Vol. 110, No. 1, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 14, 1983 Pages 140-146

Alteration of Liposome Disposition *in vivo* by Bilayer Situated Carbohydrates

F. C. Szoka, Jr.[1] and E. Mayhew[2]

1. Department of Pharmacy and Pharmaceutical Chemistry
University of California at San Francisco
San Francisco, CA 94143

2. Department of Experimental Pathology, Roswell Park
Memorial Institute, Buffalo, NY 14263

Received November 29, 1982

Summary: The inclusion of either lactosylcerebroside or dimannosyldiglyceride at a 9% molar ratio in small unilamellar vesicles increased by two-three fold the fraction of the I.V. dose that appeared in mouse liver. For lactosylcerebroside containing liposomes, the half-time for clearance from plasma was 1.2 hours compared to 5.5 hours for liposomes of similar size, charge, and composition but lacking the glycolipid. Uptake of the lactosylcerebroside containing liposomes by the liver could be significantly reduced but not eliminated by the simultaneous injection of asialoorosomucid.

The importance of specificity and selectivity for any therapeutic agent has long been recognized as a prerequisite for its successful use against a disease state. One approach to achieve such specificity is to use drug carriers to deliver the drug to its site of action. The liposome is one carrier that has been proposed as a suitable site-directed carrier (1).

Attempts to alter the *in vivo* disposition of liposomes by incorporating carbohydrates on the liposome surface as ligands for directing liposomes to the endogenous receptors ((2, 3) in

Abbreviations: Dimannosyldiglyceride, DMG; Dipalmitoyl iodophydroxybenzamidinephosphatidylethanolamine, BPE; Dipalmitoylphosphatidylcholine, DPPC; Dipalmitoylphosphatidylglycerol, DPPG; Cholesterol, chol; Palmitoyldihydrolactosylcerebroside, LC; phosphate buffered saline, PBS; small unilamellar vesicles, SUV.

0006-291X/83/010140-07$01.50/0

the liver have yielded ambiguous results (4). Recently, Ghosh et al., 1982 (5) have demonstrated that liposomes containing asialogangliosides or that have been covalently modified by the addition of p-aminophenolglycosides to a preformed liposome were able to increase the rate of uptake of liposomes into the liver. In these studies multilamellar liposomes of a heterogeneous size distribution were used. These are primarily taken up by the liver (6) and thus the effect of the surface modifications on the extent of uptake could not be determined. Herein we report that small unilamellar vesicles containing glycolipoids with either a terminal beta-galactose or alpha-D mannose residue increase both the rate of removal of the liposome from circulation and the rate and extent of uptake into the liver.

MATERIALS AND METHODS

Dipalmitoylphosphatidylcholine, dipalmitoylphosphatidylglycerol were prepared as previously described (7, 8) Cholesterol was from A.B. Fluka (Bach, Switzerland) and was recrystallized from methanol twice. Dipalmitoyliodophydroxybenzamidinephosphotidylethanolamine was synthesized as previously described (9). Dimannosyldiglyceride was isolated from micrococcus luteus as described (10). Dipalmitoyldihydrolactosylcerebroside was purchased from Sigma. Phosphate-buffered saline had the following composition: 137 mM NaCl, 2.6 mM KCl, 6.4 mM Na_2HPO_4, 1.4 mM KH_2PO_4. Asialoorosomucoid was a generous gift of Dr. D. Doyle, Roswell Park Memorial Institute.

Small unilamellar vesicles were formed from multilamellar lipid vesicles (11) by sonicating 20 umoles lipid/ml of PBS in a sealed vessel under nitrogen with an 80 watt Bath type sonicator (lab supply, Hicksville, NY) for 1 hour at 45°C for the lactosylcerebroside and nonglycolipid containing liposomes and 61°C for the dimannosyldiglycoside containing liposome. After sonication, the preparations were allowed to stand at the sonication temperature for 30 min and then were centrifuged at 100,000 X G for 90 min to remove large liposomes. The supernatants which contained >90% of the initial radioactivity were dialyzed against 100 volumes of PBS at 4°C which was changed 3 times over an 18 hr period. The following lipid compositions were used in these experiments: DPPG/DPPC/chol : 2/8/8 (molar ratio); LC/DPPG/DPPC/chol : 2/2/8/10; DMG/DPPG/DPPC/chol : 2/2/8/10. The BPE was present in the liposomes at less than 0.1 molar ratio and at a specific activity of 1×10^5 cpm per umole total lipid. The size distribution of the SUV was determined as previously described (12). To examine the effect of plasma factors on the size distribution of the liposomes, 1 umole of lipid was incu-

