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Clearance and tissue distribution of functionalized polymeric liposomes from the blood stream of rats

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Polymeric liposomes containing a synthetic porphinato-iron-imidazole complex (hemoglobin or red blood cell model) were labeled by introducing 1,2-di[1-^{14}C]palmitoyl-*sn*-glycero-3-phosphocholine into their polymerized bilayers. After intravenous injection into rats, their clearance from a blood stream was measured. The apparent half-life time (50% disappearance time) was about 14 ± 2 h. Their tissue distribution was determined with time by whole autoradiographic measurement.

Liposomes (vesicles) are expected to be useful as carriers for biologically active materials [1–3] and their behavior in the blood stream in vivo has been studied using labeled compounds loaded on or in the liposomes [4–8]. The role of plasma high density lipoprotein (HDL) on liposome degradation/integration has been also elucidated in vitro or in vivo [8,9]. It has been reported also that a type of a marker scarcely affects the apparent clearance rate of liposomes from a blood stream in vivo [5]. To make liposomes more stable in vitro and/or in vivo, a method has been suggested to polymerize lipids in bilayers to give liposomes of cross-linked membranes. These polymeric liposomes have been found mechanically stable [10] and to be stable against enzymes (phospholipases) [11,12]. Thus we may reasonably expect the polymeric liposomes to be stable in vitro and/or in vivo, perhaps in the interaction with HDL. But few reports have appeared on their behavior in vivo, although the interactions in vitro have been recently studied [12–14].

In a recent investigation on functionalized liposomes, we used them as carriers of a synthetic heme-imidazole complex and demonstrated that a stable oxygen carrier can be prepared by embedding a heme complex into the hydrophobic region of their bilayers [15–17]. The use of polymeric liposomes as carriers gave mechanically stable and concentrated heme solutions [12,18]. We have also reported their interactions with blood components in vitro [12]. To elucidate the possibility of the functionalized liposomes as a model of red blood cells, the behavior in the blood stream and the tissue distribution in vivo are studied in this report by the use of a ^{14}C-labeled phospholipid as a marker.

Labeled and single unilamellar polymeric liposomes were prepared as follows: 1,2-bis(octadeca-

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2,4-dienoyl)-*sn*-glycero-3-phosphocholine (4.0 g, 5.0 mmol) [19], hemin (tetra-*meso*-{*o*-[2′,2′-dimethyl-20′-(2″-trimethylaminoethylphosphonatoxy)eicosanamido]phenyl}porphinato iron (292 mg, 0.1 mmol), 1-dodecylimidazole (70.5 mg, 0.3 mmol) and 1,2-di[1-^{14}C]palmitoyl-*sn*-glycero-3-phosphocholine (2.5 mg, 400 μCi) purchased from Amersham Japan Co. were dissolved in benzene/methanol and freeze-dried. The powder obtained was suspended in oxygen-free saline (0.9% NaCl (w/v%), 150 ml) and ultrasonicated under Ar by a probe-type sonicator (80 W, 40 min). The suspension was filtered through membrane filters of 0.45 μm pore size and then irradiated by ultraviolet light at 40°C for 7 h. The solution was concentrated by ultrafiltration [12,18]. The concentrated solution (21.5 (w/v%), pH 7.2) was finally filtered through membrane filters of 0.22 μm pore size into a sterile glass bottle. The yield of the solution was 19.5 ml. The specific activity and the total radioactivity were 19.0 μCi/ml and 0.37 mCi, respectively. The solution was diluted with a solution, which had been prepared by the same way without the labeled compound and had the same concentration of the functionalized liposomes, to prepare the solution for injection (the final specific activity was 10.34 μCi/ml). Radioactivity measurements were done with a liquid scintillation counter (Mark III, Tracor Analytic) [20]. The weight-averaged diameters were 20 nm by a laser particle analyzer [12,18].

All experiments were performed with male Wistar rats (8 weeks) weighing 220–230 g. Three rats received intravenously the polymeric liposome solution through the tail veins with an infusion pump. The injection volume (amount was 10 ml (2150 mg)/kg body weight per rat. The animals were given food and water ad libitum through the experiment. Blood (80 μl) was drawn through external jugular vein for measuring time-dependence of the level of the particles. The sample were absorbed on Combust-Pad and after drying they were analyzed in a Packard Tri-Carb liquid scintillation counter.

