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SPECIFICITY OF LIPOSOME UPTAKE FROM LIPIDS OF TARGET CELLS

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Liposomes have been intensively studied in recent years as a new therapeutic form and a tool for biological research [11]. The most hopeful prospects are offered by the use of liposomes for directional transport of drugs [6]. The wide possibilities for choice of size, structure, and membrane composition of the vesicles provide ample scope for the search for optimal solutions to this complex problem.

One of the most important tasks in directional transport of liposomes is making their uptake by nonphagocytic cells more effective. This can be done in various ways, using affinity pairs on the contacting membranes as the model: antigen–antibody [7], hormone–receptor [4], lectin–glycolipid [13]. Manipulations of this kind are aimed primarily at making more effective contact between liposomes and cells. However, a simple increase in the binding of vesicles with the cell surface is insufficient to ensure intracellular placing of the drugs. This requires taking account of the mechanism of interaction between liposomes and the cell. Among these mechanisms, some of the most important are stable adsorption, diffusion of the internal contents of the adsorbed liposomes, endocytosis, and fusion of the contacting membranes [3].

The use of a fusion mechanism is the most promising method for cells with weak endocytosis.

One possible way of making more effective contact and subsequent fusion of liposomes with target cells may be by ensuring similarity of structural characteristics of the contacting membranes [5]. Two arguments at least can be advanced in support of this view: first, general ideas on participation of three-dimensional structures of carbohydrate components of cell membrane glycolipids in processes of intercellular recognition and adhesion; second, information showing that similarity in the phase state of the lipid bilayer of vesicles and cells facilitates fusion [10]. From our point of view, these demands are satisfied most completely by a mixture of membrane lipids of target cells.

To verify the effectiveness of this approach the writers compared uptake by target cells of liposomes made from lipids of the same cells and from lipids of other cells. Cells of ascites lymphatic leukemia NKLy/LL cells and Ehrlich's ascites carcinoma cells (EAC) were studied as models. Uptake of liposomes from homologous lipids also was compared with incorporation of vesicles from egg phosphatidylcholine (PC).

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EXPERIMENTAL METHOD

Polar lipids from target cells were obtained after removal of neutral lipids with cold acetone as described in [2]. Egg PC from Kharkov Bacterial Preparations Combine was purified on alumina [14] and cholesterol from Leningrad Meat Combine was recrystallized three times from ethanol. The fractional composition of the phospholipids and purity of PC were determined by three-layer chromatography on silica-gel in a system of chloroform – methanol – water (65 : 25 : 4). The phospholipid content was determined as lipid phosphorus [15]. Cholesterol-^{14}C-oleate with specific radioactivity of 21.5 mCi/mmole (Amersham Corporation, England) was used as the radioactive label. Unilamellar liposomes (ULL) were prepared by the ultrasonic method [12]. Liposome preparations had a specific radioactivity of about 0.1 μCi/μmole phospholipids in the mixture used.

NKLy/LL and EAC cells were collected on the 7th day after transplantation, washed with medium No. 199 with centrifugation at 500g (5 min) to remove ascites fluid, and incubated with liposomes at 37°C. Incubation was stopped at different times by addition of samples of cold physiological saline, and the cells were washed three times to remove unincorporated liposomes by centrifugation at 1000g (10 min). The cells were immobilized on nitrocellulose filters (Synpor, Czechoslovakia) with pore diameter of 1.2-1.5μ and radioactivity was counted in 10 ml of multipurpose scintillator (from Beckman) on an LKB Wallac 1210 counter. Statistical analysis was carried out by Wilcoxon's paired T test.

EXPERIMENTAL RESULTS

Plasma membranes of eukaryote cells are characterized by a definite ratio of phospholipids and cholesterol, and this is reflected in their phase state. Guided by the general consideration of aiming at similarity between structural characteristics of contacting membranes, we studied uptake of liposomes made from homologous lipids of target cells with the addition of 30 moles % cholesterol to the mixture. This ratio is characteristic of both lymphoma [8] and EAC [9] cells.