bated in 12 ml of 80% fetal calf serum in PBS at 37°C for 1 hr. After incubation, the sample was chromatographed on a 1 x 25 cm Biogel A1.5 m column eluted with 0.15 m ammonium acetate pH7.4 to separate liposomes from plasma proteins. The recovery of the lipid label from the column was 96.4% and the fractions containing the liposomes were pooled and examined by negative stain electron microscopy.

Healthy female Swiss Webster mice, 20-25 gm, were injected via the tail vein with 2.7 umol of lipid containing ^{125}I BPE in a volume of 0.2-0.3 ml. Groups of 3 or 4 animals were sacrificed at the indicated times by chloroform anaesthesia and 1.0 ml blood sample rapidly removed from the heart with a heparinized syringe. The indicated tissues were quickly removed, rinsed in PBS, weighed, and counted in a Beckmann gamma counter. Counts were corrected for liposomes in the blood remaining in the tissue. Studies with the lactosylcerebroside containing liposomes and the nonglycolipid containing liposomes were repeated three times. Studies with the diaminosyldiglyceride were repeated two times.

RESULTS AND DISCUSSION

Previous studies that have demonstrated an altered distribution of liposomes containing surface situated carbohydrate ligands have used multilamellar vesicles which have poorly characterized size distributions (11).

Thus, the effect of liposome size has made it difficult to determine the effect of the carbohydrate on altering the distribution. We have approached the problem by preparing SUV with a well defined size distribution and surface ligand density. The number average diameter of the various liposome preparations computed from negative stain electronmicrographs are listed in Table I. These liposomes have a diameter of about 500 Å that is unaffected by a 1 hr incubation with serum at 37°C. Thus, we believe that the observed differences in distribution shown in Table II, particularly the increased uptake in the liver, are due to the presence of the carbohydrate sequences at the liposome surface. Increased uptake of liposomes containing glycolipids by the liver results in decreased levels of liposome accumulation by other tissues, except in the case of the DMG SUV containing liposomes where slight elevations compared to the DPPG/DPPG/chol SUV are

TABLE I

Number Average Diameter (Å) of Liposome Preparations

Composition	After Preparation Diameter ± S.D.	n	After Incubation With Serum Diameter ± S.D.	n
DPPG/DPPC/chol	530 ± 181	152	546 ± 126	126
LC/DPPG/DPPC/chol	503 ± 172	374	526 ± 167	154
DMG/DPPG/DPPC/chol	496 ± 147	299	not done	

seen in the spleen and femur (Table II). This may be due to the presence of cell types, i.e. macrophages, with surface mannosyl receptors in these organs. The distribution for the

TABLE II

Disposition of Liposomes in Mice 1 hr post injection*

Tissue	Liposome Composition DPPG/DPPC/chol	LC/DPPG/DPPC/chol	DMG/DPPG/DPPC/chol
Liver	20.9 ± 1.1	46.6 ± 2.9	39.4 ± 2.6
Spleen	1.7 ± 0.3	0.9 ± 0.1	2.4 ± .05
Lung	1.7 ± 0.2	1.0 ± 0.1	0.8 ± 0.1
Brain	0.2 ± 0.05	0.1 ± 0.03	0.1 ± .02
Small Intestine	2.1 ± 0.3	1.5 ± 0.2	1.8 ± 0.4
Heart	0.8 ± 0.3	0.3 ± 0.1	0.4 ± 0.4
Kidneys	2.1 ± 0.2	1.5 ± 0.5	1.2 ± 0.08
Stomach	0.2 ± 0.02	0.1 ± 0.03	0.6 ± 0.03
Ovaries	0.02 ± 0.01	0.04 ± 0.02	0.06 ± 0.03
Thymus	0.3 ± 0.03	0.2 ± 0.05	0.1 ± 0.06
Lymph Node	0.3 ± 0.04	0.2 ± 0.03	0.3 ± 0.1
Rt Femur	0.5 ± 0.07	0.4 ± 0.08	0.7 ± 0.1
Plasma	32.6 ± 3.48	23.4 ± 11.8	18.1 ± 2.6

*Percent injected dose per tissue except plasma which is % dose per ml plasma.