Fig. 1 shows the clearance of the labeled polymeric liposomes after intravenous injection into rats. The apparent half-life time (50% disappearance time) in the blood stream was determined to be 14 ± 2 h. If we assume the blood volume of a rat is 6.5 ml/100 g body weight [21], the initial level of the polymerized liposome particles in blood can be calculated to be 2.9 g/dl. At such high dosage of the injected compounds, blockade of the reticuloendothelical system may contribute to the half-disappearance time [22]. Half-life times of blood substitutes in the blood stream are estimated in the initial plasma level of 2–6 g/dl in the cases of modified and/or polymerized hemoglobin solutions [23–25]. Under these conditions, the life-time is about 15–25 h corresponding to that of the present polymeric liposome particles (14 h). The curvilinear disappearance shown in Fig. 1 may indicate no saturation of the clearance mechanisms because a linear disappearance at high dosage is considered to be caused by saturation of organs [24,26].

In the present study, we used a ^{14}C-phospholipid as a marker. In this case phospholipid transfer to HDL may be caused in vivo. Two processes for the transfer from non-polymerized liposomes are known [9]. One is the transfer from cholesterol-rich liposomes involving an exchange process with high retention of entrapped solute and another is a unidirectional process leading to liposome degradation in vitro. Nevertheless, incorporation of cholesterol or sphingomyelin effectively reduces the transfer to plasma HDL [9]. But in vivo studies showed that in lipoprotein-deficient mice the clearance rate from the blood stream was reduced considerably in comparison with normal mice and in vitro only HDL was detrimental to liposome stability [8]. Because, in the present polymeric

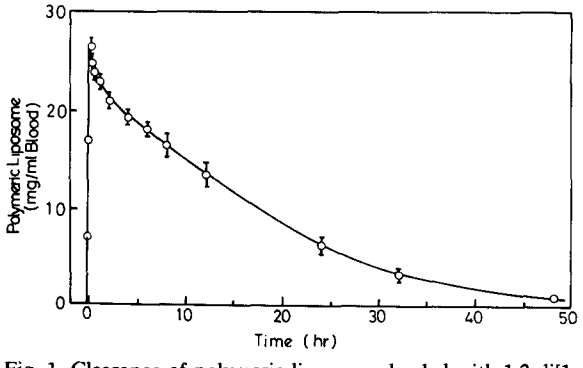


Fig. 1. Clearance of polymeric liposomes loaded with 1,2-di[1-^{14}C]palmitoyl-*sn*-glycero-3-phosphocholine in their polymerized bilayers. The bars represent standard deviations. Details are described in the text.

