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IN VITRO INTERACTION OF SIZED AND UNSIZED LIPOSOME VESICLES WITH HIGH DENSITY LIPO PROTEINS

By

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ABSTRACT

An in vitro method of interaction of liposome vesicles with high density lipoproteins (HDL) was studied. A formulation of lipids was hydrated, and homogenized with a high shear mixer and designed as "unsized" liposomes. A portion of this preparation was further processed by passing through a polycarbonate membrane and it is labelled as "sized". Both these preparations were incubated with HDL. Destabilization rate of liposome preparations in presence of HDL was determined. Time course of drug release from sized and unsized liposomes was followed upon HDL addition to liposomes. These in vitro studies clearly showed that HDL destabilized the small sized liposomes (0.25 ± 0.09 μm). The destabilization effect of HDL on unsized is not significant.

INTRODUCTION

Liposomes are formed by mechanical dispersion of dried lipids in an aqueous media (1). Destabilization of liposomes in the presence of blood has been attributed to the removal of phospholipid from the bilayers by plasma high density lipoproteins, HDL (2,3,4). Phospholipid loss from the liposome bilayers leads to the formation of pores and leakage (5). The effect of HDL on survival time of small unilamellar vesicles (SUV) prepared with neutral lipids has been shown (4,5).

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The liposome drug delivery system could render drugs to the biological system while reducing the toxic effects. The entrapped drugs are expected to remain encapsulated by the phospholipid bi-layers and slowly difuse out of bi-layers. Contrary to this desired expectation, liposomal formulation under study, leaks in presence of plasma and/or HDL.

In this study we described the preparation of "sized" and "unsized" liposomes and demonstrated the effect of human plasma HDL on sized and unsized liposomes.

EXPERIMENTAL

The lipids used in these studies were egg phosphatidyl glycerol, 95% (EPG) (Avanti Polar Lipids, Inc., Birmingham, AL) egg phosphatidyl choline, 95% (EPC) (Asahi Chemical Industry Company, Ltd., Japan) cholesterol (Chol) (Croda, Inc., Mill Hall, PA) and dl-alpha tocopherol (E) (Sigma Chemicals, St. Louis, MO) in the weight ratio of (EPC:EPG:Chol:E) 5:1:2:0·06. All constituents were dissolved in sufficient amount of chloroform. After evaporation of organic solvent under reduced pressure, the residue was slowly hydrated with aqueous solution of a drug. Metaproterenol sulfate (supplied by Luscochimica, S.p.A., Lomagna, Italy) was selected as a model drug for this work. The hydrated liposomes were homogenized with high shear mixer (Charles Ross & Son Co., Hauppauge, NY). The liposomes, 1 to 3.2 μm designed as "unsized" were collected for the release study. The liposomes, 0.15 to .34 μm, designated as "sized" were further processed by passing through 0.2 micron polycarbonate membrane (6).

Both sized and unsized liposomes were twice washed. Washing procedure is as follows: 2 g of liposome paste was mixed with 4 g of normal saline. One gram of diluted liposome suspension was weighed into each of six centrifuge tubes and spun down at 100,000 rpm, at 4°C for 30 minutes (ultracentrifuge, Model TL100, Beckman Instruments Inc., Palo Alto, CA). The supernatant and pellet were separated by decantation. The supernatant was discarded. 700 microliters of normal saline were added to the contents of each centrifuge tube and the pellet was resuspended. These tubes were then spun down again, washed again, and spun down again. The final supernatants were also discarded. The resultant pellet in the centrifuge tube is two times "washed liposomes". The encapsulation (percent trapped vs. total drug) of two times washed liposomes is greater than 90%.

Assay of metaproterenol sulfate was performed by using high performance liquid chromatography. Details of assay and percent encapsulation determination were described elsewhere (7) in detail. However, a brief note of the quantification of drug encapsulation is given here. Liposomal suspension was centrifuged at a high speed (100,000 rpm) to separate supernatant from the pellet. The supernatant and pellet were separated by decantation. Pellet was reconstituted and solubilized the liposomes in Triton-x-100 (a detergent) solution to render a clear solution. This final clear solution and supernatant (collected earlier) were analyzed by HPLC. A Whatman C-18 ODS column, a UV detector set at 278 nm, and a mobile phase consisted of 70% phosphate buffer (pH 7.0) and methanol 30% with a flow rate of 1 ml/min were the conditions of HPLC.

Known quantity of washed liposome paste was taken into centrifuge tubes. These tubes were then divided into two groups. One group was kept in 37°C. Either 0, 2, 4, or 8 mg of HDL (10 mg/ml) was added to each of the tubes. Adjusted the final volume in each tube to 1.2 ml with normal saline and incubated all tubes for 30 minutes at 37°C or 4°C. The incubation tubes that received no HDL served as controls. At the end of 30-minute incubation, all tubes were spun down to separate the liposome pellet from supernatant. The percent trapped drug in liposomes after incubation was determined.

Destabilization rate of liposomes in presence of HDL was determined. About 1 g of washed liposome paste was diluted with normal saline to make a total of 25 grams. Several vials were filled with 1 ml portions of the diluted liposomes, tightly sealed and allowed them to reach 37°C in a water bath. Normal saline and HDL were also brought to the 37°C by placing them in the water bath. 200 microliters of HDL (10 mg/ml) was added to each of the reaction tubes while the controls received 200 microliters of normal saline. Both sized and unsized liposomes were involved in the study.

