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Enhancing effect of cetylmannoside on targeting of liposomes to Kupffer cells in rats

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Summary

In order to evaluate whether surface modification of liposomes by cetylmannoside (Man) could be useful for targeting to Kupffer cells, the effect of Man on disposition of liposomes was examined after intravenous administration to rats. In the case of small unilamellar vesicles (SUV), no difference in disposition was observed between control liposomes (PC-SUV) and modified liposomes (Man-SUV). On the other hand, in the case of multilamellar vesicles (MLV), modified liposomes (Man-MLV) were rapidly eliminated from the circulation, and showed higher accumulation (51.4% of dose) in the liver as compared with control liposomes (PC-MLV, 25.7% of dose). In the spleen, splenic clearance of Man-MLV (0.068 ml/min) was comparable to that of PC-MLV (0.068 ml/min), although Man-MLV showed lower accumulation (5.7% of dose) than PC-MLV (14.7% of dose). This lower accumulation in the spleen of Man-MLV might be due to the low blood concentration caused by the high accumulation in the liver. Thus, it is considered that liposomal size is important in revealing the effects of Man, and Man-MLV is able to enhance only the affinity for the liver. The cellular distribution in the liver of Man-MLV 2 h after intravenous administration to rats gave encouraging evidence that Kupffer cells might be involved in the enhanced hepatic uptake of the liposomes. These results suggest the usefulness of Man-MLV for targeting to Kupffer cells. Furthermore, the involvement of plasma protein(s) in the uptake of Man-MLV is suspected.

Introduction

There is increasing evidence from animal studies that activation of the host mononuclear phagocyte system (MPS) by biological response modifiers (BRM) appears to be important in control of metastasis and/or metastatic tumor growth (Fidler, 1985; Sone, 1986). In cancer therapy by BRM, since they are rapidly cleared from the circulation after parenteral administration (Parant et al., 1979; Fogler et al., 1985), a high dose is necessary to achieve an efficient therapeutic effect. In clinical application, therefore, development of new BRM and novel dosage forms is required for efficient activation of MPS without side-effects.

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Recently, much attention has been paid to the use of liposomes, which are modified by appropriate receptor-recognizing ligands as drug carriers to delivery BRM to MPS in vivo (Ghosh et al., 1982; Barratt et al., 1986, 1987; Muller and Schuber, 1989). For example, the mannose receptor presenting at the surface of Kupffer cells, which recognize the terminal mannose residues (Stahl et al., 1978, 1984), is utilized for targeting liposomes to the cells of MPS. In these attempts, natural glycolipids or synthetic glycolipids have been used as the ligands for surface modification of liposomes. However, the method for incorporation of natural glycolipids into liposomes is severely limited by the fact that naturally occurring glycolipids are difficult to obtain in a highly purified form. Moreover, since synthetic glycosides are covalently coupled with lipids, such as phosphatidylethanolamine or cholesterol, it is difficult to control the coupling reaction with high reproducibility.

In previous work, we observed that long-chain alkyl glycosides formed liposome-like vesicles, and the preparation and physical properties of the vesicles were reported (Kiwada et al., 1985a,b, 1988). The structures of the glycosides are very simple and they are readily synthesized.

In the present study, we examined the effect of Man on hepatic uptake of liposomes, and evaluated the potentiality for targeting to the Kupffer cells by investigating the cellular distribution in the liver of these liposomes after intravenous administration.

Materials and Methods

Materials

Hydrogenated egg phosphatidylcholine (PC) was kindly supplied by Nippon Fine Chemicals Co., Ltd (Osaka, Japan). Dicetyl phosphate (DCP) and cholesterol (CH) were purchased from Nacalai Tesque Ltd (Kyoto, Japan) and Wako Pure Chemical Industries, Ltd (Osaka, Japan), respectively. Cetylmannoside (Man) was synthesized as described in the previous paper (Kiwada et al., 1985a). [^{3}H]Inulin was from New England Nuclear Co. (Boston, U.S.A.). Scintisol EX-H® as a scintillation cocktail was from Dojindo Laboratories (Kumamoto, Japan). Sodium pentobarbital (Nembutal®, Dainabot, Osaka, Japan), Hanks' balanced salt solution (Nissui Pharmaceutical Co., Ltd, Tokyo, Japan), collagenase (Wako Pure Chemical Industries, Ltd, Osaka, Japan) and diaminobenzidine (DAB; Dojindo Laboratories, Kumamoto, Japan) were purchased commercially. All other chemicals were reagent grade or better.

