sonicator (Bronwell Scientific Co., Rochester, N.Y.). Clearing of the cloudy suspension signaled the formation of single compartmental vesicles. The vesicles, enclosing a proportion of the buffer solution and $^{99m}TcO_4^-$,

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For reprints contact: Joseph P. Kriss, Div. of Nuclear Medicine, Stanford University Medical Center, Stanford, Calif. 94305.

* Commonwealth Fellow (1972–1974). Present address: Dept. of Medicine, University of Glasgow, Glasgow, Scotland.

TABLE 1. COMPOSITION OF SALT BUFFER

Component	Amount (mM)
Na_2HPO_4	3.5
NaH_2PO_4	1.5
NaCl	145
KCl	15
$MgSO_4$	1
$CaCl_2$	1
dextrose	10

were separated from the free radiopharmaceutical by passage through a Sephadex G-25 (fine) column. The final product is referred to as $^{99m}TcO_{4_e}^-$ -vesicles.

Technetium-99m-sulfur colloid was prepared by the method of Patton, et al, (*3*). Mouse erythrocytes were labeled with ^{51}Cr according to the method of Gray and Sterling (*4*).

All experiments involving tissue analysis were performed in C_3H mice weighing about 15–20 gm. In Experiment 1, mice received intravenously a mixture of $^{99m}TcO_{4_e}^-$ -vesicles (sonicated for 60 min) and ^{51}Cr autologous erythrocytes. This maneuver was employed to determine the amount of labeled vesicles present in an organ in excess of the amount present simply in the blood of the organ. After counting the blood and tissue samples and standards of ^{99m}Tc and ^{51}Cr at spectrometer settings optimal for both radionuclides, respectively, calculations were made as follows for a given organ:

$$A_L = total_L - total_H \times \frac{^{51}Cr\ std_L}{^{51}Cr\ std_H}$$

$$A'_L = A_L - total_H \times \frac{A/ml\ blood}{B/ml\ blood}$$

where A = net ^{99m}Tc cpm; B = net ^{51}Cr cpm = $total_H$; A' = net ^{99m}Tc cpm, corrected for blood content; L = low window setting optimal for ^{99m}Tc counting; and H = high window setting optimal for ^{51}Cr counting all counts corrected for background.

The mice were sacrificed at 5 min and the organs (liver, spleen, kidneys, stomach, heart, lung, and thyroid) and a sample of blood, fat, and muscle were removed and counted in preweighed tubes. A measured volume of the injected sample of vesicles and ^{51}Cr-labeled blood cells was retained as a counting standard. Distribution of radioactivity in the animals was expressed as percentage of injected dose per gram of tissue and percentage of injected dose per organ.

In a series of other experiments, standard $^{99m}TcO_{4_e}^-$ -vesicles sonicated for 30 min and free $^{99m}TcO_4^-$ were injected into C_3H mice through three routes of administration: intravenous (Experiment 2), subcutaneous (Experiment 3), and intraperitoneal (Experiment 4). For each route of administration studied, a pair of C_3H mice matched for age, sex, and weight were used: one mouse received vesicles, the other free $^{99m}TcO_4^-$. All mice were sacrificed at 2 hr and the percentage of injected dose per gram of tissue was calculated as above.

For scintigraphic studies, pairs of Sprague-Dawley rats matched for age, sex, and weight (about 150 mg) were used for each of a series of experiments. Animals were anesthetized with intraperitoneal pentobarbitone (10–15 mg). Intravenous injections were made through a 23-gage scalp vein catheter inserted in a tail vein. Just prior to the injection of the radioactive test substances, the rats were placed on top of the low-energy collimator of a Picker Dynacamera interfaced to a digital computer system, Hewlett-Packard Model 5407A (*5*). Data were stored in list mode. In this mode the x and y coordinates of 1,300,000 counts can be stored. After completion of the study, integration times for framing the data were selected and serial images were mapped into a 64 × 64 matrix. A series of three experiments was performed to test the in vivo distribution of the following pairs of injected materials $^{99m}TcO_{4_e}^-$ -vesicles (sonicated 60 min) versus free $^{99m}TcO_4^-$ administered intravenously (Experiment 5), $^{99m}TcO_{4_e}^-$ -vesicles (sonicated 5 min) versus ^{99m}Tc-sulfur colloid administrated intravenously (Experiment 6), and $^{99m}TcO_{4_e}^-$ -vesicles versus free $^{99m}TcO_4^-$ administered subcutaneously (Experiment 7).

