PHOSPHATIDYLCHOLINE LIPOSOMES IN THE REPAIR OF THE HEPATOCYTE PLASMA MEMBRANE

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The mechanism of action of the hepatotropic poisons tetrachloromethane (CCl_4) and heliotrine is based on their ability to damage biological membranes by disturbing their lipid bases, which is responsible for their barrier and matrix functions [1, 10]. Previous investigations have shown structural and functional disturbances of membranes of the endoplasmic reticulum, arising as a result of the action of these poisons, and they demonstrated the possibility of correcting these disturbances by the use of liposomes made from egg phosphatidylcholine (PCh) in experiments in vivo [6, 7].

Injury to plasma membranes in these investigations was assessed on the basis of release of the hepatocyte cytoplasmic enzyme fructose-monophosphate aldolase into the blood stream [4, 7]. It was shown that the hyperenzymemia arising as a result of the action of the poisons is abolished by phospholipid therapy. The possibility of repairing plasma membranes by means of phospholipids, thus revealed, was developed further in the investigation described below, the aim of which was to study the phospholipid composition and activity of membrane-bound enzymes in the plasma membranes of hepatocytes during injury and subsequent repair, with the aid of phosphatidylcholine liposomes.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 100-120 g. CCl_4 and heliotrine were injected intraperitoneally in doses of 0.25 ml and 25 mg/100 g body weight respectively. The lipid preparations were injected intraperitoneally 24 h after administration of the poison in a dose of 10 mg/100 g body weight. The animals were decapitated 48 h after administration of the poison, their blood was collected, and activity of fructose-1-monophosphate aldolase (FMA) in the serum obtained from it was determined by Braginskii's method [8]. Multilayered liposomes from egg PCh, isolated by chromatography on alumina [3], were formed in 0.9% NaCl solution by Bangham's method [11]. Plasma membranes were obtained from liver cells by centrifugation in a sucrose density gradient [5]. Total ATPase activity of the plasma membranes [5] was determined on the basis of the increase in inorganic phosphate in the course of the reaction [9]. Na,K-ATPase activity was calculated as the difference between total and ouabain-resistant ATPase activity. 5'-nucleotidase activity was determined by the quantity of hydrolyzed phosphorus [13]. Phospholipids were extracted from the plasma membrane fraction by Folch's method [14]. The phospholipid content was determined as the quantity of lipid phosphorus after mineralization [12] and the protein content by Lowry's method [15].

EXPERIMENTAL RESULTS

Two models of toxic hepatitis obtained by intraperitoneal injection of CCl_4 and heliotrine were used. The degree of toxic liver damage 48 h after injection of the poison was estimated, just as previously, as the increase in FMA activity in the blood

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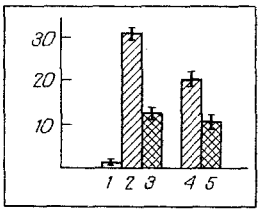

Fig. 1. Action of phosphatidylcholine on serum fructose-1-monophosphate aldolase activity. Abscissa: experimental conditions: 1) control, 2) CCl_4, 3) CCl_4 + PCh, 4) heliotrine, 5) heliotrine + PCh. Ordinate: aldolase activity (in μmoles phosphoglyceric aldehyde/ml serum/h).

serum. Normally the activity of this enzyme, localized in the cytosol of the hepatocytes, is found in rat blood serum in trace amounts (0.8 μmole phosphoglyceric aldehyde/ml serum/h). As Fig. 1 shows, aldolase activity rose sharply (40-fold) after injection of CCl_4. Heliotrine also induced hyperenzymemia, but to a less marked degree (activity rose 25-fold). The increase in aldolase activity is evidence of damage to the hepatocyte plasma membrane as a result of the action of both poisons, or even its complete destruction at the stage of necrosis. Analysis of the phospholipid composition of the damaged plasma membrane revealed a decrease in the content of total phospholipids under the influence of CCl_4 by 21% and of heliotrine by 42% compared with normal (Table 1).

The functional state of the hepatocyte plasma membranes was assessed as activity of membrane marker enzymes Na,K-ATPase and 5′-nucleotidase, whose activity, as a rule, depends on the state of the lipid matrix of the membrane. In the case of liver damage by CCl_4 a decrease in total ATPase activity and Na,K-ATPase activity by half was recorded in the plasma membrane fraction. Heliotrine poisoning was accompanied by reduction of total ATPase activity by half and total inhibition of Na,K-ATPase activity (Table 2). Simultaneously with this, activity of the other plasma membrane marker enzyme 5′-nucleotidase also decreased. In plasma membranes isolated from the liver of animals poisoned with CCl_4 or heliotrine 5′-nucleotidase activity was reduced by 50 and 60% respectively compared with the control preparations. These results also indicate the presence of marked damage to plasma membranes under the influence of heliotrine or CCl_4.