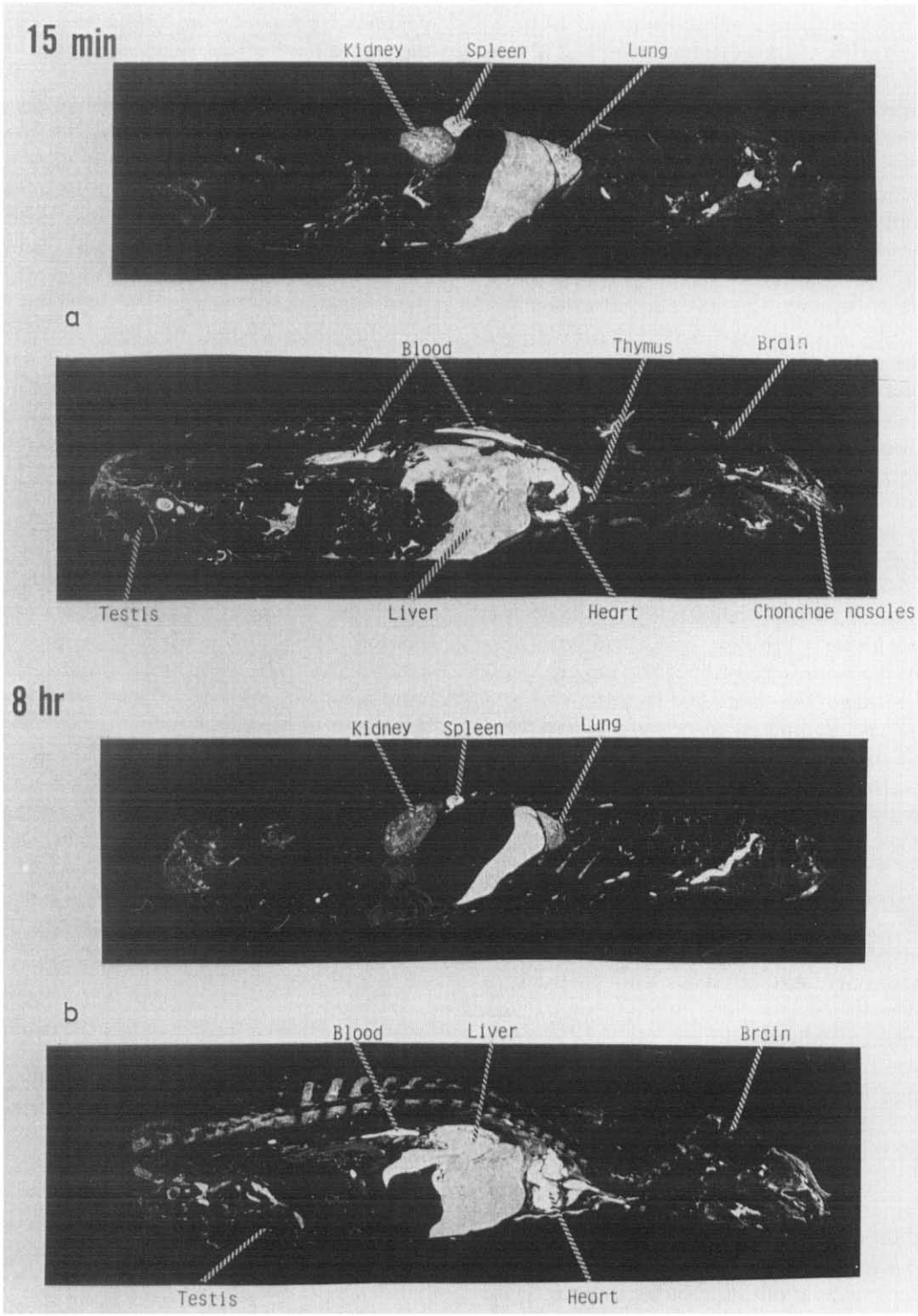
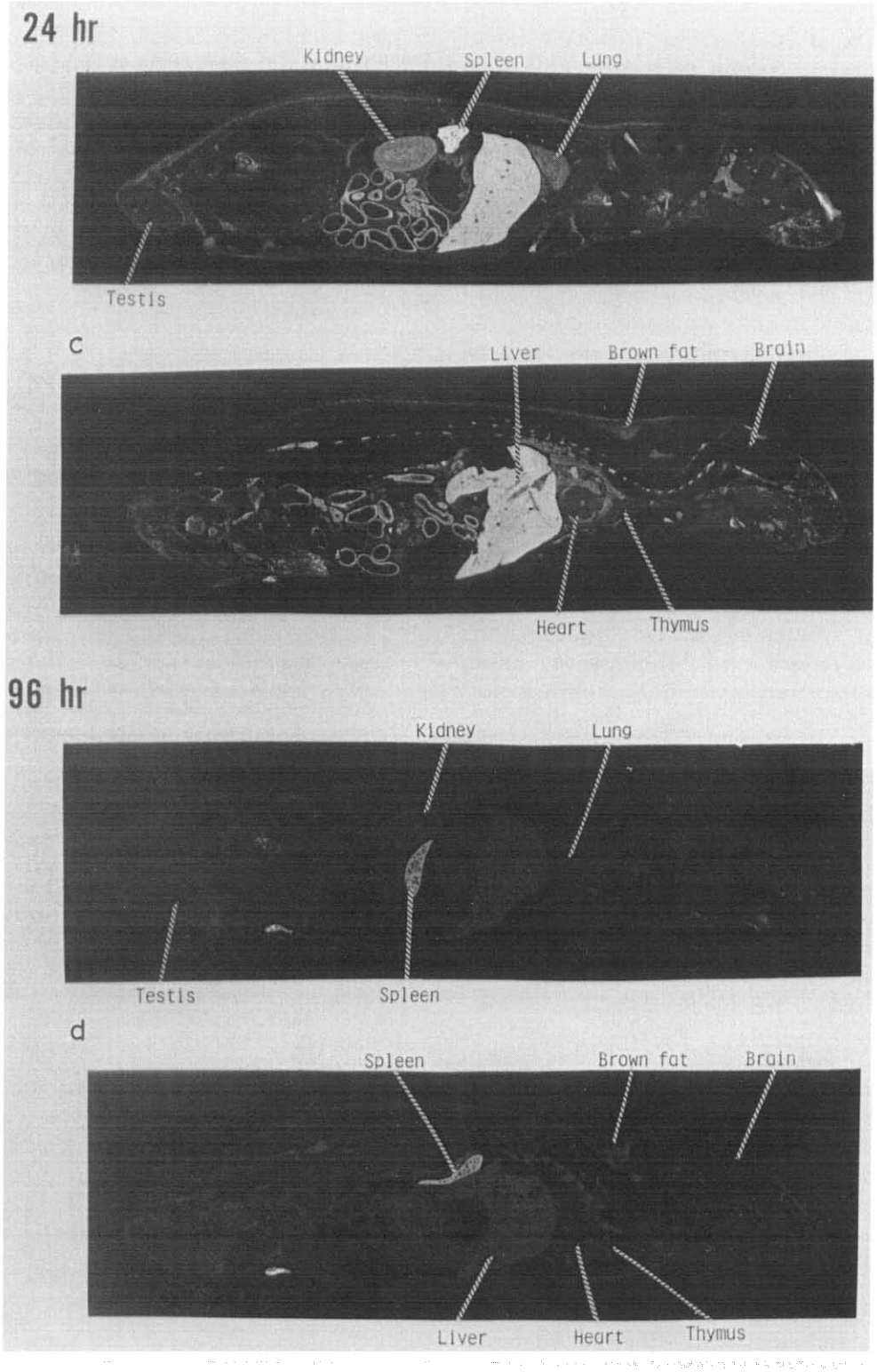


