DYNAMICS OF BLOOD CLEARANCE OF LIPOSOMES PREPARED FROM NONHYDROLYZED DIESTER ANALOG OF PHOSPHATIDYLCHOLINE AFTER INTRAVENOUS INJECTION INTO MICE

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Changes in the content of liposomes prepared from egg phosphatidylcholine and its nonhydrolyzed diester analog 1,2-dipalmitylphosphatidylcholine in the blood and liver in the course of time after intravenous injection of the liposomes into mice were studied. Cholesteryl-[1-^{14}C]-oleate was used as marked. The kinetics of blood clearance of the two types of liposomes consists of two phases: an initial rapid phase connected with removal of intact liposomes from the blood, and a subsequent slow phase. Replacement of phosphatidylcholine by its diester analog did not cause any delay in the disappearance of liposomes from the blood during the first phase, but reduced the rate of decrease of radioactivity of the blood somewhat during the slow phase. The results show that lipolytic enzymes capable of hydrolyzing the ester bonds in the 1 and 2 positions of phosphatidylcholine evidently do not participate in the mechanisms of removal of intact liposomes from the blood stream. Since liposomes prepared from the diester analog of phosphatidylcholine are rapidly cleared from the blood stream and since they cause unfavorable side effects, they appear to have no prospects for use as drug carriers capable of circulating in the blood stream for a long time.

KEY WORDS: liposomes; intravenous injection; phosphatidylcholine; diester analog of phosphatidylcholine.

One of the main obstacles to the clinical use of liposomes, which have proved to be promising carriers of drugs in vivo, is their rapid disappearance from the blood [3, 4]. To overcome this handicap it is therefore necessary to prepare liposomes with a long circulating life, but this is an extremely difficult task. Although there is reason to suppose that the main contribution to the removal of liposomes from the circulation is made by reticuloendothelial cells, chiefly in the liver, which are responsible for their endocytosis, the concrete mechanisms of the processes taking place under these circumstances have not yet been explained [2, 4].

A possible role in the removal of liposomes from the circulation is ascribed to lipolytic enzymes, which can directly or indirectly facilitate endocytosis of liposomes by the liver cells or make possible alternative processes. Evidence of this possibility is given by the delayed elimination from the blood of liposomes prepared from the 1,2-dialkyl analog of phosphatidylcholine (i.e., incapable of hydrolysis in positions 1 and 2) compared with liposomes from the ordinary phosphatidylcholine [1]. However, according to other investigations liposomes are assimilated by the liver while still intact [2, 4]. In the light of these observations, the ability or inability of liposomal lipids to be hydrolyzed should not affect the rate of blood clearance from liposomes. It has also been shown that liposomes made from phosphatidylcholine, like those made from its nonhydrolyzed analogs, can be destroyed equally by components of the blood.

In the investigation described below an attempt was made to determine the true role of the possibility of hydrolysis in positions 1 and 2 of phosphatidylcholine on the rate of removal of liposomes prepared from it from the blood stream.

EXPERIMENTAL METHODS

Chromatographically pure lipids – egg phosphatidylcholine and cholesterol (both from Sigma) – and also the diester analog of phosphatidylcholine 1,2-dipalmitylphosphatidylcholine, generously provided by A. Bushnev

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TABLE 1. Kinetics of Removal of Radioactive Label from the Blood Stream and Its Accumulation in the Liver (in % of injected dose; M ± m, n = 6)

Time after injection	Radioactivity in blood, %		Radioactivity in liver, %	
	liposomes of phosphatidylcholine	liposomes of diester analog	liposomes of phosphatidylcholine	liposomes of diester analog
2 min	82,2±3,1	76,9±3,7	11,4±1,3	14,0±1,4
5 »	65,0±4,1	59,9±5,3	23,8±1,7	26,2±2,3
1 h	19,2±1,3	22,7±2,0	42,1±1,5	49,1±3,2
3 »	5,1±0,4	12,2±2,3	35,5±1,7	40,5±2,7
24 »	0,9±0,1	4,5±0,8	6,4±0,6	14,2±1,6

(M. V. Lomonosov Institute of Fine Chemical Technology), and cholesteryl-[1-^{14}C]oleate (from the Radiochemical Centre. Amersham) were used. All solvents and components of buffer solutions were Soviet products of the analytical grade of purity.

To obtain liposomes, 20 mg lipids with a molar ratio of phospholipid:cholesterol of 2:1, as a solution in chloroform, and 5 μCi cholesteryl-[^{14}C]oleate in the form of a solution in benzene, were mixed and evaporated to dryness in a round-bottomed flask on a rotary evaporator. The resulting film of lipids was covered with 2 ml phosphate buffer (0.145 M NaCl, $5 \cdot 10^{-3}$ M NaH_2PO_4, pH 7.4) and treated with ultrasound on the UZDN-1 ultrasonic disintegrator at 20°C for 3 min, then centrifuged for 6 min at 3000g.