Preparation of liposomes

Liposomes were prepared as described previously (Kiwada et al., 1985). Briefly, appropriate lipids were mixed in chloroform, dried under reduced pressure. The lipids (250 μmol as total lipids) were then hydrated in 5 ml of phosphate-buffered saline (PBS) containing [^{3}H]inulin as an aqueous marker and mechanically vortexed at room temperature. The liposomes were extruded through polycarbonate membranes (Nuclepore Co., CA, U.S.A.) of pore size 0.8 μm. Non-encapsulated [^{3}H]inulin were removed by dialysis with polycarbonate membrane of pore size 0.2 μm against PBS. Small unilamellar vesicles (SUV) were prepared by the sonication method from MLV. The MLV were sonicated in a probe-type sonicator (UR-200P, Tomy Seikou, Tokyo, Japan) until the suspensions became clear for about 1 h. PC liposomes were composed of PC/DCP/CH in a molar ratio of 5 : 1 : 4. Man liposomes were composed of PC/Man/DCP/CH in a molar ratio of 2 : 3 : 1 : 4. Liposomal size was determined by using a laser light scattering instrument (LPA-3000, Otsuka Electronics, Osaka, Japan).

Tissue distribution study

Wistar male rats weighing 200–250 g anesthetized with ether were cannulated in the femoral vein, femoral artery and bladder. Two cannulae were inserted into the bladder, one for sampling and the other for washing. Each operated rat was placed in a Bollman cage. After recovering from the anesthesia, liposomes were injected through the cannula in the femoral vein. The injected volume was 2.5 ml/kg body weight with a dose corresponding to 125 μmol as total lipid/kg body weight. Blood samples were drained from the cannula inserted into the artery at 1.5, 3.0, 6.0, 15.0,

30.0, 60.0, and 120.0 min after injection. Urine samples were collected from the cannula inserted into the bladder at 15.0, 30.0, 60.0 and 120 min after injection. In addition, after the sampling of blood and urine at 120 min, liver, lung, spleen and kidney were subsequently removed. Radioactivities of the samples were counted with a liquid scintillation counter (Aloka LSC-602, Tokyo, Japan) as described previously (Sato et al., 1986).

Pharmacokinetic analysis

The area under the blood concentration-time curve (AUC) and area under the moment curve (AUMC) were calculated using the trapezoidal rule. Total body clearance (CL_{total}) was calculated by dividing injected dose (D) by $AUC_{0\to\infty}$. Hepatic clearance ($CL_{hepatic}$), splenic clearance ($CL_{splenic}$) and renal clearance (CL_{renal}) were calculated by dividing the hepatic uptake, splenic uptake and cumulative urinary excretion amount for 2 h by $AUC_{0\to 2h}$, respectively. Mean residence time (MRT) is calculated by dividing $AUMC_{0\to\infty}$ by $AUC_{0\to\infty}$ (Yamaoka et al., 1978).

Apparent volume of distribution at steady state (Vd_{ss}) is defined as follows.

