EFFECT OF INCREASED COPPER AND ZINC DOSES ON THE PATHOMORPHOLOGICAL LIVER CHANGES IN CHRONIC EXPERIMENTAL CARBON DISULFIDE INTOXICATION

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CARBON disulfide is a potent hepatotoxic agent. Centrilobular necroses and fatty infiltration of the liver have been demonstrated pathomorphologically following continuous exposure of rats to carbon disulfide /6, 8, 10/. Presumably, elementary sulfur, released in the liver during carbon disulfide biotransformation, and forming covalent bonds with the microsomal proteins of liver cells, is to be considered as the underlying cause of such disorders /5, 12/.

On the other hand, the carbon disulfide metabolites (dithiocarbamates, trithiocarbamates, xanthogenates and thiasolidon) exert a chelating effect on some metal ions - zinc, copper, iron, manganese, cobalt etc. - as a result of which depletion of these microelements from their biologically active centers in the organism occurs /7/.

It is the purpose of this work to assay under experimental conditions the effect of compensating the deficit of the microelements copper and zinc in the organism of white rats on the development of histological and ultrastructural changes in the liver against the background of chronic carbon disulfide intoxication.

MATERIAL AND METHODS

To gain better insight into the problem, a 90-day-long experiment was conducted on forty male white rats of the Wistar line, with initial body mass (bm) 130 ± 10 g, distributed into five groups as follows:

Group one - control - fed standard diet.
Group two - standard diet plus exposure to carbon disulfide.
Group three - zinc enriched diet (12.4 mg/kg bm) plus exposure to carbon disulfide.
Group four - copper enriched diet (4.5 mg/kg bm) plus exposure to carbon disulfide.
Group five - diet enriched with zinc (12.4 mg/kg bm) and copper (4.5 mg/kg bm) plus exposure to carbon disulfide.

The carbon disulfide concentration selected for experimental animals' exposure - 50 mg/m³ atmospheric air - was conditioned by the existing
mean concentrations of this toxic agent in the production of artificial fibers in the “Sviloza” Works - Svishtov, established by the Research Institute of Hygiene and Occupational Diseases, being five times higher than the maximum allowable one on a nationwide scale (MAC = 10 mg/m³ air). The animals were exposed to carbon disulfide for six hours daily in inhalatory chambers, five times per week. For comparison the controls were likewise placed in inhalatory chambers, but treated with air only.

Composition of the control standard diet was based on literature data /4/, and was very close to the normatives for physiological requirements for white rats. With prophilactical purpose the amount of zinc in the experimental diet was increased as much as 5 times, and that of copper - 10 times, by comparison with the standard one. Our goal was to compensate the deficit of these microelements in chronic copper disulfide poisoning, with simultaneous abiding to their balanced ratio in the diet. Microelements were fed in the form of carbonate salts, ZnCO₃ and CuCO₃ respectively.

At termination of the experiments, the animals were sacrificed by decapitation. Pieces measuring 1 cm³ were obtained from the liver, and fixed in 10 per cent neutral formula for histological study. Staining was done with hematoxylin eosin after the classical histomorphological method.

Electron microscopic study was carried out on liver sections, measuring 1/1 mm, fixed in 4 per cent glutaraldehyde in cacodylate buffer, followed by postfixation in osmium tetroxide, embedding in durecopan, cutting in ultramicrotome Reichert-OM₂, contrast staining with uranyl acetate and lead nitrate, and observation and examination in electron microscope Hitachi-H7S.

RESULTS

Histological and ultrastructural study in the control group of animals did not show any pathological changes in hepatocytes.

In the carbon disulfide-treated group the histological findings disclosed: strong congestion, particularly of venae centrales; evidence of fatty, vacuolar and parenchymatous dystrophy, as well as centrilobular atrophy of the hepatic trabeculae in part of the lobules; enlargement of periportal spaces by proliferating connective tissue, or small-cell infiltrates from lymphocytes, plasmocytes, monocytes and histiocytes.

The electron-microscopic findings in the liver of these animals were characterized by: pyknotic nuclei in the periphery of hepatic lobules; ununiform chromatin dispersion in the nuclei; undulated and smudged (blurred) nuclear membrane; slight degranulation of granular endoplasmic reticulum; polymorphous mitochondria, eventually distended or ballooned,
part of which presenting a severe form of dystrophy with complete de¬
letion of the structure and formation of myelin figures (Fig.1); dislocation,
disruption and reduction of cristae mitochondriales; increased number of lysosomes with predomination of secondary forms; zones of focal cell degeneration and lesions in the hepatocytes; small- and medium-sized droplets.

Fig. 1: Electron microscope picture of the liver of animals, given standard diet and exposed to carbon disulfide. Magnif - 25000

In animals receiving larger zinc dose, and exposed to carbon disulfide, histologically in the liver were observed: atrophic zones between the hepatic trabeculae: markedly expressed vacuolar fatty and parenchymatous dystrophies; periportal spaces expanded by lymphocyte, plasmocyte, monocyte and histiocyte infiltrates, and connective tissue proliferation; proliferations and dilations of bile ductules, often obstructed by biliary thrombi; acidophilic necrosis foci in hepatocytes, mainly centrilobularly and periportally; formation of granulation tissue abounding of capillaries.

