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Interaction of liposomes of different phospholipid and ganglioside composition with rat hepatocytes

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Summary

The studies of the effect of phospholipids and gangliosides from different tissues on the interaction between liposomes and rat hepatocytes in the monolayer culture have been carried out. Gangliosides purified from the liver upon their incorporation into liposomal membrane increase the capture of liposomes by these cells in a concentration-dependent manner. The effect of incorporated gangliosides on liposome capture by target cells depends on the phospholipid composition of liposomes. Liposomes with phospholipid–ganglioside composition which most closely resemble that of target cells demonstrate the highest affinity toward these cells.

Introduction

Liposomes are widely studied as potential carriers to deliver biologically active substances in in vivo systems (Weinstein and Lesserman, 1984). The successful use of liposomes as drug carriers depends on the affinity of liposomes to target cells. A certain success in targeted liposome transport is achieved by the incorporation of proteins or carbohydrates possessing specific affinity toward a target into the liposomal membrane (Wolff and Gregoriadis, 1984; Torchilin, 1985). It is known that molecules bearing oligosaccharide chains and located on the surface of plasmatic membranes in particular, gangliosides are the recognizing structures of a cell (Tumanova, 1978). As different cells have a unique set of gangliosides, we supposed that liposomes containing gangliosides purified from different sources (organs or cells) would also possess the unique surface topography and, possibly, the increased affinity toward certain tissues. The first data supporting this approach have been already published by us and by other authors (Burkhanov et al., 1985; Rosenberg et al., 1984).

In this work we studied the effect of liposome–phospholipid composition and liposome-incorporated gangliosides from different sources (rat liver, human aorta, bovine brain) on the interaction between liposomes and rat hepatocytes in the monolayer culture.

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Materials and Methods

To prepare liposomes the following reagents were used: egg lecithin (Kharkov bacterial preparations plant), cholesterol-[^{14}C]oleate (Amersham), bovine brain gangliosides (Sigma) and phospholipids and gangliosides from different tissues obtained in our laboratory according to Folch method (Folch et al., 1957) as described previously (Saatov et al., 1984).

Small unilamellar liposomes were prepared from phospholipids and gangliosides in a molar ratio of 10 : 1.5 (in experiments on the dependence of liposome capture by rat hepatocytes on gangliosides content the ratio was varied from 10 : 0 to 10 : 3) with the trace amounts of cholestrol-[^{14}C]oleate (Amersham) added to the mixture. Lipid mixture dissolved in chloroform–methanol (1 : 1 v/v) was vacuum dried on rotary evaporator. Phosphate buffer, pH 7.4 was added to the lipid film obtained up to the final lipid concentration 1.7 mg/ml. Upon shaking, the emulsion formed was sonicated using the ultrasound disintegrator Branson at room temperature until the solution became transparent.

To obtain rat hepatocytes two-step liver perfusion with collagenase was done (Seglen, 1976). Cells were seeded using the medium RPMI-1640 (additionally containing 10% of pure fetal calf serum (Gibco), 10^{-6} M insulin and 10^{-8} M hydrocortisone) into wells ($S = 2$ cm^2) of plastic plate. Cell density was 1.25×10^5 cells/cm^2. The cultivation was performed in the mixture of 5% CO_2 and 95% air at 37 °C. The experiments were carried out on cell monolayer after 48 h of cultivation. 20 μl (2×10^5 dpm. of cholesterol-[^{14}C]oleate) of liposome suspension was added into each well containing 0.5 ml of serum-free medium. After the incubation, unbound liposomes were removed by washing the cells 3 times with PBS. Cells with bound liposomes were lysed and transferred into scintillation vials, and then the radioactivity was measured on Rackbeta 1215 liquid scintillation counter (LKB).

Results and Discussion

Table 1 shows that the efficacy of liposome capture depends on both phospholipid and ganglioside composition of liposomes. The lowest capture was observed in case of liposomes made of pure egg lecithin. Rat liver gangliosides do not practically affect the capture of lecithin liposomes by hepatocytes, but they enhanced the capture of liposomes made of rat aorta phospholipids by 2-fold, and liposomes made of rat liver phospholipids by 3-fold. The effect of aorta and brain gangliosides on liposome capture by hepatocytes differs considerably from that of liver ganglio-

TABLE 1

The capture of liposomes of different lipid composition by rat hepatocytes (nmol lipid / mg protein, mean ± S.E.M. n = 4)

Liposome composition	Capture		Endocytosis fraction from total capture (%)
	37 °C	4 °C	
Lecithin	0.50 ± 0.02	0.37 ± 0.04	26
Lecithin + liver ganglioside	0.55 ± 0.05	0.40 ± 0.06	27
Liver phospholipids	1.18 ± 0.03	0.77 ± 0.06	36
Liver phospholipids + liver gangliosides	3.88 ± 0.31	1.09 ± 0.20	72
Liver phospholipids + aorta gangliosides	1.06 ± 0.08	0.81 ± 0.09	24
Liver phospholipids + brain gangliosides	1.14 ± 0.06	0.99 ± 0.13	13
Aorta phospholipids	1.08 ± 0.06	0.73 ± 0.07	32
Aorta phospholipids + aorta gangliosides	1.56 ± 0.05	1.13 ± 0.12	28
Aorta phospholipids + liver gangliosides	2.63 ± 0.10	1.23 ± 0.15	53

