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INTERACTION BETWEEN LIPOSOMES WITH DIFFERENT LIPID COMPOSITION AND HEPATOCYTES IN VITRO

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The successful use of liposomes as containers for targeting drugs to affected organs depends on the degree of affinity of the liposomes for cells of the target organ [2]. Progress in the direction of transport of liposomes has been made through the incorporation of proteins possessing specific affinity for receptors of target cells into the liposomal membrane [4]. In some investigations, in order to increase the affinity of liposomes, carbohydrate-containing compounds are incorporated into that membrane [3, 7].

Data on interaction of liposomes composed of ovolecithin, phospholipids, and rat liver gangliosides and rat hepatocytes in experiments in vitro are described.

EXPERIMENTAL METHOD

To obtain liposomes ovolecithin (from the Olaine Pharmaceutical Chemical Factory), cholesterol (from Sigma, USA), cholesteryl-^{14}C-oleate (from Amersham Corporation, England), and total phospholipid and ganglioside fractions of rat liver, obtained by the method in [6], were used to obtain liposomes.

Liposomes were prepared by the standard method [5], by making up solutions of phospholipids, cholesterol, and gangliosides in the molar ratio of 7:3:0.3. To introduce the radioactive label into the liposomal membrane, cholesteryl-^{14}C-oleate was added to a mixture of lipids (0.5 mg of lipids in 1 ml of buffer).

To isolate rat hepatocytes the method of two-stage perfusion of the liver with collagenase [8] was used. Cells were seeded in medium RPMI-1640 containing 10% embryonic calf serum, insulin (10^{-6} M), hydrocortisone (10^{-8} M), in wells (S = 2 cm^2) of a plastic culture dish with a density of $1.25 \cdot 10^5$ cm^{-2}, and cultured in an at-

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TABLE 1. Uptake of Liposomes by Hepatocytes (amount of label associated with cells, in cpm; M ± m, n = 4-6)

Composition of liposomes	Hepatocytes
Lecithin	673±14
Lecithin + liver gangliosides	705±16
Liver phospholipids	1298±38
Phospholipids + liver gangliosides	1514±92

TABLE 2. Effect of Iodoacetamide on Uptake of Liposomes by Hepatocytes $\{[(\text{uptake}_0 - \text{uptake}_{\text{inhib}})/\text{uptake}_0] \cdot 100\,\%\}$

Composition of liposomes	Hepatocytes
Lecithin	30,8
Lecithin + liver gangliosides	51,8
Liver phospholipids	54,0
Phospholipids + liver gangliosides	75,7

Legend. Uptake_0) Uptake of liposomes by cells in absence of iodoacetamide, $\text{uptake}_{\text{inhib}}$) uptake of liposomes by cells in response of iodoacetamide, cells preincubated for 40 min at 37°C with iodoacetamide in concentration of 100 μM.

TABLE 3. Effect of Preincubation with Gangliosides on Uptake of Liposomes by Hepatocytes $\{[(\text{uptake}_0 - \text{uptake}_{\text{inhib}}) \cdot (\text{uptake}_0)^{-1}] \cdot 100\%\}$

Composition of liposomes	Hepatocytes
Lecithin	22,2
Lecithin + liver gangliosides	21,5
Liver phospholipids	39,7
Phospholipids + liver gangliosides	59,9

Legend. Uptake_0) Uptake of liposomes by cells in absence of gangliosides, $\text{uptake}_{\text{inhib}}$) uptake of liposomes by cells in presence of gangliosides; cells incubated with 25 μg of gangliosides at 37°C for 40 min.

mosphere of 5% CO_2 + 95% air at 37°C. Experiments were carried out on a cell monolayer after culture for 48 h. To each well, containing 0.5 ml of the above medium without serum, 20 μl ($8 \cdot 10^4$ cpm of cholesteryl-^{14}C-oleate) of liposomes was added. After incubation at 37°C for 1 h the unbound liposomes were removed by washing the cells three times with physiological saline. Cells with bound liposomes were transferred to scintillation flasks containing dioxan scintillator and counted on a Rack-Beta 1215 counter (LKB, Sweden).

EXPERIMENTAL RESULTS

To determine the effect of the phospholipid and ganglioside composition of the liposomal membranes on their interaction with the target cells binding of liposomes made from lecithin, lecithin with liver gangliosides

or liver phospholipids, and also from phospholipids and rat liver gangliosides with hepatocytes in a monolayer were investigated. Table 1 shows that liposomes whose phospholipid basis consisted of total liver phospholipids were bound significantly more than lecithin liposomes. Gangliosides have virtually no effect on uptake of lecithin liposomes by hepatocytes. Gangliosides added to liver phospholipid liposomes significantly increased affinity of the liposomes for hepatocytes. Differences in the effects of gangliosides on liposomes made from lecithin and liver phospholipids were evidently due to differences in the degree of their insertion into the liposomal membrane and differences in exposure of the carbohydrate residues on the surface of these liposomes [7].

The ability of liposomes to penetrate inside cells by endocytosis has been demonstrated by several investigations [1, 9].

During interaction between liposomes and cells, besides endocytosis, adsorption of the liposomes on the cell surface, their fusion with the cell membrane, and exchange of lipids between liposomes and cells also take place. To study the contribution of active endocytosis to the total uptake of liposomes by hepatocytes, the effect of glycolytic inhibitor of endocytosis, iodoacetamide, on binding of liposomes with hepatocytes was studied. Table 2 gives values reflecting the contribution of endocytosis to total uptake of liposomes by the cells. Endocytosis of liposomes was least marked in the case of lecithin liposomes. Addition of gangliosides to lecithin liposomes increased endocytosis a little. The greatest contribution of endocytosis to uptake of liposomes by hepatocytes was found in the case of liposomes from liver phospholipids and gangliosides. Endocytosis is known to be preceded by "recognition" of liposomes on the cell surface. It can be postulated that gangliosides facilitate this "recognition." This process can be seen most clearly for liposomes with lipid composition similar to that of the cells.

To determine the role of gangliosides in interaction between liposomes and cells the effect of pre-incubation of the cells with gangliosides on binding of liposomes with hepatocytes was investigated. It will be clear from Table 3 that inhibition of uptake of liposomes by the cells was greatest in the case of interaction between liposomes from liver phospholipids and gangliosides and hepatocytes. The effect of gangliosides on interaction of liposomes from liver phospholipids with hepatocytes was rather weaker. In the remaining cases, gangliosides had a negligible effect. The action of gangliosides thus exhibited greatest specificity during interaction between liposomes and cells with similar lipid composition.

These data show that binding of liposomes with cells is determined by the phospholipid and glycolipid composition of the liposomes; affinity of liposomes for an organ, moreover, can be enhanced by creating liposomes with lipid composition similar to that of the target cells.

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