$$Vd_{ss} = \frac{D \times MRT}{AUC_{0\to\infty}}$$

Fractionation of liver cells

Liposomes (125 μmol/kg) were injected intravenously into the jugular vein of male Wistar rats of 200–250 g body weight, which were anesthetized by an intraperitoneal injection of sodium pentobarbital, 60 mg/kg body weight. Liver cells were dispersed from rat liver according to Shimaoka et al. (1987) with some modifications. The liver was perfused in situ through the portal vein with Ca^{2+}-free Hanks' balanced salt solution containing 5 mM EGTA at 37 °C for 5 min and then perfused with 0.05% collagenase solution for about 10 min. The perfused liver was excised and dispersed in Hanks' solution and filtered through a double layer of stainless steel mesh (160 mesh). The liver cells were separated into four subfractions by differential centrifugation. First, the liver cells were centrifuged at $40 \times g$ for 1 min. The precipitated cells were washed twice with Hanks' solution and used as fraction 1. The supernatant obtained by the first centrifugation and the washing solution were combined and centrifuged at $70 \times g$ for 1 min. The precipitated cells were washed twice with Hanks' solution and used as fraction 2. Similarly, fractions 3 and 4 were separated by centrifugation at $140 \times g$ and $850 \times g$ for 1 min and 5 min, respectively. The viabilities of parenchymal and non-parenchymal cells were examined by trypan-blue exclusion test, and were more than 90%. Parenchymal and non-parenchymal cells were distinguished by their morphological differences. Furthermore, Kupffer cells and endothelial cells were distinguished by their peroxidase activities. For the enzyme assays, cells were suspended in 5 ml of 0.05 M Tris-HCl, pH 7.4, containing 7% (w/v) sucrose and 5 mg DAB. The reaction was started by adding 5 μl of 30% (v/v) H_2O_2. After incubation for 30 min at 37 °C, cells were centrifuged at $300 \times g$ for 5 min and examined by optical microscopy. Peroxidase-positive Kupffer cells were brownish in color, while endothelial cells were negative. Cell numbers were counted in a Burker-Turk chamber. The radioactivity in cell preparation was measured as follows. 0.2 ml of 30% (v/v) H_2O_2 and 0.2 ml of 2 N KOH in isopropanol were added to 0.5 ml of cell preparation in a liquid scintillation vial. The mixture was stirred gently but sufficiently, then left at room temperature overnight. 10 ml of scintillation cocktail (Scintisol EX-H®) was added, and after mixing, 1 ml of 10% (v/v) acetic acid was added for neutralization. The mixture was allowed to stand at room temperature for at least 3 h to dissipate chemiluminescence, and the radioactivity was counted with a liquid scintillation counter.

Statistical analysis

Statistical significance was evaluated by means of Student's t-test and $p = 0.05$ was taken as the minimal level of significance. Data were expressed as the mean ± S.E.

Results and Discussion

Characteristics of liposomes

The mean diameters and encapsulation ratios of the liposomes used in this study are sum-

TABLE 1

Characteristics of liposomes

Lipid compositions [a] (PC : Man : DCP : CH)	Mean diameters [b] (nm)	Encapsulation ratio of [^{3}H]inulin (%)
PC-MLV (5 : 0 : 1 : 4)	793 ± 31	20.3 ± 1.2
Man-MLV (2 : 3 : 1 : 4)	828 ± 53	27.1 ± 0.9 [c]
PC-SUV (5 : 0 : 1 : 4)	73 ± 4	2.3 ± 0.3
Man-SUV (2 : 3 : 1 : 4)	68 ± 4	1.9 ± 0.3

[a] Molar ratio.

[b] Liposome size was determined by dynamic laser light scattering instrument.

[c] Significant difference from PC-MLV at $p < 0.01$. Each value represents the mean ± S.E. of three experiments.

marized in Table 1. The mean diameter of MLV surface-modified by Man was almost equal in comparison with PC-MLV because of sizing by the extrusion technique. The encapsulation ratio of [^{3}H]inulin encapsulated in Man-MLV was greater than that in PC-MLV as reported previously (Barratt et al., 1986). For this reason, it is speculated that the bulky hydrophilic head groups of Man result in larger inter-bilayer spaces than those of PC. On the other hand, the mean diameter and encapsulation ratio of Man-SUV were almost equal as compared with those of PC-SUV.

Effect of Man on blood elimination of liposomes

After intravenous administration of liposomes containing [^{3}H]inulin as an aqueous marker to rats, the blood elimination of liposomes of both MLV and SUV was determined as shown in Fig. 1. As reported previously (Sato et al., 1986), free inulin was eliminated very rapidly from the blood and excreted into the urine. Over 90% of injected dose was excreted within 2 h after injection. Therefore, the radioactivity observed in the blood represents intact liposomes in the blood. The blood elimination of Man-SUV from the circulation was almost equal to that of PC-SUV, and was remarkably slower than those of both PC-MLV and Man-MLV. Man-MLV was eliminated from the circulation more rapidly than PC-MLV. This rapid blood elimination of Man-MLV is as a result of instability in vivo and/or enhanced hepatic uptake. Therefore, it is considered that liposomal size is important to reveal the effects of Man.

