A POSSIBLE MECHANISM OF THE RESISTANCE OF ENDOPLASMIC MEMBRANES OF THE CIRRHOTIC LIVER TO ACTIVATION OF LIPID PEROXIDATION

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The harmful effects of chronic (3-6 weeks) poisoning of rats with CCl_4 on the structure of the microsomal membrane and content of cytochrome P-450 was studied. The disturbances investigated were found to be partially reversible during repeated exposure to the toxic factor, indicating loss of the ability of CCl_4 to activate peroxidation of lipids in preparations of microsomes from the cirrhotic rat liver. The hypothesis is put forward that the mechanism of molecular adaptation to the chronic action of CCl_4 consists of a change in the fatty acid spectrum of the microsomal phospholipids through replacement of highly saturated fatty acids by fatty acids with a minimal number of double bonds.

The study of the molecular mechanisms of action of toxic doses of carbon tetrachloride has shed light on the primary reactions of activation of lipid peroxidation (LPO) in the endoplasmic membranes of the liver, induced by the toxic agent and, in the opinion of some investigators, leading to marked disorganization of the structure and function of the liver cells [11, 12]. Meanwhile, Hartman et al. [3] have postulated a possible role of LPO reactions in the development of cirrhosis of the liver caused by prolonged poisoning of animals with carbon tetrachloride. It is also known that a single administration of a large dose of CCl_4 to rats with cirrhosis of the liver does not lead to the appearance of classical signs of acute poisoning: central lobular necrosis and fatty degeneration of the liver [17].

The facts described above suggested that the liver develops a definite resistance to the action of CCl_4 during the development of cirrhosis. It seemed likely that this resistance could be due to a change in the sensitivity of the phospholipid component of the membranes of the endoplasmic reticulum to induction of the LPO reaction. To test this hypothesis experimentally, the model of vital activation of the lipid-peroxidase reaction suggested by Hochstein and Ernster [4] was used; the destructive effect of this reaction on the intact microsomal system has been thoroughly investigated [13, 16].

EXPERIMENTAL METHOD

Male Wistar rats were poisoned by repeated inhalations of CCl_4 by the method of Rabinovici and Wiener [10]. Animals of the control (without CCl_4 in the experimental poisoning chamber) and experimental groups were sacrificed after poisoning for 21 and 42 days. Histological examination after CCl_4 poisoning of the animals for 3 weeks revealed evidence of subacute toxic hepatitis, and after 6 weeks marked cirrhotic changes were found in the liver. The microsomal fraction of the liver was isolated by ultracentrifugation of the mitochondrial supernatant at 105,000 g for 60 min in a medium containing 0.35 M sucrose, 0.025 M KCl, 0.01 M $MgCl_2$, and 0.05 M tris-HCl (pH 7.5). The content of microsomal cytochrome P-450 was determined by the method of Omura and Sato [9] with a model 356 Hitachi differential dual-beam spectrophotometer. The intensity of fluorescence of the aniline naphthalene-sulfonic acid (ANS) in the cell containing the microsomal suspension was recorded by means of a spectrofluorometer.

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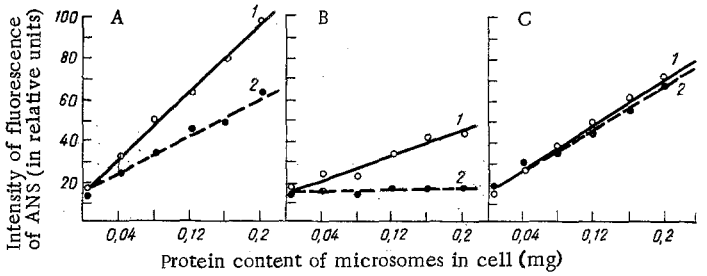

Fig. 1. Effect of activation of enzymic LPO reaction of microsomal membrane on degree of fixation of ANS in control samples (A) and in samples of liver microsomes from rats receiving CCl_4 for 3 (B) and 6 (C) weeks. The intensity of fluorescence of ANS at "zero time" (1) and after peroxidation (2) is shown. Wave lengths of excitation 300 nm, of fluorescence 470 nm. Volume of cell 3 ml; ANS added 10 μM.

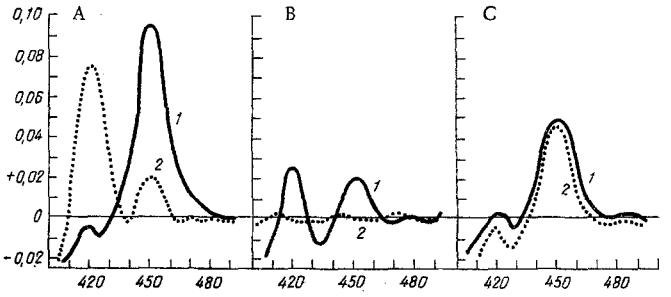

Fig. 2. Effect of peroxidation of microsomal lipids on spectral characteristics of cytochrome P-450. Legend as in Fig. 1. 1) "Zero time"; 2) after enzymic peroxidation for 10 min. Protein in control and experimental samples 2.4 mg in 3 ml medium. Abscissa, wave length (in nm); ordinate, absorption.

The activity of the LPO systems in the microsomal membranes was determined by the thiobarbiturate reaction from the quantity of malonic dialdehyde (MDA) formed [1]. The composition of the incubation medium of the NADP·H_2-dependent and ascorbate-dependent LPO systems was identical with that used by Hochstein et al. [5] to achieve the maximal reaction velocity. The incubation time was calculated from the moment of addition of the excess NADP·H_2 (0.8-1 mM) and all the above parameters before the addition of the reduced NADP were taken as the indices of "zero time." The specimens were incubated at 37° for 10 min with constant agitation. At the end of this time the reaction was stopped by the addition of 300 M EDTA. The optical density of the microsomal suspension was measured during incubation spectrophotometrically at 520 nm [13]. The basic incubation medium contained: 125 mM KCl + 20 mM tris-HCl (pH 7.4). Protein was determined by the method of Lowry et al. [6].

