The need to "reprocess" the surface energy mainly with a view to its long-term accumulation in the form of high-molecular-weight compounds [6], evidently was responsible for the increase in bulk and numerical density of peroxisomes (Table 2).

During consumption of a low-protein diet, besides evidence of adaptive structural changes in the liver parenchyma (proliferation of hepatocytes, an increase in the number of secondary lysosomes, undertaking mobilization and redistribution of endogenous reserves) [7], which evidently took place in the early period of consumption of the diet, atrophic changes thus also were observed. These latter evidently predominated in the later periods of consumption of the low-protein diet by the animals. If the protein intake was excessive, on account of the increased functional load on the liver, adaptive structural changes in its parenchyma were expressed as hypertrophy of hepatocytes and hyperplasia of their subcellular structures.

LITERATURE CITED

1. V. Ya. Brodskii, N. N. Tsireksedze, M. E. Kogan, et al., Tsitologiya, 25, 260 (1983).
2. V. A. Konyshev, Diet and Regulating Systems of the Organism [in Russian], Moscow (1985).
3. B. N. Kudryavtsev, M. B. Kudryavtseva, E. É. Zavadskaya, et al., Tsitologiya, 24, 431, (1982).
4. É. M. Kurashov, Tsitologiya, 18, 1406 (1976).
5. N. N. Lapteva, The Pathophysiology of Protein Metabolism [in Russian], Moscow (1970).
6. L. F. Panchenko, A. M. Gerasimov, and V. D. Antonenkov, The Role of Peroxisomes in Cell Pathology [in Russian], Moscow (1981).
7. A. A. Pokrovskii and V. A. Tutel'yan, Lysosomes [in Russian], Moscow (1976).
8. Z. A. Ryabinina and V. A. Benyush, Polyploidy and Hypertrophy of Cells in Growth and Restoration Processes [in Russian], Moscow (1973).
9. P. Fabry, Čsl. Fysiol., 8, 529 (1959).
10. K. Fletcher, Amer. J. Clin. Nutr., 19, 170 (1966).
11. A. V. Loud, J. Cell Biol., 37, 27 (1968).
12. P. B. Lazarow, in: The Liver: Biology and Pathobiology, New York (1982).
13. M. M. Mucekler and H. C. Pitot, J. Cell Biol., 90, 495 (1981).
14. D. S. Sarma, E. Verney, and H. Sydransky, Lab. Invest., 27, 48 (1972).

EFFECTIVENESS OF COLCHICINE IN EXPERIMENTAL ALCOHOL-INDUCED LIVER DAMAGE

A. S. Loginov, K. D. Dzhalalov,
É. A. Bendikov, Yu. E. Blok,
and S. M. Chebanov

UDC 616.36-004.4-085.277.3:
547.944.6]-035.1

KEY WORDS: liver; alcohol-induced damage; colchicine.

An important role in the pathogenesis of the toxic action of alcohol on the liver is played by disturbances of a number of enzymic and metabolic processes in hepatocytes. Induction of mono-oxygenases and mobilization of cytochrome P-450-dependent hydroxylation reactions (including the microsomal ethanol-oxidizing system), initiation of free-radical reactions and processes of lipid peroxidation (LPO), as well as activation of mesenchymal reactions (collagen synthesis, fibrillogenesis), deserve attention in this context [3].

The first two mechanisms are provided by a single NADPH-dependent electron and proton transport system in membranes of the endoplasmic reticulum (ER) of the hepatocytes [1]. Under the influence of oxidation of ethanol, conditions are created in ER for intensification of free-radical reactions on induced cytochrome P-450 and an increase in the activity of peroxidation of cell membrane phospholipids [2, 9, 11]. As a result of enzymic and metabolic modifications in the hepatocytes under the influence of ethanol mesenchymal reactions are activated.

Central Scientific-Research Institute of Gastroenterology, Moscow.

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 101, No. 5, pp. 559-562, May, 1986. Original article submitted April 25, 1985.