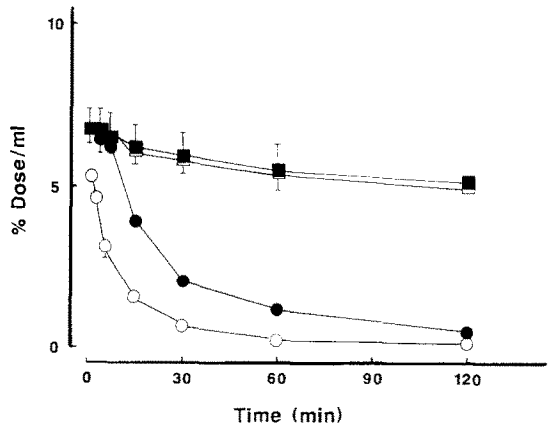

Fig. 1. Time courses of blood concentrations of [^{3}H]inulin as an aqueous marker of liposomes after intravenous administration to rats. Liposomes were injected at a dose of 125 μmol/kg body weight. Each value represents the mean ± S.E. of three rats. (●) PC-MLV, (○) Man-MLV, (■) PC-SUV, (□) Man-SUV.

Effect of Man on urinary excretion of [^{3}H]inulin

As shown in Fig. 2, the urinary excretion of [^{3}H]inulin after injection of PC-SUV and Man-SUV was almost equal, and the amounts for 2 h of [^{3}H]inulin were 5.5 and 6.3% of dose, respectively. The radioactivity observed in the urine represents inulin that has leaked from liposomes and the excretion rate reflects the rate of the leakage in

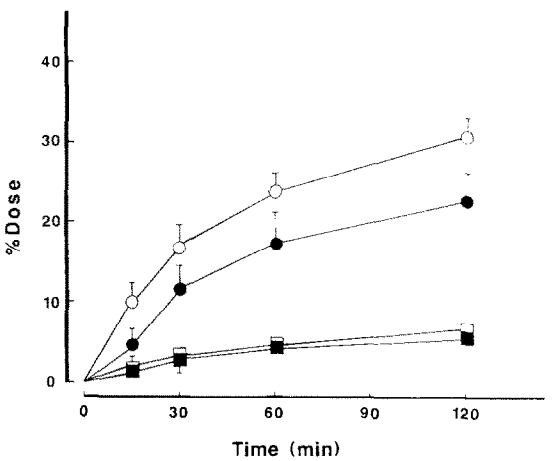

Fig. 2. Time courses of cumulative urinary excretions of [^{3}H]inulin as an aqueous marker of liposomes after intravenous administration to rats. Liposomes were injected at a dose of 125 μmol/kg body weight. Each value represents the mean ± S.E. of three rats. Symbols are the same as those in Fig. 1.

vivo, because [^{3}H]inulin encapsulated in liposomes was not excreted by glomerular filtration. Therefore, both PC-SUV and Man-SUV were stable in vivo. In the case of MLV, the urinary excretion amounts of [^{3}H]inulin 30 min after injection of PC-MLV and Man-MLV were 11.6 and 16.5%, respectively. On the other hand, blood concentrations of PC-MLV and Man-MLV observed in the blood 30 min after injection were 2.03 and 0.65% dose/ml, respectively. If blood volume is estimated as 7.7% of body weight (250 g) according to Allen et al. (1988), the remaining amounts of PC-MLV and Man-MLV in the blood are calculated as 39.1 and 12.5% of dose, respectively. Therefore, the rapid blood elimination of Man-MLV mentioned above cannot be explained only by the difference in urinary excretion of [^{3}H]inulin after injection of the liposomes.

Effect of Man on tissue distribution of liposomes

The tissue distribution of MLV (panel A) and SUV (panel B) 2 h after intravenous administration is shown in Fig. 3. Man-MLV showed higher accumulation (51.4% of dose) in the liver as compared with PC-MLV (25.7% of dose). Thus, the rapid blood elimination of Man-MLV is considered to be due to the higher accumulation in the liver. In the spleen, Man-MLV showed lower accumulation (5.7% of dose) than PC-MLV (14.7% of dose). In the lung and kidney, both PC-MLV and Man-MLV showed very low distribution. On the other hand, there was no difference in tissue distribution observed between Man-SUV and PC-SUV. As described by Darnell et al. (1990), the macromolecules and particles are internalized by endocytosis (pinocytosis or receptor-mediated endocytosis) or phagocytosis. Intracellular membrane-limited vesicles about 0.1 μm in diameter were formed by endocytosis, whereas they were about 1–2 μm or larger by phagocytosis. Therefore, it is considered that SUV having a mean diameter of about 0.07 μm is mainly internalized by endocytosis, and MLV having a mean diameter of about 0.8 μm is internalized predominantly by phagocytosis. Since the enhancing effect of Man on hepatic uptake of liposomes is observed only in the case of MLV, this suggests that the effect of

