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Influence of chronic stress and long-term administration of zinc on morphology of the rat liver: histological and morphometrical studies

Among other effects, stress is known to alter various drug-metabolizing enzymes *in vivo* and *in vitro*, which may, in turn, result in severe health problems (9). Little is known about the biochemical mechanisms by which stress affects liver xenobiotic metabolism. Most studies have focused on alterations at the level of hypothalamo-pituitary-adrenal (HPA) axis. Glucocorticosteroids (GCs), which are produced by the stress-responsive HPA axis, are among the most important modulators of cytochrome P450s and several other enzymatic systems that play a central role in the metabolism of a large number of exogenous compounds, including drugs and environmental chemicals. Only few earlier investigations described morphological changes in the liver after chronic stress. Animal studies demonstrated alterations in hepatic blood flow, reduction in hepatic microsomal activity and reduction in hepatic excretion process in animals subjected to chronic stress (17). It was also shown that long-term stress decreases the liver resistance to the action of hepatotoxic chemicals and delays the process of repair of the liver parenchyma after its damage (16).

Zinc is an essential trace element and has important biological functions that control many cell processes, including DNA synthesis, normal growth, brain development, reproduction, behavioral development, fetal development, bone formation and wound healing (1). It is essential for the function of more than 200 enzymes. Zinc supplementation is known to be beneficial in a few disease and stress conditions (18). Zinc has been reported to act as antioxidant. Zinc repletion was known to reduce the free radical production in mice, and intravenous injection of zinc to guinea pigs was reported to inhibit superoxide production. (18). Zinc has been shown to protect *in vivo* from a variety of hepatotoxic agents, including carbon tetrachloride, bromobenzene, pentobarbital, alkylating agents, acetaminophen, copper, lead, manganese, nickel and cadmium (18). On the other hand, exposure to zinc, especially at higher doses, may produce toxic effects on various tissues and organs (13). Zinc excess produces toxicity in the liver. As shown by the ultrastructural examination, there is evidence of toxic injury to the hepatocytes of zinc treated mice (2).

Stress-induced behavioral disturbances such as motor activity deficit, reduced food and water consumption and decrease of responsiveness to rewarding stimuli resemble clinical symptoms of human depression and are often used as animal model of depression. The data obtained from clinical observations indicate lower serum zinc level in depressed patients. Moreover, the negative correlation between serum zinc level and Hamilton scale was observed (10). Clinical studies demonstrated a beneficial effect of zinc supplementation in antidepressant therapy (12). Regarding the necessity of

chronic zinc supplementation in depressed patients it seems needful to investigate the influence of chronic zinc therapy on the structure of internal organs, especially the liver.

The present study investigated the influence of chronic stress on the morphological structure of the rat liver. The experiments were done on Wistar male rats using the CUMS (chronic unpredictible mild stress) procedure. We also assessed the influence of two zinc compounds - zinc aspartate and zinc sulphate – on the stress-induced changes in the liver morphology. Histological examination of liver slices stained with hematoxylin and eosin, Masson's technique and PAS method were performed with the use of light microscope. Ouantitative analysis of morphological changes was carried out using a computer analyser of histological pictures (Lobophot 2, Nicon).

MATERIAL AND METHODS

The experiments were carried out on male Wistar rats weighing 180–200g at the beginning of the experiment. Care and treatment of the animals were in accordance with the guidelines for laboratory animals, the Local Ethical Committee of the Medical University of Lublin No. 388/02. The animals were kept under standard laboratory conditions, with free access to granular standard diet and tap water. Experiments were performed between 8.00 and 15.00 h.

Animals were subjected to chronic unpredictable mild stress (CUMS). The model of CUMS consisted of several stressors, which were performed every day for 16 weeks. CUMS procedure included the following kinds of stressors (one stressor per day): 14h period of 45 cage tilt, 2h period of immobilization at 20°C or at 4°C, 5 min exposure to electric bell, 3 min period of swimming in cold water (12°C) or 5 min period of illumination (80±1 klx) and 48h period of food deprivation. These stressors were repeated during the 16-week stress procedure.

The following drugs were used: zinc aspartate (Farmapol, PL) and zinc sulphate (POCH, PL). The drugs were administered intraperitoneally (ip) once daily during 16-weeks CUMS procedure 1h before every stress session. Zinc aspartate was administered at a dose of 15mg/kg/24h (elemental zinc dose of 2.7mg/kg/24h) and zinc sulphate at a dose of 15mg/kg/24h (elemental zinc dose of 3.0mg/kg/24h).