Drug release from sized and unsized liposomes was determined at several time points upon HDL addition to liposomes. At the end of 24-hour incubation, the liposomes in reaction tubes were washed with normal saline and reincubated with 200 microliters of additional HDL (10 mg/ml). Figure 1 shows the time course of drug loss from sized and unsized liposomes in presence of HDL.

RESULTS AND DISCUSSION

The percent drug remained in the liposomal vesicles is shown in Table 1. Sized liposomes showed a reduction in percent encapsulation while the unsized liposomes were not effected by HDL.

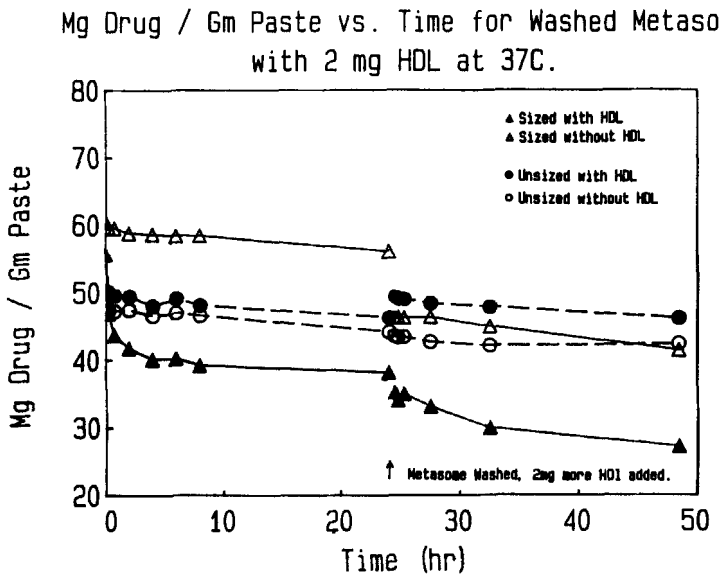

FIGURE 1

Table 1

Percent Encapsulated Drug After 30-Minute Incubation of Liposomes With Various Amounts of HDL is Shown. Sized (0.242 μm) and Unsized 2.598 μm) Liposomes Were Incubated at 37°C and 4°C

Concentration of HDL in Reaction Mixture mg/ml	Amount of HDL Added to Incubation Vessel	% Encapsulation of Sized Liposomes 0.246 ± 0.93 μm		% Encapsulation of Unsized Liposomes 2.146 ± 1.094 μm	
		4°C	37°C	4°C	37°C
0.67	0 mg	89.99	89.99	91.41	91.41
	2 mg	83.53	41.39	89.70*	88.48
3.33	4 mg	87.16	52.48	95.48	85.25
6.67	8 mg	69.80	49.60	92.70	93.20

* Incubated for 60 minutes

At the studied conditions, no HDL concentration dependency on drug loss from sized or unsized liposomes was observed. Thus, rate studies were designed to perform with 2 mg HDL in reaction. Unsized vesicles maintained about 90% encapsulation before and after incubation with HDL. Destabilization effect of HDL was retarded at 4°C.

Vesicle lamellarity determined by ^{31}P NMR technique has been compared to the vesicle mean diameter determined by quasi elastic light scattering technique (8). The mean vesicle diameter of 0.18 ± 0.15 micrometers indicate unilamellar or bilamellar characteristics. Based upon this information, the sized liposomes (0.246 ± 0.093 μm) can be characterized as unilamellar vesicles (ULV) or bilamellar vesicles (BLV) while the unsized (2.146 ± 1.094 μm) liposomes show multilamellar vesicle (MLV) character.

Figure 1 clearly demonstrated that sized and unsized liposomes when incubated with HDL, the sized vesicles discharged about 30% of drug in first 24 hours while the unsized vesicles and controls (sized and unsized vesicles incubated without HDL) maintained drug within the vesicles. Additional 24-hour incubation with HDL repeated the earlier observed phenomena of drug loss from sized liposomes but no destabilization effect on unsized liposomes.

These in vitro studies clearly showed that HDL destabilized, EPC/EPG/Chol derived, small (0.246 ± 0.093 μm) liposomes. The destabilization effect of HDL on unsized (MLV) is not significant. The unsized liposomes (MLV) exhibit a tightly packed "onion skin" configuration of concentric bilayers (9). The interlamellar spaces between bilayers carry aqueous drug solution. We speculate that when the phospholipids in the bilayers are attacked by HDL only drug solution in that layer of MLV leaks out. Since the unsized liposomes maintained their drug encapsulation with minimal loss, it is conceivable that the HDL attacks only the outermost layer of liposomal vesicle. On the other hand, sized liposomes (ULV and BLV) lost incremental amounts of drug vesicles during the first and second incubation periods with HDL. We attempt to explain the loss of encapsulation in sized liposomes during the first 24-hour incubation is primarily due to destabilization of ULV and the outermost layer of MLV. HDL attacks phospholipids in the outermost bilayer and destabilizes the vesicles. When there are only two bilayers in a vesicle, one can assume that the first layer ruptures in the initial incubation and the second round of incubation with HDL, further destabilizes the bilayers of liposome and the result is additional loss of drug from vesicles.

Extrapolation of this concept to in vivo situations will have profound effects on rate of availability of drug from liposome formulations.

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