This combined clinical and experimental study thus showed that the development of EpA in animals and man is accompanied by a fall in activity of antioxidant enzymes in the blood. This fall is evidence that disturbances of LPO regulation during epileptogenesis are linked, to a certain degree, with insufficiency of the antioxidant system, and in particular, with insuffiency of the antioxidant system and, in particular, with insufficiency of the enzymes composing it.

LITERATURE CITED

1. V. A. Voronko, E. V. Nikushkin, I. G. Pronina, et al., Current Problems in Disease and Recovery [in Russian], Moscow (1983), p. 72.
2. V. E. Kagan, R. N. Kopaladze, L. L. Prilipko, et al., Byull, Éksp. Biol. Med., No. 12, 16 (1983).
3. V. M. Kovalenko, G. N. Kryzhanovskii, V. S. Kovalenko, et al., Zh. Nevropatol. Psikhiat., No. 6, 892 (1984).
4. G. N. Kryzhanovskii, E. V. Nikushkin, V. A. Voronko, et al., Zh. Nevropatol. Psikhiat., No. 6, 806 (1984).
5. G. N. Kryzhanovskii, E. V. Nikushkin, V. A. Voronko, et al., Byull, Éksp. Biol. Med., No. 11, 36 (1983).
6. V. Z. Lankin, A. K. Tikhadze, A. A. Kovalevskaya, et al., Dokl. Akad. Nauk SSSR, 261, No. 6, 1467 (1981).
7. V. G. Mkhitaryan, A. A. Megrabyan, G. E. Badalyan, et al., Zh. Éksp. Klin. Med., No. 3, 237 (1983).
8. E. V. Nikushkin, V. E. Braslavskii, and G. N. Kryzhanovskii, Zh. Nevropatol. Psikhiat., No. 6, 810 (1981).
9. I. R. Typeev, M. M. Bordyukov, G. N. Kryzhanovskii, et al., Byull. Éksp. Biol. Med., No. 11, 538 (1985).
10. V. A. Shaternikov and L. A. Savchuk, Lab. Delo, No. 10, 598 (1964).
11. C. Beauchamp and I. Fridovich, Analyt. Biochem., 44, 276 (1971).
12. J. Maral, K. Puget, and A. M. Michelson, Biochem. Biophys. Res. Commun., 77, 1525 (1977).
13. S. L. Taylor, M. H. Lamden, and A. L. Tappel, Lipids, 11, 530 (1976).
14. L. J. Willmore, M. Hiramatsa, H. Kochi, et al., Brian Res., 277, 393 (1983).
15. Y. Yoshihito and R. Konichi, Blood, 43, 99 (1974).
16. Z. Zöllner and K. Kirsch, Z. Ges. Exp. Med., 135, 545 (1962).

EFFECT OF LIPID COMPOSITION OF LIPOSOMES ON THEIR CLEARANCE FROM THE BLOOD STREAM AND ACCUMULATION IN THE MOUSE LIVER

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KEY WORDS: liposomes; phospholipids; gangliosides; liver

Incorporation of drugs inside lipid vesicles (liposomes) has a marked effect on their pharmacodynamic and therapeutic properties. By the use of liposomes it is possible to control the circulation time of a drug or its selective accumulation in particular organs. Targeted transport of liposomes to particular tissues of cells is a fundamental task in the use of liposomes as containers for drug transport in the body. To accomplish this task, liposomes must satisfy at least two demands: they must have access to the target cells and must bind preferentially with these cells. The liver is an organ (one of several) in which the parenchymal cells (hepatocytes) are directly accessible for the blood stream. We know, moreover, that the liver cells are the location of many diseases [5]. Development of methods of effective delivery of drugs to the liver cells is thus an important problem.

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TABLE 1. Distribution of Liposomes in Mouse Blood and Liver 5 Min after Intravenous Injection (in % of injected label in 1.5 ml blood and 1 g liver, M ± m, n = 5)

Composition of liposomes	Blood	Liver
Lecithin	25,8±2,4	38,6±4,4
Lecithin + liver gangliosides	24,2±0,8	39,3±2,7
Liver phospholipids	6,5±0,6	53,4±1,8
Liver phospholipids and gangliosides	2,0±0,3	76,3±8,1
Liver phospholipids and gangliosides after preliminary injection of 500 μg of gangliosides	5,7±2,3	48,3±4,2

The writers showed previously [1] that the liver gangliosides stimulate uptake of liposomes by rat hepatocytes *in vitro*. In the investigation described below the effect of the phospholipid and glycolipid composition of the liposomes on their elimination time from the circulation and on their accumulation in the mouse liver was investigated.

EXPERIMENTAL METHOD

Liposomes were obtained from ovolecithin (Khar'kov Bacterial Preparations Factory), cholesterol (Sigma, USA), and cholesteryl-^{14}C-oleate (Amersham International, England). Total phospholipid and ganglioside fractions were obtained from the liver by Folch's method [3].