0007-4888/86/0005-0610$12.50

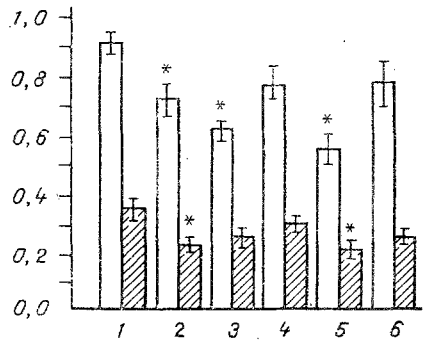

Fig. 1. Concentrations of cytochromes P-450 and b_5 (nanomoles/mg protein) in rat liver during chronic alcohol poisoning and treatment with colchicine. 1) Control; 2) ethanol; 3) colchicine; 4) ethanol + colchicine; 5) ethanol + CCl_4; 6) ethanol + CCl_4 + colchicine. Here and in Fig. 2, asterisk indicates statistically significant differences from control.

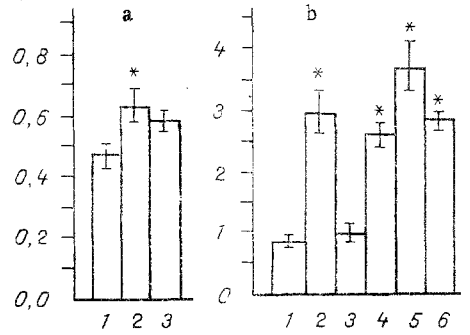

Fig. 2. Concentrations of lipid hyperperoxides (in relative units) and fluorescent LPO products (in relative units/mg lipid) in rat liver during chronic alcohol poisoning and treatment with colchicine. a) Concentration of lipid hyperperoxides A_{232}/A_{215}; 1) control; 2) ethanol; 3) ethanol + CCl_4 + colchicine; b) concentration of fluorescent LPO products: 1) control; 2) ethanol; 3) colchicine; 4) ethanol + colchicine; 5) ethanol + CCl_4; 6) ethanol + CCl_4 + colchicine.

The aim of this investigation was experimental verification of the possible role of inhibition of cytochrome P-450-dependent hydroxylation reactions, enzyme-dependent LPO, and collagen synthesis in the hepatoprotective effect of the antifibrotic agent colchicine [6], in alcohol-induced liver damage.

EXPERIMENTAL METHODS

Experiments were carried out on 77 noninbred male albino rats weighing 200-220 g. The animals were divided into seven groups: 1) control (n = 15), 2) rats (n = 10) receiving ethyl alcohol once daily by gastric tube in the morning before feeding, in a dose of 0.9 g/100 g body weight, for 10 days, 3) ethyl alcohol given to the rats (n = 12) in the same dose for