Note: endocytosis fraction was calculated according to the following formula:

$$\frac{\text{capture}_{37\,^\circ\text{C}} - \text{capture}_{4\,^\circ\text{C}}}{\text{capture}_{37\,^\circ\text{C}}}$$

TABLE 2

The effect of preincubation with rat gangliosides on liposome capture by rat hepatocytes

Liposome composition	Inhibition of liposome capture(%)
Lecithin	22
Lecithin + liver gangliosides	22
Liver phospholipids	39
Liver phospholipids + liver gangliosides	60

Note: inhibition was calculated according to the formula:

$$\frac{\text{capture}_0 - \text{capture}_{inh}}{\text{capture}_0}$$

where capture_0 = liposome capture by cells in the absence of gangliosides; and capture_{inh} = liposome capture in the presence of 25 μg of gangliosides.

sides. Brain gangliosides do not affect the capture of liposomes made of liver phospholipids. Aorta gangliosides slightly increase the capture of liposomes prepared from aorta phospholipids and do not affect the interaction of hepatocytes with liposomes made of liver phospholipids. As far as the endocytosis is considered to be an important way of intraliposomal material delivery into endocytotic cells, we have studied the effect of gangliosides on liposome endocytosis by hepatocytes. For this purpose we followed liposome capture by hepatocytes at 4° C, when the endocytosis does not take place, and only liposome adsorption on the cell surface occurs. Then we can calculate roughly the portion of endocytosis in the total liposome capture by hepatocytes. Table 1 shows that liposomes prepared from rat liver phospholipids and gangliosides are internalized more effectively than liposomes of any other composition, which points out that gangliosides enhance namely endocytosis (probably, receptor-mediated one) of liposomes consisting of the "proper" phospholipid–ganglioside mixture.

We have also studied the effect of rat liver ganglioside content on the capture of liposomes made of rat liver phospholipids. Fig. 1 shows that with the increase of ganglioside content liposome capture by hepatocytes at 37° C quickly increases, which gives us additional evidence of ganglioside role in liposome–cell interaction. At 4° C when we follow mainly liposome adsorption, the dependence curve of liposome capture by hepatocytes on the ganglioside content quickly reaches the plateau and reflects, apparently, the saturation of ganglioside binding centers.

According to the data obtained we can conclude that rat liver gangliosides effectively increase liposome capture by rat hepatocytes, the effect depending on their content in phospholipid mixture and on phospholipid composition of liposomes.

The difference in the effect of gangliosides on liposome–hepatocyte interaction upon their incorporation into liposomes made of different phospholipids is associated, most probably, with a different degree of ganglioside incorporation into liposomal membrane and the exposition of appropriate carbohydrate residues on the liposome surface.

It follows also from the data presented that liposomes with a phospholipid–ganglioside composition which most closely resembles that of target cells (liver hepatocytes in our particular

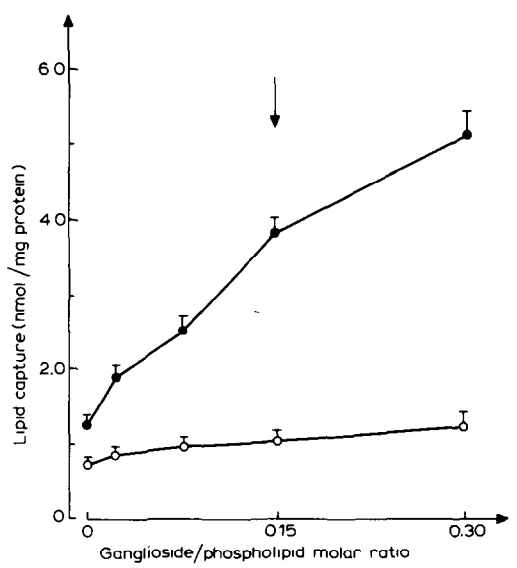

Fig. 1. The dependence of liposome capture by rat hepatocytes on rat liver ganglioside content. 1 h incubation at 37° C (●) and 4° C (○). Liposomes are made of rat liver phospholipids. Arrow corresponds to the composition of liposomes used in the majority of experiments (Tables 1 and 2).

case) demonstrate the highest affinity toward these cells. The mechanism of the phenomenon observed can be similar to the mechanism of cell-to-cell recognition which can proceed via specific interaction between carbohydrate residues and their specific binding sites. In other words, specific liposomes can recognize target cells just like similar cells can recognize each other and form separate colonies in the mixed suspension containing several cell types (Moscona, 1963). The liposomes being much smaller than cells within this specific binding means that they can be easily endocytosed. This is additionally confirmed by the data of Table 2 where it is shown that the preincubation of hepatocytes with liver gangliosides drastically affects liposome capture (60% decrease in the case of liposomes made of liver phospholipids and liver gangliosides).

Thus, the use of liposomes made from the mixture of phospholipids and gangliosides characteristic of the target cells allows one to achieve preferential liposome accumulation by these cells via ganglioside-dependent receptor-mediated endocytosis. The binding can be useful for obtaining liposomes specific towards different cells and organs. Further studies in this direction are now in progress in our laboratory.

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