REPAIR OF HEPATOCYTE MITOCHONDRIAL MEMBRANES WITH THE AID OF PHOSPHATIDLCHOLINE LIPOSOMES

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The ability of various phospholipid preparations to repair liver membranes in vivo when injured in toxic hepatitis is widely known [4, 7, 12]. The reparative effect of phospholipids has been discovered both for membranes of the endoplasmic reticulum [8, 12, 13] and for plasma membranes of liver cells [9]. Restoration of the phospholipid matrix of the membrane helps to restore its functional properties, i.e., normalizes the activity of membrane lipid-dependent enzymes, such as, in particular, glucose-6-phosphatase, cytochrome P-450, and K^+,Na^+-ATPase [5, 7, 13, 15]. In all forms of hepatitis it is the energy-forming apparatus of the cell that is first to be damaged [1, 2]. The aim of this investigation was to study the possibility of repair of mitochondrial membranes in the liver, injured by tetrachloromethane (CCl_4).

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing 90-180 g. Damage to the membranous structures of the liver cells was induced by a single intraperitoneal injection of 0.2 ml CCl_4/100 g body weight. Lipids, in the form of multilayered liposomes were injected intraperitoneally in a dose of 10 mg lipid/100 g body weight, 24 h after injection of CCl_4. The animals were killed 48 h after injection of the poison. Egg phosphatidylcholine was isolated by a modified method [3]. The mitochondrial fraction was obtained by differential centrifugation [11] H^+-ATPase activity was determined by the method described in [12]. The rate of oxygen consumption by the mitochondria was determined polarographically [12]. The phospholipid composition of the mitochondrial membranes was studied by thin-layer chromatography on silica-gel [10]. The phospholipid content was determined from the amount of lipid phosphorus after mineralization of the samples [14]. The microviscosity of the mitochondrial membranes was measured by pulse photometry, using the fluorescent probe trimethoxybenzanthrone, as described in [6], with the probe in a concentration of 2.6 mM, the protein concentration 0.56 mg/kg, and the temperature 25°C. Protein was determined by Lowry's method [16].

EXPERIMENTAL RESULTS

To assess the state of the mitochondrial membrane in the normal and injured state we measured H^+-ATPase activity and calculated the respiratory control (RC), after Chance. H^+-ATPase is an enzyme determining the basic bioenergetic function of the mitochondria. Activity of this enzyme depends both on the lipid composition of the membrane and on its microviscosity.

As Table 1 shows, the hydrolytic activity of H^+-ATPase, when the membranes are modified by CCl_4, is increased fourfold. Evidence of considerable disturbances in electron transmission along the respiratory chain and the absence of regulation of oxidative phosphorylation is given by the results of polarography. After injection of CCl_4 into the animals RC

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TABLE 1. Functional Parameters of Mitochondria during Injury and Repair

Parameter	Control	Action of CCl_4	Repair by PCh
H^+-ATPase, μmoles P_i/min/mg protein	4,56	17,76	7,84
RC after Chance	3,5	1,0	1,65

Legend. Here and in Tables 2 and 3, PCh denotes phosphatidylcholine.

TABLE 2. Content of Total Phospholipids and Microviscosity of Mitochondrial Membrane during Injury and Repair

Parameter	Control	Action of CCl_4	Repair by PCh
Phospholipids, μg/mg protein	0,33±0,01	0,22±0,02	0,28±0,01
per cent of control	100	67	85
Rotary correlation time, msec	12,2±0,5	9,3±0,6	12,9±0,6
Microviscosity, mPa·sec	700±28	530±35	740±50

TABLE 3. Phospholipid Composition of Mitochondrial Membranes during Injury and Repair

Parameter	Control		Action of CCl_4		Repair by PCh	
	μg P_i/mg protein	%	μg P_i/mg protein	%	μg P_i/mg protein	%
CL	24,9±1,0	11	26,0±1,3	13	25,2±0,7	11
PEA	49,1±1,8	21	33,9±2,6	16	45,6±3,9	20
PC	98,3±1,7	42	61,6±6,0	30	81,9±4,2	36
P+PI	8,0±0,6	3	7,0±0,8	3	7,7±0,7	3
PC	10,7±0,2	5	19,1±1,6	9	16,7±0,6	7
SPH	14,4±0,3	6	15,8±1,1	8	14,6±0,9	6
UF 1	28,9±1,4	12	24,9±2,6	12	20,2±1,5	9
UF 2	0	0	18,5±1,7	9	16,3±0,9	7

Legend. CL) Cardiolipin, PEA) phosphatidylethanolamine, PS) phosphatidyl-serine, PI) phosphatidylinositol, LPC) lysophosphatidylcholine, SPH) sphingomyelin, UF1) unidentified fraction 1, UF2) unidentified fraction 2.

after Chance was reduced from 3.5 to 1.0. A single dose of liposomes from egg phosphatidylcholine after CCl_4 poisoning reduced H^+-ATPase activity by half and increased RC after Chance to 1.65. Thus after injection of egg phosphatidylcholine, coupling of respiration and phosphorylation of ADP in the mitochondria was partly restored, as shown by restoration of the normal values of RC and H^+-ATPase activity.

The results of evaluation of structural parameters of the mitochondrial membrane: such as the phospholipid/protein ratio, phospholipid composition, and microviscosity, are given in Tables 2 and 3.

It will be clear from Table 2 that the phospholipid—protein ratio during injury by CCl_4 fell to 67% of the control level. Injection of phosphatidylcholine had a beneficial effect, restoring the total phospholipid content in mitochondrial membranes to 85% of normal.

This analysis of the phospholipid composition of the mitochondria revealed both the pattern of injury to the lipid matrix and the possibility of its correction by the use of liposomes made from egg phosphatidylcholine in vivo (Table 3). Incidentally this damage is accompanied by quantitative redistribution of the phospholipid fractions. The decrease in the percentage of phosphatidylcholine and phosphatidylethanolamine in membranes and the increase in the quantity of lyso-

phosphatidylcholine were most marked. The action of CCl_4 not only was reflected in the phospholipid content, but also caused a qualitative change in the set of phospholipid fractions: additional minor components appeared (fraction 2). As a result of their appearance, in CCl_4 injury 21% of the lipid phosphorus belonged to the unidentified fractions (1 and 2). The mitochondrial membranes responded to injection of egg phosphatidylcholine by a fall in this value to 16% and also by partial restoration of the normal content of phosphatidylcholine, phosphatidylethanolamine, and lysophosphatidylcholine.

The change in the lipid composition under the influence of CCl_4 leads to a change in the physicochemical state of the membrane, as shown by a decrease in microviscosity of the mitochondrial membranes from 700 to 530 mPa·sec and shortening of the rotary correlation time of the fluorescent probe from 12.2 to 9.3 nsec (Table 2). Injection of phosphatidylcholine restores these parameters to normal.

The possibility of repairing damaged mitochondrial membranes with the aid of exogenous phosphatidylcholine, introduced in the form of multilayered liposomes, was thus demonstrated unambiguously. Normalization of the structural parameters of the mitochondria is the prelude to restoration of their functional activity.

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