TABLE 2. DISTRIBUTION OF ^{99m}Tc AND ^{51}Cr AFTER INJECTION OF $^{99m}TcO_4{}^-{}_e$-VESICLES AND ^{51}Cr RED BLOOD CELLS*

Organ	^{99m}Tc activity corrected for activity in organ blood (%)	^{51}Cr activity (%)
Liver	47.7 ± 6.0	8.6 ± 1.5
Spleen	7.5 ± 0.2	1.9 ± 1.2
Kidney (L)	0.4 ± 0.1	1.5 ± 0.7
Kidney (R)	0.5 ± 0.1	1.4 ± 0.4
Stomach	0.6 ± 0.2	0.9 ± 0.5
Heart	0.0 ± 0.0	1.2 ± 0.3
Lung	0.3 ± 0.2	2.3 ± 0.9

* Values expressed as percent injected dose per organ (mean ± s.d.). C_3H mice were sacrificed 5 min after i.v. injection.

RESULTS

When ^{51}Cr-labeled red blood cells were injected simultaneously with $^{99m}TcO_{4_e}^-$ -vesicles (Experiment 1), the calculated distribution of ^{99m}Tc in mouse organs, corrected for the blood content of the organ, showed net tissue accumulation of the radionuclide in liver, spleen, kidneys, stomach, and lung (Table 2). However, the magnitude of tissue accumulation in the latter three organs was very low compared with the blood content of these organs as shown by their respective ^{51}Cr activities. In contrast, levels of ^{99m}Tc in liver and spleen were relatively high, undoubtedly due to accumulation in reticuloendothelial cells. The data suggest that transcapillary passage of vesicles is of a low order of magnitude.

The effect of route of administration on subsequent tissue distribution of $^{99m}TcO_{4_e}^-$ -vesicles and free pertechnetate is shown in Table 3 (Experiments

TABLE 3. DISTRIBUTION OF ^{99m}Tc AFTER INJECTION OF $^{99m}TcO_4{^-}_e$-VESICLES AND FREE $^{99m}TcO_4^-$ 2 HR AFTER INTRAVENOUS, INTRAPERITONEAL, AND SUBCUTANEOUS ADMINISTRATION*

	Intravenous administration		Intraperitoneal administration		Subcutaneous administration	
Organ	$^{99m}TcO_4{^-}_e$-vesicles	$^{99m}TcO_4^-$	$^{99m}TcO_4{^-}_e$-vesicles	$^{99m}TcO_4^-$	$^{99m}TcO_4{^-}_e$-vesicles	$^{99m}TcO_4^-$
Liver	25.1	14.1	3.3	19.1	1.2	18.2
Blood	3.7	12.5	4.8	22.0	1.9	17.7
Spleen	45.2	7.1	6.0	2.2	0.9	9.8
Kidney (L)	3.7	7.2	3.5	10.8	2.0	9.8
Kidney (R)	4.0	7.4	3.8	11.7	2.2	10.2
Stomach	6.7	205.0	10.8	189.9	4.5	153.6
Heart	1.0	15.6	0.9	9.1	0.5	6.8
Lung	2.7	11.5	1.7	16.8	1.3	15.2
Thyroid	17.6	131.3	11.0	—	9.7	409.2
Muscle	0.6	4.7	6.6	6.0	1.3	5.6
Fat	1.7	0.7	2.2	1.2	1.9	1.5

* Values expressed as percent injected dose per gram tissue.