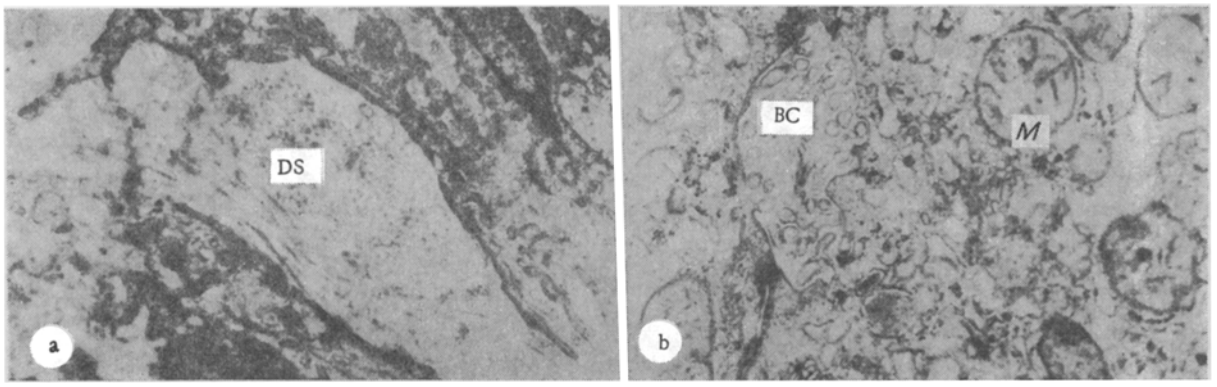

Fig. 3. Ultrastructural changes in rat liver during chronic alcohol poisoning (a) and treatment with colchicine (b). a) Electron micrograph of rat liver; DS is Disse's space with many collagen fibers; b) the same, after injection of ethanol + CCl_4 + colchicine; single collagen fibers in hepatocytes, structure of mitochondria (M) and of ER is preserved. BC) Biliary canaliculus.

3 months, 4) an aqueous solution of colchicine in a dose of 0.001 mg given once a day to the animals (n = 10) before feeding for 3 months, 5) ethyl alcohol given to the animals (n = 9) once daily and colchicine given for 3 months in the same dose, 6) besides ethanol, rats (n = 11) given CCl_4 intramuscularly in a dose of 0.1 ml/100 g body weight every 3 days for 1 month, 7) besides ethanol and CCl_4, rats (n = 9) receiving colchicine once a day in the same doses for 1 month. The liver tissue was investigated morphologically by light and electron microscopy.

Concentrations of cytochromes P-450 and b_5 in the microsomal-cytosal fraction of rat liver was estimated by differential spectrophotometry [8]. Total lipids were extracted from liver homogenate with a mixture of chloroform and methanol [7]. The concentration of LPO products in rat liver homogenate was measured as accumulation of hydroperoxides with conjugated double bonds and of fluorescent compounds [10]. The concentrations of hydroperoxides were determined by means of a Perkin-Elmer 555 differential spectrophotometer (Sweden), and fluorescent LPO products were estimated by means of a Hitachi-850 spectrofluorometer (Japan).

RESULTS

Comparison of the cytochrome P-450 concentration in the liver of the control rats and of animals receiving ethanol for 10 days (group 2) revealed and enzyme induction effect. The mean increase in the cytochrome P-450 concentration in these cases was 22%. Electron-microscopic investigation of the liver of the rats of group 2 revealed moderate hyperplasia of the smooth ER of the hepatocytes.

In the animals of group 3, on the other hand, the concentrations of cytochromes P-450 and b_5 in the liver were lowered to 0.73 ± 0.05 and 0.23 ± 0.02 nmole/mg protein respectively ($P < 0.05$; Fig. 1). Meanwhile a marked increase in the concentration of fluorescent LPO products was observed in the liver of the rats of this group ($P < 0.001$; Fig. 2). On histologic investigation of the liver tissue of the rats of group 3, in most cases diffuse fatty degeneration in the form of tiny droplets, microfocal infiltration with lymphocytes, sclerosis of the central veins, and moderate pericellular fibrosis were found. Ultrastructural changes were characterized by lipid inclusions in the cytoplasm of most hepatocytes, disturbance of the integrity of the outer mitochondrial membranes, and degranulation of the rough and vacuolation of the smooth ER. Accumulation of collagen fibers was observed in Disse's space (Fig. 3).

In animals receiving the hepatotoxine (ethanol + CCl_4) combined with colchiine, degenerative changes in the organelles of the hepatocytes were much less marked than in rats receiving the same hepatotoxic compounds but without colchicine (Fig. 3b). Colchicine also had a marked inhibitory effect on fibrillogenesis in the liver, induced by alcohol in conjunction with CCl_4.

Meanwhile colchicine had a marked hepatoprotective effect on the rats with liver damage induced by alcohol poisoning, reflected in a decrease in the degree of fatty infiltration of the hepatocytes and injury to the membranes of the mitochondria and ER. Meanwhile colchicine

exhibited definite antifibrotic properties, inhibiting accumulation of mature collagen fibers in the liver (Fig. 3b). There is reason to suppose that colchicine blocks the intracellular stage of collagen synthesis and inhibits its secretion at the microtubule level as a result of binding of specific proteins (tubulins) [4, 6].