In this group of animals the electron microscopic findings presented nuclei with undulated membranes: unevenly dispersed chromatin; pyknotic nuclei mainly in the periphery of hepatic lobuli where the nuclear membrane integrity was disrupted; intranuclear lipid inclusions in single hepatocytes; reduced in quantity and degranulated granular endoplasmic

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reticulum, represented by dilated and swollen vacuoles; polymorphous mitochondria with considerable atrophic and dystrophic changes - edematous, distended and myelinated with a diminished cristae (Fig. 2); increased number of lysosomes with a prevalence of secondary forms - cytolysosomes, cytosegrosomes; lesion zones within the cytoplasm, filled up with homogeneous structureless material; small-, medium- and large-sized lipid droplets, located intra- and extracellularly; activated Kupffer's cells containing profuse amount of phaged material.

Fig. 2: Electron microscope picture of the liver in animals given increased zinc dose and exposed to carbon disulfide effect. Magnif - 15000

Histological findings in animals treated with a larger copper dose plus carbon disulfide exposure: there was evidence of parenchymatous, fatty and vacuolar dystrophy; single hepatocytes with acidophilic necrosis; at some points proliferations of the epithelium of biliary ductules in the periportal spaces, occasionally associated with small cell infiltrates. Here the electron microscope study of liver cells revealed the usual form and size of the nuclei; chromatin dislocated unevenly; granular endoplasmic reticulum represented by tubules parelelly arranged one on top of the other, mainly granular mitochondria, some of them dystrophic with a reduced number of irregularly dislocated cristae; equally increased primary and secondary forms

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of lysosomes; sparsely grouped lipid droplets in groups of 2-3, eventually extracellularly also; - and local cell degeneration; moderately activated Kupffer's cells.

In the liver of animals given simultaneous treatment with elevated doses zinc and copper plus carbon disulfide exposure, the histological study revealed: strong congestion, of venae centrales in particular; evidence of weakly pronounced fatty and parenchymatous dystrophy; periportal spaces seldom dilated by small-cell infiltrates, as well as by connective tissue proliferation, moderate atrophy of liver trabeculae situated centrilobularly.

Here the electron microscope picture of the liver was characterized by normal structure of the nuclei of hepatocytes; granular endoplasmic reticulum represented by tubules some of which dilated; hyperplastic smooth endoplasmic reticulum with moderately increased number of small to medium-sized vesicles; predominance of granular mitochondria (Fig. 3) with lipid inclusions in some of them; increased number of lysosomes regarding secondary forms; lipid droplets in individual hepatocytes; proliferation of collagen fibers in the vicinity of sinusoids; moderate activation of Kupffer's cells with augmented number of pinocytotic vesicles and phaged material.

Fig. 3: Electron microscope picture of the liver of animals, given increased doses of copper and zinc, and exposed to carbon disulfide effect. Magnif - 19500.
DISCUSSION

Our results about the effect exerted by carbon disulfide on rat liver (cytoplasmic degeneration documented histologically and electron-microscopically, mitochondrial lesions, reduced number of ribosomes bound to the granular endoplasmic reticulum, increased number of lysosomes, necrotic foci etc.) are consistent with data reported by other authors /11, 13/. Presumably, elementary sulfur, released in the organism during carbon disulfide metabolism and damaging a number of cell structures, is the underlying cause of the dystrophic and necrotic processes in hepatocytes. According to Kundiev et al. (1978), this is exactly the mechanism inhibiting the hepatic monooxigenase system with substrate involving numerous endogenic products (cholesterol, fatty acids, prostaglandins, steroid hormones) which in turn, leads to their excessive accumulation in hepatocytes and to the pathomorphological changes observed.

In our study one is impressed by the fact that the increased amount of zinc, given to carbon-disulfide-poisoned animals, accounts for intensification of the histological and ultrastructural changes in the liver, whereas when a larger dose of copper is added to the diet (alone or in combination with zinc), the deleterious effects are somewhat restricted. These data correlate well with our results, pointing to increased levels of total fats and cholesterol in the blood serum of carbon-disulfide-poisoned animals, given additionally zinc, and to normalization of the listed serum lipid indicators in rats receiving high copper diet (alone or in combination with zinc) /2/.

Depletion of the microelement copper from the sites of exerting its biological effect in the organism is a well established fact in carbon disulfide intoxication /7/. Along with that, in rat copper deficit (induced by deficiency in this particular bioelement, or by altered zinc: copper ration with a considerable prevalence of zinc in the diet) many investigators demonstrate an elevated content of total fats in the liver and hypercholesterolemia, reversible following adequate import of the bioelement in question /9/. Using labelled mevalonic acid, Allen and Klevay (1978) were successful in proving that in case of copper deficit cholesterol synthesis in the liver is enhanced, but since its clearance to peripheral tissues is accelerated at higher rate, no rise in cholesterol content in the organ occurs.

Hence, the assumption is warranted that besides by inhibiting the hepatic monooxigenase system, copper deficit in the organism against the background of chronic carbon disulfide intoxication also contributes to dystrophic and necrotic changes development in the liver by stimulating the synthesis and accumulation of certain lipid fractions in hepatocytes.
CONCLUSIONS

The experiment performed confirms the substantial pathomorphological changes (proved histologically and electron-microscopically) taking place in the liver of white rats under the effect of carbon disulfide, already established by other authors.

Administration of larger doses of the microelement zinc, in conjunction with carbon disulfide intoxication, further intensifies the pathological changes in the liver of white rats.

Ingestion of increased copper doses (alone or in combination with zinc) against the background of carbon disulfide poisoning in white rats restricts to some degree the pathomorphological changes in the liver.

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