Experiments were carried out on 60 male CBWA albino mice weighing 20-22 g. The preparation of labeled liposomes, containing 0.5 mg lipids in 50 μl and with a radioactivity of about 0.12 μCi, was injected into the caudal vein. At definite times after the injection the animals were decapitated, blood was collected into heparinized test tubes, and the liver was removed, washed with physiological saline, and gently dried. Samples of 0.1 ml blood and of approximately 100 mg liver (two samples from each animal) were introduced into scintillation flasks and solubilized by Neame's method [8]. The concentration of radioactive label was determined by the Mark III (Nuclear Chicago) scintillation spectrometer.

EXPERIMENTAL RESULTS

The fate of liposomes prepared from egg phosphatidylcholine and liposomes prepared from its diester analog, injected into the blood stream, was studied (Table 1). Liposomes of these two types behaved identically. They disappeared quickly from the blood and accumulated mainly in the liver. The kinetics of disappearance of liposomes from the blood stream consists of two phases, fast followed by slow, in agreement with data in the literature [1, 5, 6, 9]. No significant differences could be found in the rates of disappearance of the two types of liposomes from the blood or the rates of their accumulation in the liver during the first few hours. This observation contradicts the results obtained by Deshmukh et al. [1] and is evidence that enzymes capable of hydrolyzing ester bonds in positions 1 and 2 of phosphatidylcholine evidently have no appreciable effect on the rate of elimination of liposomes from the blood stream.

After 24 h, however, the level of radioactivity in the blood after injection of liposomes prepared from the diester analog of phosphatidylcholine was appreciably higher than after injection of ordinary phosphatidylcholine, in qualitative agreement with the observations of Deshmukh et al. [1]. But even in the case of the diester analog, this level was only a small proportion (under 5%) of the initial level. This means that even if the liposomes remained intact throughout the period of the experiment, even under the most favorable circumstances their total number was negligibly small and could not be of any real value for clinical application. Moreover, whereas the nature of the rapid phase of the kinetics of disappearance of liposomes from the blood stream is sufficiently well understood, and is evidently connected with primary elimination of intact liposomes from the circulation, the nature of the slow stage has received much less study. The presence of radioactive label in the blood 24 h after injection does not necessarily mean that intact liposomes still remained in the blood stream at that time (as Deshmukh et al. assumed [1]). It is more likely [2, 4, 5] that this radioactivity was connected with breakdown products of liposomes, which could, for example, be discharged into the blood stream gradually as the liposomes undergo destruction in the liver and other organs.

In conclusion, it must be pointed out that mice tolerated the injections of liposomes prepared from the diester analog of lecithin badly. The animals became drowsy, developed hypokinesia, and refused to eat. No such phenomena were observed in the case of ordinary egg lecithin.

It can be concluded from all these facts that, in order to create liposomes capable of circulating for a long time in the blood stream, it is necessary to use other lipids, or approaches based on a different principle, on a search for which the authors are currently engaged.

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EFFECT OF PSYCHOTROPIC DRUGS ON RNA SYNTHESIS IN BRAIN CELL NUCLEI

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Changes in the distribution of biogenic amines in the brain were produced by intraperitoneal injection of psychotropic drugs – reserpine (80 μg/100 g) and chlorpromazine (300 μg/100 g) – during chronic experiments on rats. Under these conditions reserpine reduced the endogenous RNA polymerase activity of types I and II of the rat brain cell nuclei on average by 61 and 34%, and chlorpromazine did so by 32 and 38% respectively. In a cell-free system reserpine and chlorpromazine in concentrations of 0.1 and 1 mM had no effect on the RNA-synthesizing activity of isolated rat brain cell nuclei. It is suggested that the action of psychotropic drugs on the genetic apparatus may be mediated through either a decrease in cyclic AMP production or inhibition of RNA-synthesizing activity.

KEY WORDS: RNA polymerase; psychotropic drugs; biogenic amines; rat brain.

Changes in the distribution of biogenic amines in the brain can be produced by means of psychotropic compounds. These changes, in turn, are reflected in behavioral actions [4].

The object of this investigation was to study the role of the genetic apparatus in these phenomena.

EXPERIMENTAL METHODS

Experiments were carried out on male albino rats weighing 100-120 g. Psychotropic drugs – reserpine in a dose of 80 μg/100 g and chlorpromazine in a dose of 300 μg/100 g – were injected intraperitoneally into the rats daily for 3 days. Physiological saline was injected into control animals. On the 3rd day, 2 h after the injection, the rats were killed and the cell nuclei isolated from their brain by the method of Chauveau et al.

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