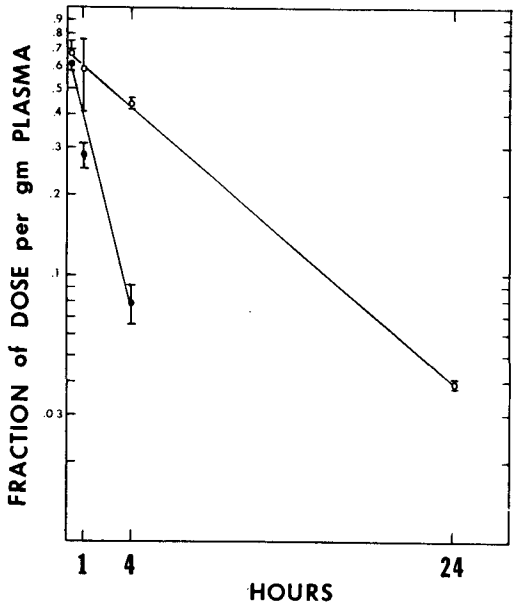

Fig. 1. Clearance of Liposomes from the circulaton. (●), LC/DPPG/DPPC/chol SUV; (o) DPPG/DPPC/chol SUV; mean ± S.D., n=4

DPPG/DPPC/chol SUV are in close agreement to published values for small unilamellar vesicles with a negative surface charge (6).

The disappearance of both DPPG/DPPC/chol and LC SUV from plasma appears to follow first order kinetics (Figure 1). The half-time for disappearance of the DPPG/DPPC/chol SUV is 5.5 hrs, while the LC SUV are removed with a half-life of 1.2 hrs. The liver uptake of the DPPG/DPPC/chol SUV plateaus after 1 hr whereas the uptake of the LC SUV continues to increase up to four hours (Figure 2). Total recovery of label from both types of SUV was 87% at 1 hr, 80% at 4 hrs, and 50% at 24 hrs. A similar loss of iodinated label from the animal was observed when multilamellar vesicles were used (9). The appearance of the LC SUV in the spleen peaks at 1 hr and declines over the next 23 hours as compared to the DPPG/DPPC/chol SUV which continued to increase over the 24 hour time period (Figure 3). At the end of the 24 hours the DPPG/DPPC/chol SUV had accumulated to a four-fold

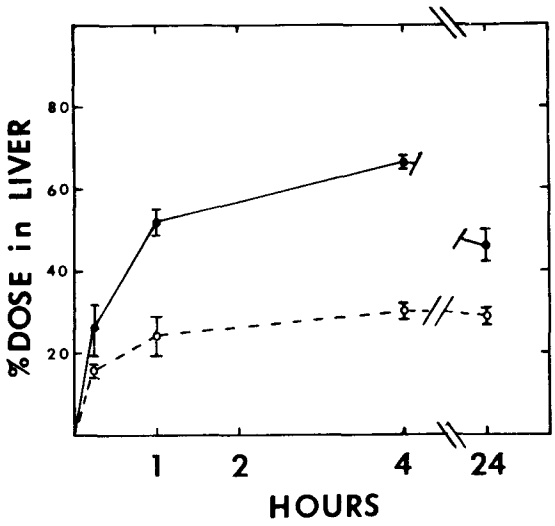

Fig. 2. Accumulation of Liposomes in the liver. (●), LC/DPPG/DPPC/chol SUV; (o) DPPG/DPPC/chol SUV; mean ± S.D., n=4

greater extent than the LC SUV. Although only 3% of the total dose of DPPG/DPPC/chol SUV was found in the spleen on a per gram of tissue basis, this is twice as high as the dose in the liver.