EXPERIMENTAL RESULTS AND DISCUSSION

Prolonged poisoning of the animals with CCl_4 led to structural disturbances in the microsomes as shown by a decrease in the hydrophobic properties of the microsomal membrane recorded [15] as a decrease in intensity of fluorescence of ANS (Fig. 1:1). In addition, the quantity of cytochrome P-450 incorporated into the endoplasmic membranes and responsible for oxygenase reactions of mixed type [7] was reduced by 80% after poisoning for 3 weeks and by 53% after 6 weeks (Fig. 2:1). It is particularly important to note that between the 4th and 6th weeks of poisoning inclusive, i.e., during continued exposure to the action of the toxic factor, the progressive development of cirrhosis of the liver was accompanied by a relative increase in the hydrophobic properties of the microsomal membrane (Fig. 1) and in the content of P-450 (Fig. 2), indirect evidence of the formation of definite resistance of the microsomal membrane to the harmful action of CCl_4.

TABLE 1. Changes in Lipid-Peroxidase Activity and Decrease in Optical Density of Microsomal Suspension at Different Stages of Cirrhosis of the Liver Induced by CCl_4

	Activity of NADP·H_2-dependent lipid-peroxidase reaction (in mμM MDA/min/mg protein)	Decrease in optical density of microsomal suspension after peroxidation of lipids for 10 min (in % of value at "zero time")
Control Samples	4.8± 0.4	35± 5
Samples from rat liver:		
after inhalation of CCl_4 for 3 weeks	6.3± 0.5	58± 3
after inhalation of CCl_4 for 6 weeks	0.2± 0.02	2±0.5

*Livers of six rats were used for each microsomal sample. Results given are mean values for 8-9 experiments.

The results given in Fig. 2 show that the spectrum of cytochrome P-450 underwent changes of a fundamentally different character in the experimental and control samples of microsomes on activation of the enzymic LPO reaction. In the latter conversion of cytochrome P-450 into its inactive form (P-420) was observed (see Fig. 2A), evidently as the result of a change in the hydrophobic properties of the microsomal membrane through the destructive action of lipid peroxides on its phospholipid component. According to the observations of Tam and McCay [13], the decrease in optical density of the microsomal suspension, the accumulation of an LPO product, namely MDA (Table 1), and also the decrease in fluorescence of ANS (Fig. 1A) observed in these experiments were evidence of damage to the membrane phospholipids.

By contrast with the control samples, in microsomal samples from the liver of rats inhaling CCl_4 for 3 weeks initial damage to the membrane has been followed by complete disorganization of themicrosomes on activation of the enzymic LPO reactions, manifested by the absence of the spectral characteristics of cytochrome P-450 or even P-420 (Fig. 2B) and by minimal activity of the microsomal membrane with respect to ANS fixation (Fig. 1B). Peroxidized specimens of microsomes at this period showed a greater degree of accumulation of MDA and a greater decrease in optical activity than in the control (Table 1). Finally, microsomes from the cirrhotic liver were completely resistant to induction of the LPO reaction, as shown by the minimal accumulation of MDA, by the very slight decrease in optical density of the suspension (Table 1), and also by the absence of changes in the spectrum of cytochrome P-450 (Fig. 2B) and the identical degree of fluorescence of ANS before and after activation of the enzymic LPO reaction (Fig. 1C). The results described in this paper thus indicate that induction of the lipid-peroxidase reaction in the microsomes of the liver with developing cirrhosis is sharply impeded. Remembering that the decrease in hydrophobic properties of the membrane, the decrease in optical density of the microsomal suspension, and the conversion of P-450 into P-420 are manifestations of damage to the microsomes it can be concluded that the development of cirrhosis in the liver through CCl_4 poisoning leads to the appearance of resistance of the microsomal membranes to LPO reactions.

The work of Tam and McCay [8] showed that MDA formation during LPO activation in the microsomes is connected with destruction of highly unsaturated fatty acids of the phospholipids of the microsomal membrane, which account for 15% of the total fatty acid content. This fact and the results of the present experiments evidently provide a sufficiently firm basis for two conclusions. The first is that the highly unsaturated fatty acids of the membrane phospholipids play a decisive role in maintaining the hydrophobic properties of the membrane and also the functional and spectral characteristics of a vital component of the microsomal electron transport chain – cytochrome P-450. The second conclusion is that the structural changes taking place in the microsomes in the course of chronic CCl_4 poisoning and the development of cirrhosis of the liver are aimed at reducing the degree of unsaturation of the fatty acids of the membrane phospholipids. This process is evidently a manifestation of molecular adaptation at the level of the microsomal membranes to the repeated action of the toxic factor on the cell.

A change in the fatty-acid composition of the subcellular membranes toward a higher level of saturation has been recorded in certain situations. In particular, Van Deenen et al. [14] showed that a diet containing saturated fatty acids leads to a predominance of fatty acids with fewer double bonds than in the control in the phospholipids of the liver. A similar process is observed during regeneration, when 30 h after hepatectomy the content of arachidonic acid ($C_{20:4}$) in the phospholipids of the liver falls while the content

of oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) acids rises [2]. It can be concluded from these examples that the suggested mechanism of molecular adaptation during chronic exposure to a harmful factor, involving structural reorganization of the subcellular membranes, is not artificial and has definite analogies in animal tissues.

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