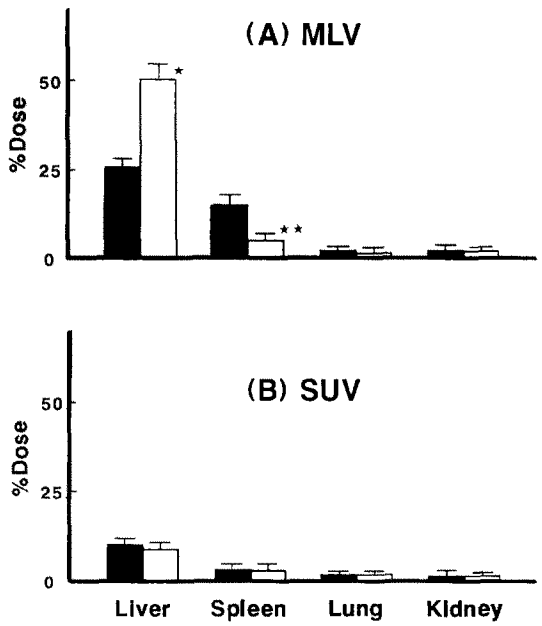

Fig. 3. Tissue distribution of [^{3}H]inulin encapsulated in MLV (A) and SUV (B) 2 h after intravenous administration to rats. Liposomes were injected at a dose of 125 μmol/kg body weight. Each value represents the mean ± S.E. of three rats. Significant differences from PC-MLV; * $p < 0.05$, ** $p < 0.01$. (■) PC liposomes, (□) Man liposomes.

Man might be mainly revealed effectively in the phagocytosis process.

Pharmacokinetic parameters of PC-MLV and Man-MLV

The affinities for liver and spleen are not always suitable for an exact discussion by comparison of the accumulation in the liver and spleen, respectively. Therefore, pharmacokinetic parameters were calculated and shown in Table 2. Both $AUC_{0\rightarrow 2h}$ and $AUC_{0\rightarrow\infty}$ of PC-MLV were about 3-fold higher than that of Man-MLV. In addition, the total clearance of Man-MLV (1.113 ml/min) was higher than that of PC-MLV (0.429 ml/min). MRT of Man-MLV (34.24 min) was a little shorter than that of PC-MLV (43.58 min). The apparent volume of distribution at steady state (Vd_{ss}) of Man-MLV (38.22 ml) was about 2-fold higher than that of PC-MLV (18.30 ml), which was about 2-times the whole blood volume. The enhancement of Vd_{ss} of Man-MLV indicates the existence

TABLE 2

Pharmacokinetic parameters of PC-MLV and Man-MLV

Parameters	PC-MLV	Man-MLV
$AUC_{0 \rightarrow 2h}$ (%dose min ml^{-1})	218.34 ±23.49	83.69 ±4.84 [a]
$AUC_{0 \rightarrow \infty}$ (%dose min ml^{-1})	239.91 ±30.48	90.32 ±4.62 [a]
MRT (min)	43.58 ±4.31	34.24 ±2.65
Vd_{ss} (ml)	18.30 ±0.68	38.22 ±4.21 [a]
CL_{total} (ml min^{-1})	0.429±0.005	1.113±0.057 [a]
$CL_{hepatic}$ (ml min^{-1})	0.135±0.008	0.619±0.080 [a]
$CL_{splenic}$ (ml min^{-1})	0.068±0.010	0.068±0.007
CL_{renal} (ml min^{-1})	0.104±0.002	0.367±0.053 [a]
CL_{other} (ml min^{-1})	0.123±0.042	0.059±0.082

[a] Significant difference from PC-MLV at $p < 0.01$. Each value represents the mean ± S.E. of three rats.