The most marked decrease in the concentrations of cytochromes P-450 and b_5 and the greatest rise in the levels of lipid hydroperoxides and fluorescent LPO products in the liver were observed in the rats of group 6 (Figs. 1 and 2). These changes in enzyme activity were accompanied by correspondingly more marked ultrastructural disturbances in the hepatocytes.

On the basis of the study of changes in enzyme systems in alcohol-induced liver damage it can therefore be concluded that initial induction of monooxygenases by ethanol and mobilization of cytochrome P-450-dependent hydroxylation reactions play a role in the accumulation of free radicals on cytochrome P-450. This state of affairs is confirmed by the ethanol-dependent increase in the concentration of primary LPO products, namely hydroperoxides with conjugated double bonds, which appear in the stage of free radical formation in the molecule of polyunsaturated fatty acids [11]. Probably the high hepatotoxic effect of CCl_4 [1], which subsequently cause destruction of ER membranes and a fall in the mono-oxygenase level, also is connected with induction of cytochrome P-450 and enhanced formation of the CCl_3 radical.

Meanwhile administration of colchicine to the rats for 3 months (group 4) caused a significant fall in the concentrations of cytochromes P-450 and b_5 in the liver in the absence of any changes in the ultrastructural organization of the hepatocytes, in agreement with data in the literature [5]. In addition, colchicine lowered the level of formation of initial and end products of enzyme-dependent LPO and their accumulation in the liver under the influence of ethanol alone (group 5) and of ethanol combined with CCl_4 (group 7; Fig. 2). It can be tentatively suggested that under these circumstances of limitation of mono-oxygenase induction and of free radical formation on cytochrome P-450, colchicine prevents the development of peroxidation of phospholipids of the endoplasmic membranes, by reducing the degree of degradation of microsomal hemoproteins (Fig. 1). However, the concentration of LPO products in chronic alcohol poisoning combined with the action of CCl_4 did not reach the control values under the influence of colchicine, for the latter evidently does not inhibit components of the NADPH-dependent electron transport system responsible for enzyme-induced lipid peroxidation.

These results thus provide a basis for the further clinical study of the action of colchicine on patients with alcohol-induced liver damage.

LITERATURE CITED

1. A. I. Archakov, Microsomal Oxidation [in Russian], Moscow (1975), p. 161.
2. Yu. A. Vladimirov and A. I. Archakov, Lipid Peroxidation in Biological Membranes [in Russian], Moscow (1972).
3. A. S. Loginov, K. D. Dzhalalov, and Yu. E. Blok, Pathogenesis, Diagnosis, and Treatment of Alcohol-Induced Liver Damage [in Russian], Moscow (1984), p. 67.
4. A. Frey-Wissling, Comparative Organography of the Cytoplasm [Russian translation], Moscow (1976), p. 111.
5. H. Denk and R. Eckerstorfer, Lab. Invest., 36, 563 (1977).
6. A. Floridi, C. Fini, C. Palmerini, et al., Gastroenterology, 84, 1371 (1983).
7. J. Folch, M. Lees, and S. Sloane-Stanley, J. Biol. Chem., 226, 497 (1957).
8. T. Omyra and R. Sato, J. Biol. Chem., 239, 2370 (1969).
9. T. Slater, in: International Workshop on (+)-Cyanidanol-3 in Diseases of the Liver, ed. by H. O. Cohn (1981), p. 11.
10. M. L. Tappel, Ann. New York Acad. Sci., 203, 12 (1972).
11. J. Videla, A. Fernandes, et al., in: International Workshop on (+)-Cyanidanol-3 in Diseases of the Liver, ed. by H. O. Cohn (1981), p. 11.