Fig. 2. Autoradiograms of rats at 15 min (a), 8 h (b), 24 h (c) and 96 h (d) after the intravenous injection of ^{14}C-labeled polymeric liposomes.


24 hr
Kidney
Spleen
Lung
Testis
c
Liver
Brown fat
Brain
Heart
Thymus
96 hr
Kidney
Lung
Testis
Spleen
d
Spleen
Brown fat
Brain
Liver
Heart
Thymus

liposomes, the membrane embedding ^{14}C-phospholipid is cross-linked, we may reasonably expect the liposomes to prevent the marker lipid from transferring to HDL. But this will have to be examined and discussed elsewhere.

It is known that the life-time of non-polymerized liposomes is rather short and strongly depends on the composition of membrane lipids [4,5,27]. Firstly, the important role of cholesterol on the stability of liposomes in vitro and in vivo is well known [27]. Cholesterol makes the bilayer more stable by lowering the membrane fluidity of egg yolk phosphatidylcholine liposomes. The polymerization (cross-linking) in bilayers is reasonably expected to give the same effect for membrane stabilization physically. Secondly, positively charged liposomes are retained in the circulation longer than neutral or negatively charged ones [4]. Thus stearylamine as one of membrane lipids prolonged the half-life time most effectively [4]. The amine membrane component, 1-dodecylimidazole (5.6 mol%), which binds to the fifth coordination position of a heme and makes a heme complex bind oxygen reversibly [16–18], may have the same effect and contribute partly to the prolongation of the life-time.

For autoradiographic measurements, rats were killed at 15 min, 8 h, 24 h and 96 h after injection. Whole body sections (20 μm thickness) were prepared according to the modified method of Ullberg [28] and exposed to ^{3}H-type X-ray film (Konishiroku Photo Co.) for 10 days at 5°C. A pair of sections had to be prepared because it was impossible to observe the distribution of all the organs concerned in one section. Fig. 2 shows the representative photos. Immediately after the injection, at 15 min, the radioactivity was observed largely in the vascular system, whereas no activity was found in the heart because the section was mistakenly prepared on the whole heart muscle. At 8 h the activity in hepatic cells increased simultaneously with the decrease in activity in the vascular system. The spleen also showed high activity at 8 h, especially in its red pulps. At 24 h the activity decreased in all the organs except the spleen, in which some activity remained even at 96 h after the injection. The cumulative excreted amounts of radioactivity were found to be: time = 24 h: respiration (22.4%), urine (0.7%), feces (0.2%); 48 h: 48.1%, 1.5%, 0.6%; 72 h: 63.1%, 2.0%, 0.9%; 168 h: 73.8%, 2.4%, 2.2%.

Thus it was found that the functionalized polymeric liposomes with a marker (^{14}C-phospholipid) were cleared from the blood stream of rats with a half-disappearance time of 14 ± 2 h and that these labeled particles were found largely in the reticuloendothelical system or related organs.