In two types of experiments specificity of liposomal uptake from homologous lipids by target cells was found. In the first case incorporation of liposomes from lipids of both types of cells by EAC cells was investigated (Fig. 1), in the second case, liposomes from lipids of NKLy/LL cells by two types of cells (Fig. 2). Uptake of liposomes from homologous lipids was found to be 1.5-2 times more effective than that of vesicles from a natural mixture of lipids of the other tumor, and comparison with uptake of liposomes from egg PC showed even sharper differences. Their uptake compared with uptake of liposomes from homologous lipids (incubation of vesicles for 3 h with cells) was three times less for EAC cells and eight times less for NKLy/LL cells (Figs. 1 and 2).

To study the causes of the differences found in uptake of liposomes from homologous lipids and lipids from egg PC, attempts were made to assess the contribution of the various mechanisms of their interaction with cells to the total uptake. Molecular exchange of cholesteryl oleate between vesicles and cells, incidentally, is negligible [1], and the level of its transfer into the cells evidently reflects adsorption, endocytosis, and fusion. Incubation of cells with trypsin or EDTA is known to reduce or eliminate adsorption of liposomes [1, 6]. We found a marked decrease in incorporation of liposomes from egg PC during this kind of treatment, but no effect of adsorption on incorporation of liposomes from homologous lipids by the cells (Table 1). It is also well known that endocytosis is an energy-dependent process, and for that reason its contribution can be estimated from the decrease in uptake of liposomes under the influence of glycolysis inhibitors or of lowering the incubation temperature [1, 6]. Experiments with 2-deoxy-D-glucose and incubation of the cells at 4°C showed that in both cases there was a quantitatively similar fall in the level of uptake of liposomes from egg PC, whereas incorporation of liposomes from homologous lipids was inhibited only on cooling (Table 1). This relationship to temperature in the absence of the inhibitory effect of 2-deoxy-D-glucose undoubtedly points to a metabolically independent process of incorporation of liposomes from homologous lipids. The reduction in incorporation of liposomes from homologous lipids by a fall of incubation temperature was most probably connected with different kinds of change in the phase state of the membrane of vesicles and cells, and indicates that uptake of these liposomes is realized by a fusion mechanism [1, 6]. Evidence that a fusion mechanism is basic in uptake of liposomes from homologous lipids also was given by the duration of the process and the absence of saturation of incorporation of vesicles at physiological temperatures (Figs. 1 and 2).

It can be concluded from these data that the low level of incorporation of liposomes from egg PC is due to the fact that they are taken up by a mechanism of endocytosis, which in the cells studied is weakly represented. Meanwhile the increased incorporation of liposomes from homologous lipids compared with incorporation of liposomes from egg PC was due to the fusion mechanism, which is more effective for nonphagocytic cells.

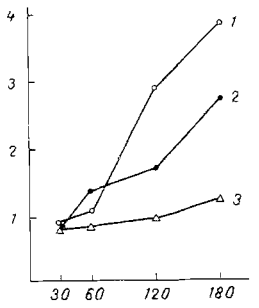

Fig. 1

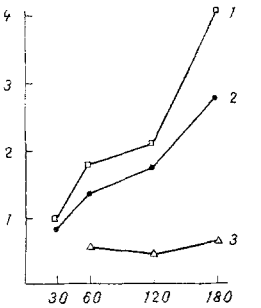

Fig. 2

Fig. 1. Uptake of liposomes of different lipid composition by EAC cells. Abscissa, time (in min); ordinate, uptake of liposomal cholesterol-^{14}C-oleate (in % of dose in incubation mixture). Values given are means for 3-5 experiments. Conditions of incubation: 10^6 cells were incubated with liposomes (0.2 μmole calculated as phospholipids) in 1 ml medium No. 199 at 37°C. Curve 1) liposomes from lipids of EAC cells; 2) liposomes from lipids of NKLy/LL cells; 3) liposomes from egg PC without cholesterol. P = 0.05 for all curves.