The simultaneous injection of asialoorosomucid at 1 mg/mouse with the LC liposomes reduced the fraction of the dose in the

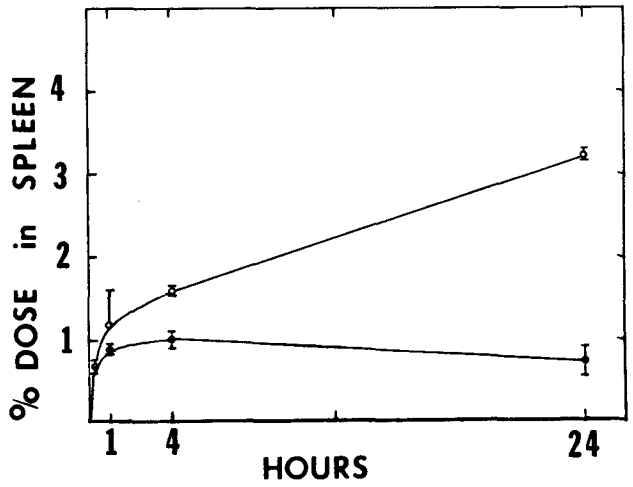

Fig. 3. Accumulation of Liposomes in the spleen. (●), LC/DPPG/DPPC/chol SUV; (o) DPPG/DPPC/chol SUV; mean ± S.D., n=4

liver from 46.0 ± 2.3% to 27.6 ± 2.7% (n=3), which is significant at the 0.001 level using the Student's T test.

Previous attempts to target liposomes by the inclusion of carbohydrate containing ligand have produced conflicting results (4, 5, 13, 14, 15, 16). The results herein show that appropriate carbohydrates on the surface of SUV enhance both the rate and extent of SUV uptake into the liver. We are currently determining if this enhanced rate of uptake is associated with an enhanced delivery of vesicle contents into liver cells.

ACKNOWLEDGMENTS

We gratefully acknowledge helpful discussions with members of the Drug Delivery Research Group of the Department of Pharmacy, UCSF. This research was supported by NIH grant GM 30163-01.

REFERENCES

1. Gregoriadis, G.(1977) Nature 265, 407-411.
2. Ashwell, G. and Morell, A.G. (1974) Adv.Enzymol. 41, 99-128.
3. Achord, D.T., Brot, F.E., and Sly, W.S. (1977) Biochem. Biophys. Res. Commun. 77,409-417.
4. Ghosh, P. and Bachhawat, B.K. (1980) Biochim. Biophys. Acta 632, 562-572.
5. Ghosh, P., Das, P.K., and Bachhawat, B.K. (1982) Arch. Biochem. Biophys. 213, 266-269.
6. Kimelberg, H.K. and Mayhew, E.G. (1978) Critical Rev. Toxicol. 6, 25-78.
7. Papahadjopoulos, D., Cowden, M., and Kimelberg, H.K. (1973) Biochem. Biophys. Acta 330,8-26.
8. Mayhew, E., Rustum, Y.M., Szoka, F.C., and Papahadjopoulos, D. (1979) Cancer Treatment Reports 63, 1923-1928.
9. Abra, R.M., Schreier, H., and Szoka, F.C. (1982) Res. Commun. Chem. Pathol. Pharmacol. 37, 199-213.
10. Lennarz, W.J. and Talamo, B. (1966) J. Biol. Chem. 241,2707-2719.
11. Szoka, F.C. and Papahadjopoulos, D. (1980) Ann. Rev. Biophys. Bioeng. 9, 467-508.
12. Olson, F.C., Hunt, C.A., Szoka, F.C., Vail, W.J., and Papahadjopoulos, D. (1979) Biochim. Biophys. Acta 557,9-23.
13. Gregoriadis, G. and Neerunjun, D.E. (1974) Eur. J. Biochem. 47,179-185.
14. Surolia, A. and Bachhawat, B.K. (1977) Biochim. Biophys. Acta 497,760-765.
15. Jonah, M.M., Cerny, E.A., and Rahman, Y.E. (1978) Biochim. Biophys. Acta 541,321-333.
16. Mauk, M. and Gamble, R.C. (1979) Proc. Natl. Acad. Sci. USA 76,765-769.