References

1 Gregoriadis, G. (1979) in Drug Carriers in Biology and Medicine (Gregoriadis, G., ed.), pp. 278–341, Academic Press, London
2 Gregoriadis, G., ed. (1983) Liposome Technology, Volume II, CRC Press, Inc., Boca Raton, FL
3 Tirrell, D.A., Donaruma, L.G. and Turek, A.B., eds. (1985) Macromolecules as Drugs and as Carriers for Biologically Active Materials, The New York Academy of Sciences, New York
4 Gregoriadis, G. and Neerunjun, D.E. (1974) Eur. J. Biochem. 47, 179–185
5 Juliano, R.L. and Stamp, D. (1975) Biochem. Biophys. Res. Commun. 63, 651–658
6 Tanaka, T., Taneda, K., Kobayashi, H., Okumura, K., Muranishi, S. and Sezaki, H. (1975) Chem. Pharm. Bull. 23, 3069–3074
7 McDougall, R.I., Dunnick, J.K., Goris, M.L. and Kriss, J.P. (1975) J. Nucl. Med. 16, 486–491
8 Senior, J., Gregoriadis, G. and Mitropoulos, K.A. (1983) Biochim. Biophys. Acta 760, 111–118
9 Damen, J., Regts, J. and Scherphof, G. (1981) Biochim. Biophys. Acta 665, 538–545
10 Juliano, R.L., Regen, S.L., Singh, M., Hsu, M. and Singh, A. (1983) BioTechnology 1, 882–885
11 Bueschl, R., Hupfer, B. and Ringsdorf, H. (1982) Makromol. Chem. Rapid Commun. 3, 589–596
12 Hasegawa, E., Eshima, K., Matsushita, Y., Nishide, H. and Tsuchida, E. (1986) Biochim. Biophys. Acta 862, 235–242
13 Juliano, R.L., Hsu, M.J., Peterson, D., Regen, S.L. and Singh, A. (1983) Exp. Cell. Res. 146, 422–427
14 Hayward, J.A. and Chapman, D. (1984) Biomaterials 5, 135–142
15 Hasegawa, E., Matsushita, Y., Kaneda, M., Ejima, K. and Tsuchida, E. (1982) Biochem. Biophys. Res. Commun. 105, 1316–1419
16 Tsuchida, E., Nishide, H., Yuasa, M., Hasegawa, E. and Matsushita, Y. (1984) J. Chem. Soc., Dalton Trans., 1147–1151
17 Tsuchida, E., Nishide, H., Yuasa, M., Hasegawa, E., Matsushita, Y.and Eshima, K. (1985) J. Chem. Soc., Dalton Trans., 275–278
18 Tsuchida, E., Hasegawa, E., Matsushita, Y., Eshima, K., Yuasa, M. and Nishide, H. (1985) Chem. Lett., 969–972
19 Hupfer, B., Ringsdorf, H. and Schupp, H. (1981) Makromol. Chem. 182, 247–253
20 Nagayama, S., Tsuda, M., Shindo, T., Kawaguchi, Y.,

Shigematsu, A., Hatori, A. and Ogawa, M. (1986) Pharmacometrics (Jap.) 32, 537–552
21 Mitruka, B.M. and Rawmsley, H.M. (1977) Clinical, Biochemical and Haematological References in Normal Experimental Animals, Masson Pub. USA Inc., New York
22 Juliano, R.L. (1982) in NATO Adv. Study Inst. Ser., Ser. A 47, 285–300
23 Mok, W., Chen, D. and Abraham, M. (1975) Fed. Proc. 34, 1458–1460
24 De Venuto, F. and Zega, A. (1983) J. Surg. Res. 34, 205–210
25 Iwashita, Y., Iwasaki, K., Ajisaka, K., Ikeda, K. and Uematsu, T. (1984) Prog. Artificial Organs-1983, 867–870
26 Zuck, T.F., De Venuto, F., Neville, T.R. and Friedman, H.I. (1978) in Blood Substitutes and Plasma Expanders (Jamieson, G.A. and Greenwalt, T.J., eds.), pp. 111–1147, Alan R. Liss, New York
27 Gregoriadis, G., Kirby, K., Large., P., Meehan, A. and Senior, J. (1982) in NATO Adv. Study Ser. Inst., Ser. A 47, 155–184
28 Ullberg, S. (1954) Acta Radiol. Suppl. 118, 1–110