Fig. 2. Uptake of liposomes from lipids of NKLy/LL cells by two types of tumor cells. Legend and conditions of incubation the same as Fig. 1. Curve 1) NKLy/LL cells; 2) EAC cells; 3) NKLy/LL cells and liposomes from egg PC without cholesterol. P = 0.05 for all curves.

TABLE 1. Effect of Various Procedures on Level of Liposomal Uptake by Cells

ULL	Experimental conditions			
	EDTA, 0,02 %	Trypsin, 0,25 %	2-deoxy-D-glucose, 1 M	4 °C
From egg PC	66,5	50,5	36,6 -	48,9
From lipids of NKLy/LL cells	91,4	100,0	85,3	42,5

Legend. Values show level of uptake of liposomes by NKLy/LL cells after incubation (30 min) in % of uptake in control ($1 \cdot 10^6$ cells, 0.3 μmole lipids calculated as phospholipids, with 30 moles % cholesterol in 1 ml medium 199 at 37°C). 2-Deoxy-D-glucose (0.1 ml) added 15 min before addition of liposomes, trypsin (0.1 ml) added before and after incubation of liposomes with cells for 20 min. Liposomes were added to cold samples 15 min after incubation of cells at 4°C, and incubation continued thereafter at the same temperature.

Some light on the causes of the observed differences could be shed by knowledge of the composition of the phospholipids of the lipid mixtures used. It will be recalled that the presence of perceptible quantities of lysolecithin makes fusion more effective [3, 6]. It was found that phospholipids of NKLy/LL cells contain 2.5% of lysolecithin, whereas EAC cells contain 0.44%. This lysolecithin level can partly explain differences in uptake of liposomes from homologous lipids compared with vesicles from egg PC (Figs. 1 and 2), but it does not explain the predominant uptake of "homologous" liposomes compared with liposomes from lipids of the other tumor. Most probably the increased effectiveness of fusion of these liposomes with target cells is connected with the unique character of structure of the lipid bilayer of the contacting membranes. This unique quality is determined by many parameters: specificity of the class composition of the individual mixture of lipids and specificity of its fatty-acid representation, the mosaic character of the lipid domains with different phase states of microregions of contacting membranes, the mosaic character of structure due to lateral spacing of the

lipids in the membrane, the unique composition of lipids with receptor functions, and so on. The importance of each of the above parameters for effectiveness of fusion calls for further investigation.

In conclusion, it should be emphasized that despite the lack of clarity in the causes of the differences found, increased uptake of liposomes from homologous lipids by nonphagocytic cells compared with liposomes of egg lecithin and selective uptake of "homologous" liposomes in preference to incorporation of vesicles from a natural mixture of lipids of other cells can be used to make the selective application of therapeutic substances to target tissues more effective.

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THYROID HORMONES AND ELECTRICAL STABILITY OF RAT LIVER MITOCHONDRIAL MEMBRANES

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Administration of thyroid hormones to animals is accompanied by their rapid binding with liver mitochondria [10], activation of endogenous phospholipase of liver mitochondria [4], stimulation of the process of $Ca^{++}/2H^{+}$ exchange through the mitochondrial membrane [3], and acceleration of lipid peroxidation (LPO) reactions in these organelles [6]. In electron micrographs of the liver of animals with hyperthyroidism the mitochondria appear more swollen than normally [14]. Resistance of the barrier systems of mitochondria isolated from animals with different thyroid states to Ca^{++} is reduced in the order hypothyroidism > normal > thyrotoxicosis [1, 2, 5]. These data can be interpreted as evidence of a connection between membrane stability and hormonal state. The general stability of a membrane is characterized by its electrical stability, determination of which in the case of artificial phospholipid membranes has found widespread application [6, 7].

The aim of this investigation was to determine the electrical stability of mitochondrial membranes in animals in different thyroid states.

EXPERIMENTAL METHOD

Male Wistar rats were used. Thyroidectomy as a model of hypothyroidism was performed on animals weighing 100-120 g. By the beginning of the experiment (2 months after the operation) the animals weighed 140-

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