Small single-layered liposomes were obtained from phospholipids and cholesterol (and gangliosides) in a molar ratio of 7:3 (0.3). The mixture of lipids was evaporated to dryness on a rotor evaporator, and the resulting film was covered with borate buffer, pH 7.4, in the ratio of 0.7 mg of phospholipids to 1 ml of buffer. After shaking the emulsion was treated on an ultrasonic disintegrator at room temperature until the solution was clarified. To incorporate a radioactive label into the liposomal membrane, cholesteryl-^{14}C-oleate was added to the mixture of lipids.

An injection of 200 μl of liposome suspension ($3 \cdot 10^4$ cpm of cholesteryl-^{14}C-oleate) was given into the caudal vein of the mice. The mice were killed by decapitation 5 min later and the blood and liver were removed. After lysis the tissues were transferred to scintillation plaques containing dioxan scintillator and radioactivity was measured on a RackBeta 1215 counter (LKB, Sweden).

EXPERIMENTAL RESULTS

To compare the effects of the phospholipid and ganglioside components of liposomes on their clearance from the circulation and accumulation in the liver, the content of liposomes made from lecithin, lecithin and liver gangliosides, liver phospholipids, and also of liver phospholipids and gangliosides after intravenous injection, in the blood and liver of the mice was studied. Data showing the concentration of cholesteryl-^{14}C-oleate, associated with lipid vesicles, in the liver and blood 5 min after injection of liposomes are given in Table 1.

At the time of recording about 25% of lecithin liposomes were circulating in the blood stream. Liver gangliosides had virtually no effect on clearance of the lecithin liposomes from the blood stream or their accumulation in the liver. The distribution of liposomes changed significantly if they were obtained from total liver phospholipids. The concentration of such liposomes in the circulating blood of the mice 5 min after injection was significantly less than that of lecithin liposomes (6.5 compared with 25.8%). Meanwhile their content in the liver increased significantly (up to 53.4%). Incorporation of liver gangliosides into the composition of liposomes made from liver phospholipids led to an even greater increase in the rate of elimination of the vesicles from the circulation and their accumulation in the mouse liver. This effect could be abolished: injection of liposomes made from liver phospholipids and gangliosides 10 min after injection of an excess of gangliosides had the result that the distribution of liposomes was virtually indistinguishable from the distribution of liposomes made from liver phospholipids only.

Rapid elimination of liposomes made from total liver phospholipids from the circulation and their accumulation in the liver is evidently the result of specific interaction of these liposomes with hepatocytes. As the writers showed previously in experiments *in vitro* [1], this interaction is accompanied by increased endocytosis of liposomes by hepatocytes. The specificity of uptake of liposomes from phospholipids of target cells has been established also for Ehrlich's ascites carcinoma cells and ascites lymphatic leukosis cells [2]. In the opinion of the authors cited this specificity is due to the more effective fusion of the liposomes with the cells, because of similarity in the phase state of their membranes. Differences in the mechanisms of interaction of liposomes with cells may perhaps be connected with the different phagocytic capacity of the cells used.

It can be tentatively suggested that the increase in the rate of elimination of liposomes made from liver phospholipids from the circulation and their accumulation in the liver as a result of incorporation of gangliosides in them is due to the presence of specific receptors for carbohydrates on the surface of the hepatocytes. This is confirmed by the fact that preliminary intravenous injection of gangliosides into mice reduced the uptake of phosphoglycolipid liposomes by the liver and their clearance from the circulation. This effect evidently arises due to blocking of the corresponding binding sites on the liver cells. The different effects of gangliosides when introduced into liposomes made from lecithin and from liver phospholipids are evidently due to differences in the degree of exposure of their carbohydrate residues on the surface of the liposomes.

Increased uptake of liposomes with carbohydrate-containing compounds incorporated into their membranes by the liver has been demonstrated in a number of investigations [4, 6]. The increase, moreover, is due to the greater uptake of liposomes by parenchymal cells coupled with a very small increase in uptake by nonparenchymal cells. It can be concluded from these results that liposomes obtained from total liver phospholipids and with the addition of gangliosides from the liver can, in principle, be used for supplying physiologically active substances rapidly and efficiently to the liver cells.

LITERATURE CITED

1. S. A. Brukhanov, E. V. Dormeneva, V. A. Kosykh, et al., Byull. Éksp. Biol. Med., No. 6, 679 (1985).
2. O. A. Rozenberg, V. Yu. Bekreneva, L. V. Loshakova, et al., Byull, Éksp. Biol. Med., No. 6, 670 (1984).
3. J. Floch, M. Lees, and G. H. Sloane-Stanley, J. Biol. Chem., 226, 497 (1957).
4. P. Ghosh and B. K. Backhawat, Biochim. Biophys. Acta, 632, 562 (1980).
5. Progress in Liver Disease, Vol. 6, New York (1979).
6. H. J. Spanjer and G. L. Scherphof, Biochim. Biophys. Acta, 734, 40 (1983).