Previous investigations of the endoplasmic reticulum of the liver showed that egg PCh, in the form of multilayered liposomes, can exert a reparative action in vivo if administered to the animal against the background of frank liver damage, i.e., 2 h after injection of CCl_4 [1, 4].

In the present experiments phosphatidylcholine liposomes were used when the liver cells were already damaged, namely 24 h after injection of the hepatotropic poisons, the metabolism of these xenobiotics having already been completed in the first 3 h after their administration. The efficacy of phospholipid therapy was evaluated 24 h after its use. In this particular model, liposome therapy also gave a beneficial effect, based on assessment of the serum hyperenzymemia level. After injection of phosphatidylcholine liposomes the aldolase activity fell by 2.4 and 1.9 times respectively in the case of liver damage by CCl_4 and heliotrine (Fig. 1).

It will be clear from Table 2 that after injection of multilayered egg PCh liposomes the plasma membranes showed a higher level of total ATPase activity than during the original damage. Na,K-ATPase activity, completely inhibited by heliotrine increased up to 67% of the control value. In membranes damaged by CCl_4 Na,K-ATPase activity was restored up to 89% of the control level. The use of phosphatidylcholine liposomes restored activity of the other plasma membrane enzyme, 5′-nucleotidase, to 30-35% of its normal value. This effect was observed on both models of liver damage.

The reparative action of multilayered phosphatidylcholine liposomes on activity of the enzyme systems is in agreement with data on changes in the content of phospholipids in the plasma membranes obtained by both models of toxic hepatitis. As Table 1 shows, injection of liposomes restored the normal phospholipid content in the membrane.

The results are evidence that hepatotropic poisons (heliotrine and CCl_4) cause considerable damage to the structure and function of the hepatocyte plasma membrane. Intraperitoneal injection of egg PCh liposomes partly or completely restored these parameters to normal. Egg PCh is utilized by liver cells to repair the damaged lipid bilayer, thus contributing to restoration of the barrier and matrix functions of the plasma membranes. As a result, on the one hand the passive permeability of the mem-

TABLE 1. Phospholipid Content (in mg P/mg protein) in Hepatocyte Plasma Membrane during Injury and after Repair by PCh ($M \pm m$)

Experimental conditions	Injection of CCl_4		Injection of heliotrine	
	absolute	%	absolute	%
Control	0,29±0,01	100	0,26±0,01	100
Damage	0,23±0,02	79	0,15±0,03	58
Rapair	0,29±0,02	100	0,25±0,02	96

TABLE 2. Enzyme Activity (in μmoles P/mg protein/min) of Hepatocyte Plasma Membrane during Injury and Subsequent Repair by PCh

Membrane preparation	Total ATPase		Na,K-ATPase		5'Nucleotidase	
	absolute	%	absolute	%	absolute	%
Control	0,46±0,04	100	0,27±0,01	100	0,16±0,02	100
CCl_4	0,27±0,02	58	0,14±0,02	52	0,08±0,01	50
CCl_4	0,46±0,03	100	0,24±0,01	89	0,13±0,02	81
Control	0,43±0,08	100	0,18±0,03	100	0,20±0,03	100
Heliotrine	0,21±0,03	49	0	0	0,08±0,01	40
Heliotrine + PCh	0,30±0,03	69	0,12±0,01	67	0,15±0,01	75

brane for cytosol enzymes is reduced, as shown by the lowering of the hyperenzymemia. On the other hand, the repaired matrix leads to normalization of protein–lipid interactions in a membrane, and in turn this promotes restoration of activity of the membrane protein enzymes ATPase and 5'-nucleotidase. Interaction of the injected multilayer liposomes with the plasma membrane of the hepatocytes most likely takes place by a mechanism of insertion of the external bilayer of the liposome into the membrane matrix, with release of the internal phospholipid spheres of the liposome into the cell cytosol [16], where this phospholipid material can be utilized for subsequent repair of intracellular membranous structures, as has been demonstrated for the endoplasmic reticulum [2, 6, 7].

Analysis of the results thus indicates that damaged hepatocyte plasma membranes can be repaired in vivo with the aid of egg PCh liposomes.

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