2–4). Whether given intravenously, intraperitoneally, or subcutaneously, the distribution pattern of ^{99m}Tc (given enclosed in vesicles) differed from that of free $^{99m}TcO_4^-$. The data indicate that intact vesicles are absorbed from the peritoneal cavity and subcutaneous sites and are distributed through the circulation to other organs and tissues, albeit at slower rates. The stomach and thyroid radioactivity levels observed after intraperitoneal administration or subcutaneous administration of $^{99m}TcO_{4e}^-$ -vesicles are low, which shows that radioactivity enclosed in vesicles does not distribute in the same manner as the free radiopharmaceutical.

Scintigraphic studies in rats demonstrating in vivo the differences in distribution of vesicles and free radiopharmaceutical or radiocolloids are shown in Figs. 1 and 2. Figure 1 shows a series of scintigraphs (A–D) of a pair of rats, one receiving $^{99m}TcO_{4e}^-$ -vesicles (60 min sonication—animal on left) and the other free $^{99m}TcO_4^-$ (Experiment 5). The images encompass a time span of 28 min from the moment of injection. In the rat injected with the $^{99m}TcO_{4e}^-$ -vesicles, hepatic uptake was evident within the first few minutes after injection. A small amount of radioactivity was observed in the bladder during the latter part of the study (Frames C and D). In the rat that received free $^{99m}TcO_4^-$, generalized body activity plus later concentration in the stomach and urine were noted. The fate of $^{99m}TcO_{4e}^-$ -vesicles (sonicated for 5 min) was compared with that of

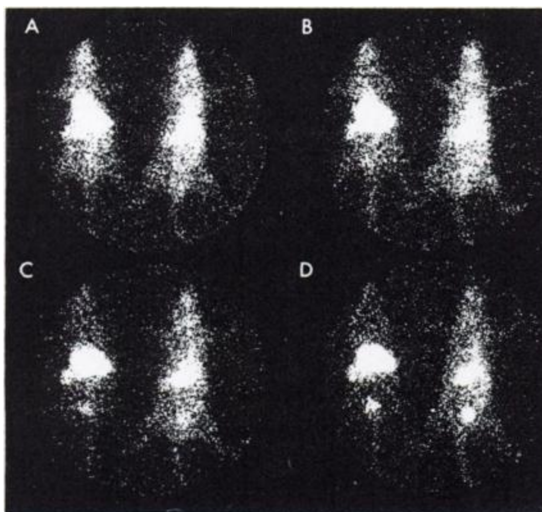

FIG. 1. Whole-body scintigraphs after intravenous injection in rats of $^{99m}TcO_4{^-}_e$-vesicles (left) and free $^{99m}TcO_4^-$ (right). Animal receiving vesicles shows predominantly hepatic concentration of radioactivity: animal receiving free $^{99m}TcO_4^-$ shows stomach and bladder concentration of radioactivity. (A) 1–7 min image after intravenous injection, (B) 8–14 min, (C) 15–21 min, and (D) 22–28 min.

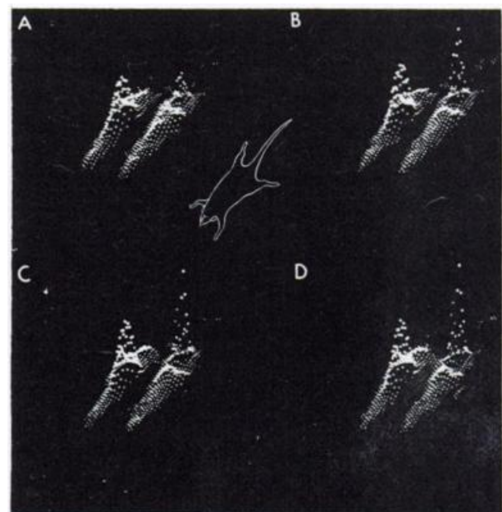

FIG. 2. Whole-body scintigraphs after intravenous injection in rats of $^{99m}TcO_4{^-}_e$-vesicles (left) and ^{99m}Tc-sulfur colloid (right). Images (A–D) each represent isometric display of radioactivity in pair of rats. Doses of radioactivity were equal. Sharp peaks of activity represent hepatic uptake, which is noticeably greater and faster in animal receiving radiocolloid (right). (A) 0–100 sec after injection, (B) 100–200 sec, (C) 200–300 sec, and (D) 300–400 sec.