of reversible binding of the liposomes with liver cells. The hepatic clearance of Man-MLV (0.619 ml/min) was about 4.5-fold higher than that of PC-MLV (0.135 ml/min), although hepatic uptake of Man-MLV was enhanced only about 1.5-fold as compared with that of PC-MLV (Fig. 3). In addition, the renal clearance of Man-MLV (0.367 ml/min) was higher than that of PC-MLV (0.104 ml/min). These results indicate that Man is not able to enhance the hepatic uptake to a degree comparable with that of the affinity for the liver, because it is more unstable in vivo than PC-MLV. In the spleen, splenic clearance of Man-MLV (0.068 ml/min) was comparable to that of PC-MLV (0.068 ml/min), although Man-MLV showed lower accumulation than PC-MLV as indicated in Fig. 3. The lower accumulation in the spleen of Man-MLV is not due to the low affinity for the spleen of Man-MLV but, rather, to the low blood concentration caused by the high accumulation in the liver. Although macrophages in the spleen probably have mannose receptors on the surface, Man-MLV was unable to enhance the splenic uptake. These results suggest that Man-MLV is useful for targeting to Kupffer cells, and raise the possibility that Man-MLV might not be taken up by mannose receptor-mediated endocytosis. Furthermore, the mechanism of Man-MLV uptake by Kupffer cells might be different from that by splenic macrophages. Moghimi and Patel (1989) have reported the existence of organ-specific opsonins, which are liver-specific and spleen-specific. Therefore, the possibility that Man-MLV might be taken up by Kupffer cells via liver-specific opsonin(s) in serum is considered.

Effect of Man on cellular distribution in the liver of MLV

As mentioned above, we demonstrated that Man-MLV was taken up by liver to a greater extent than PC-MLV. However, it is not clear what kind of cells in the liver contribute to the enhancement of hepatic uptake of Man-MLV. We investigated the cellular distribution in the liver of Man-MLV 2 h after intravenous administration to rats. First, the liver cells were fractionated into four subfractions by differential centrifugation, since it is difficult to separate completely parenchymal and non-parenchymal cells with high recovery. Parenchymal and non-parenchymal cells were distinguished by their morphological differences, such as cell size. Fractions 1 and 2 consisted of parenchymal cells (hepatocytes), which contained more than 80% of total cell numbers. Fractions 3 and 4 mainly consisted of non-parenchymal cells, which contained about 67 and 98% of total cell numbers, respectively. In fraction 1, consisting mainly of hepatocytes, uptake of MLV was not observed to be very large as shown in Fig. 4. This result indicates that parenchymal cells are not closely involved in the uptake of MLV. In fractions 3 and 4, consisting mainly of non-parenchymal cells, Man-MLV was taken up more than PC-MLV. Therefore, it is apparent that the enhancement of hepatic uptake of Man-MLV is due to the contribution of the non-parenchymal cells in fractions 3 and 4.

To ascertain the contribution of Kupffer cells to hepatic uptake, we examined the cell population of four subfractions. As described by Roerdink et al. (1981), Kupffer cells and endothelial cells, which are non-parenchymal cells, could be easily discriminated by their difference in peroxidase content. Namely, Kupffer cells are peroxidase-positive cells, while endothelial cells are peroxidase-negative cells. As shown in Fig. 5, fraction 3 consisted of hepatocytes (33%), endothelial cells (44%) and Kupffer cells (23%) (percentage of

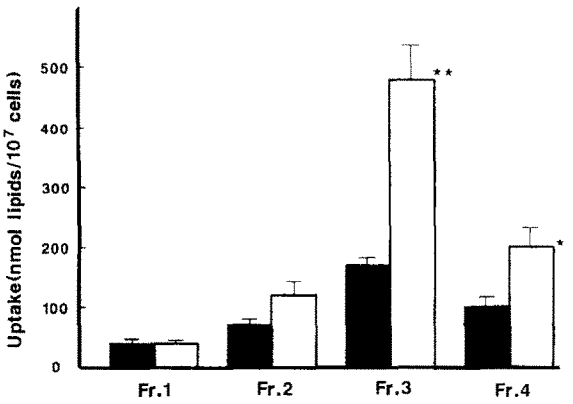

Fig. 4. Effect of Man on cellular distribution in the liver of MLV 2 h after intravenous administration to rats. Liposomes were injected at a dose of 125 μmol/kg body weight. Fraction 1 (Fr. 1), fraction 2 (Fr. 2), fraction 3 (Fr. 3) and fraction 4 (Fr. 4) were centrifuged at $40\times g$, $70\times g$, $140\times g$ and $850\times g$, respectively. Each value represents the mean ± S.E. of three rats. Significant difference from PC-MLV; * $p < 0.05$, ** $p < 0.01$. (■) PC-MLV, (□) Man-MLV.