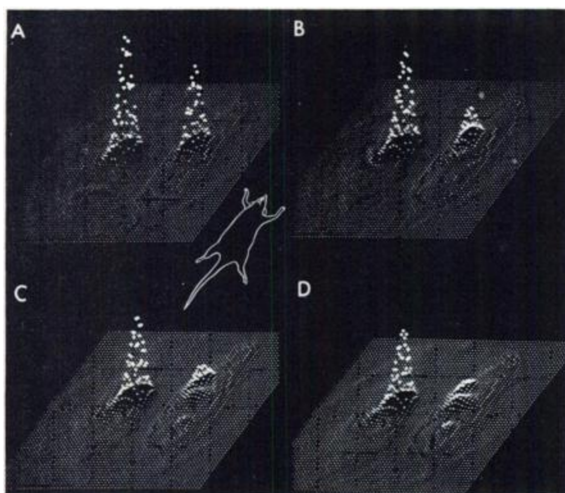

FIG. 3. Whole-body scintigraphs after subcutaneous injection in rats of $^{99m}TcO_4{}^-{}_e$-vesicles (left) and free $^{99m}TcO_4^-$ (right). Activities are presented in isometric mode. Animal receiving free $^{99m}TcO_4^-$ shows concentration of activity in stomach and bladder, while animal receiving vesicles shows persistent activity at injection site. (A) 1–10 min after injection, (B) 10–20 min, (C) 30–40 min, and (D) 50–60 min.

^{99m}Tc-sulfur colloid in another pair of rats (Fig. 2) (Experiment 6). Both animals received the same amount of radioactivity. The radiocolloid was rapidly accumulated in the liver while the hepatic localization of ^{99m}Tc enclosed in vesicles, although appreciable, was less.

Scintigraphic data on comparing the fate of subcutaneously administered $^{99m}TcO_{4_e}^-$-vesicles and free $^{99m}TcO_4^-$ in rats are presented in Fig. 3 (Experiment 7). Note that in the animal injected with vesicles, most of the activity remains at the site of the injection in contrast with fast clearance from the injection site and increased body activity in the animal that received free radiopharmaceutical.

In none of the animals used in our experiments have we observed any adverse short-term effects from vesicle administration. Data on possible subtle biochemical or tissue effects are not yet available.

DISCUSSION

Earlier experiments in our laboratory using vesicles containing the spin label tempocholine have proven that a proportion of injected vesicles remain intact in the circulation in mice for as long as 46 min after their intravenous injection (*1*). The experiments described in this paper show that vesicles, single-compartment spherules composed of lipid bilayers, might also be useful as carriers of diagnostic or therapeutic agents when injected subcutaneously or intraperitoneally. The results indicate that $^{99m}TcO_4^-$ enclosed within vesicles has a distribution pattern in vivo different from that of the respective free radiopharmaceutical or ^{99m}Tc-sulfur colloid. The $^{99m}TcO_{4_e}^-$-vesicles injected subcutaneously were cleared from the injection site, albeit slowly, and distributed quite differently from free $^{99m}TcO_4^-$. Following their intraperitoneal injection, $^{99m}TcO_{4_e}^-$-vesicles were absorbed from that site and distributed in a pattern similar to that of vesicles injected intravenously. These data suggest that vesicles can traverse endothelial membranes and lymphatic channels and remain intact. Our data also suggest that transcapillary passage of vesicles of this given composition is restricted but probably not absolutely so. Such restriction might pose serious obstacles to the delivery of vesicles to target sites other than liver and spleen. The degree of vesicle permeability and other physical characteristics can be tailored by altering the basic lipid components or by altering the length of sonication (*1,6*). Thus there remains the potential that uses for vesicles in vivo may be found for either diagnostic or therapeutic purposes. Gregoriadis and Ryman (*7,8*) have predicted that liposomes could be used to transport enzymes to cells which might be congenitally deficient in the enzyme. Our studies suggest also that vesicles could be used as vehicles to permit the regulated slow release of drugs from subcutaneous sites.

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