total cell numbers). Fraction 4 consisted mainly of endothelial cells (79%), with some Kupffer cells (19%). The relationships between uptake of Man-MLV and cell populations of liver cells were analyzed by multiple regression (Data Desk Professional, Odesta Corp., U.S.A.). If the uptake of Man-MLV by liver cells contributes to Kupffer cells, the correlation coefficient is extremely high ($r^2 = 0.991$), compared with that in the case of

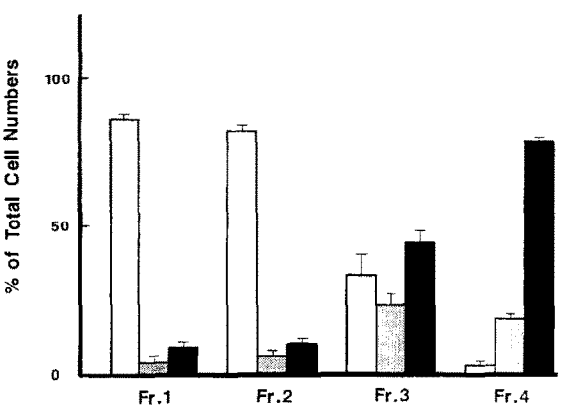

Fig. 5. Cell population of four subfractions by differential centrifugation of live cells. Each value represents the mean ± S.E. of six rats. (□) Hepatocytes, (▨) Kupffer cells, (■) endothelial cells.

other cells. This result suggests that the uptake of Man-MLV mainly contributes to Kupffer cells, but not endothelial cells. These observations are consistent with those of Roerdink et al. (1981) who reported that large liposomes were not taken up by endothelial cells, but preferentially taken up by Kupffer cells, and taken up only to a limited extent by hepatocytes. Although Magnusson and Berg (1989) have reported the existence of mannose receptors in sinusoidal endothelial cells, we could not obtain clear evidence that endothelial cells are involved in the uptake of Man-MLV. This result raises the possibility that Man-MLV is taken up by a different mechanism except for mannose receptor-mediated endocytosis. Although the percentages of Kupffer cells are not greatly different between fractions 3 and 4, there is a large difference between them in uptake of Man-MLV. As one of the reasons for this discrepancy, the existence of the heterogeneity of Kupffer cells is considered as reported by Daemen et al. (1989). They have demonstrated that Kupffer cells fractionated according to size into five subfractions showed different liposome uptake. Recently, much attention has been paid to the mannose-binding protein (MBP) in serum, which plays a key role in host defense against some microorganisms by recognizing certain configurations of mannose oligosaccharides (Ezekowitz and Stahl, 1988; Ezekowitz et al., 1988; Schweinle et al., 1989; Ohta et al., 1990). MBP shares similar binding characteristics and cross-reactive epitopes with the mannose receptor on the cell surface of MPS, and has functions as an opsonin (Kuhlman et al., 1989; Super et al., 1990). Although the mechanism of uptake of Man-MLV by Kupffer cells has not yet been clarified, Man-MLV may possibly be taken up by MBP-mediated phagocytosis rather than mannose receptor-mediated endocytosis. The involvement of MBP in hepatic uptake of Man-MLV is currently being investigated.

It is known that MBP has an analogous structure to the first complement component, C1q (Ezekowitz and Stahl, 1988), and activates the complement system via the classical (Ohta et al., 1990) or alternative (Schweinle et al., 1989) pathway. Taking into account the involvement of MBP in the complement system, it is possible that the

plasma protein interacts with the complement receptor or its analog, directly and/or indirectly. Moreover, Juliano (1988) has reported that phagocytosis by splenic macrophages was mainly mediated by the Fc receptor, whereas that for Kupffer cells was by the complement receptor. In this study, we have demonstrated that the surface-modification of liposomes by Man enhances hepatic clearance, but not splenic clearance. Therefore, it is hypothesized that Man-MLV is opsonized by MBP and preferentially taken up by Kupffer cells rather than splenic macrophages via the receptor for complement receptor or its analog.

In conclusion, the enhancing effect of Man on hepatic uptake of liposomes was observed to be marked, but only in the case of MLV. The affinity for the spleen was not enhanced by the surface modification by Man. It was suspected that the enhancement of hepatic uptake of Man-MLV mainly involved Kupffer cells, which mediated by opsonization with plasma protein(s). Man-MLV is expected to be a useful drug carrier to activate Kupffer cells selectively and efficiently by BRM.

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