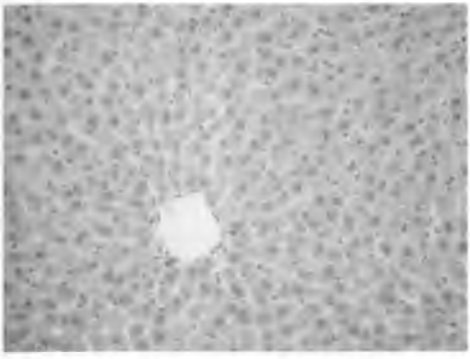
The animals were divided into six groups (including 6 animals each): control group – distilled water (ip 2 ml/24h) for 16 weeks, experimental group I – CUMS+H_2O dest, experimental group IIA – zinc aspartate, experimental group IIB – CUMS+zinc aspartate, experimental group IIIA – zinc sulphate, experimental group IIIB – CUMS+zinc sulphate.

48h after the end of the experiment all animals were decapitated and their livers were taken for histological examinations. Liver specimens fixed in 4% formalin were dehydrated in graded ethanol solutions and embedded in paraffin. Seven-ěm thick paraffin slices were stained with hematoxylin and eosin (H+E), Masson's technique, PAS method and assessed using a light microscope. Ouantitative analysis of morphological changes was carried out by measuring of the thickness of the liver external connective tissue capsule in slides stained with Masson's technique and measuring of the surface area of sinusoidal capillaries in the subcapsular region using a computer analyser of histological pictures (Lobophot 2, Nicon). All data are presented as means±SEM. The statistical significance of the difference between groups was assessed by Student's test. Statistically significant differences were designated at $P<0.05$.

RESULTS

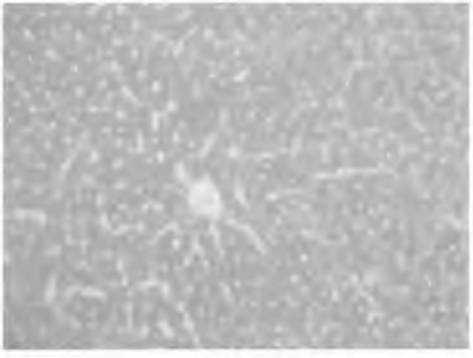
THE CONTROL GROUP (FIG.1)

Liver stained with hematoxylin and eosin evidenced a regular architectonics of the liver lobules. The hepatocytes were clearly contoured and formed quite regular trabeculas. The hepatocytes nuclei were regular in shape (round or oval) with quite regularly distributed chromatin. Most hepatocytes presented one nucleus, sometimes two. Hepatocytes cytoplasm showed an affinity to acidic stains and possessed thick basophilic granules regularly located in the liver cells. Single erythrocytes were found in the sinus lumen. The endothelial cells were flattened and Browicz-Kupffer cells were observed in the walls of the sinuses. Staining by the Masson's technique revealed a small amount of connective tissue in the vicinity of the liver triads and between liver lobules.



1A. H+E staining

1B. Masson's staining

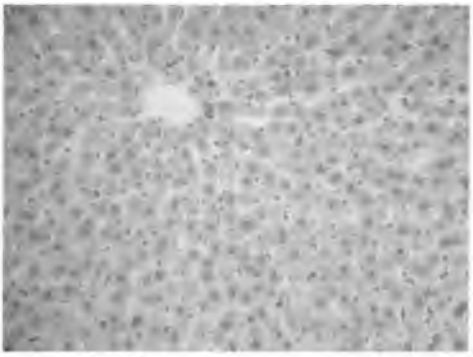


1C. PAS method

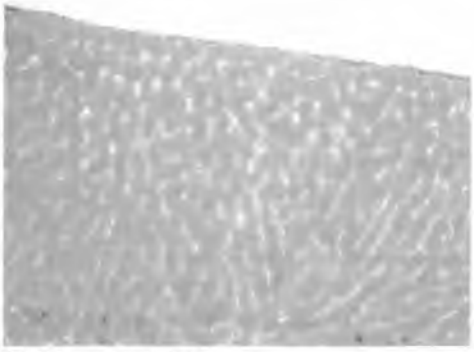
Fig. 1. Control group. Regular structure of the liver. The upper panel presents the H+E staining, the middle panel presents staining with Masson's technique and the lower panel presents the PAS method. Magn. 200x

THE EXPERIMENTAL GROUP I – CUMS+H$_2$O DEST. (FIG. 2)

Slight histological changes were observed in animals subjected to chronic stress. The general architectonics of the lobules was preserved. In most hepatocytes one or two nuclei were found. Nuclei



2A. H+E staining



2B. Masson's staining

2C. PAS method

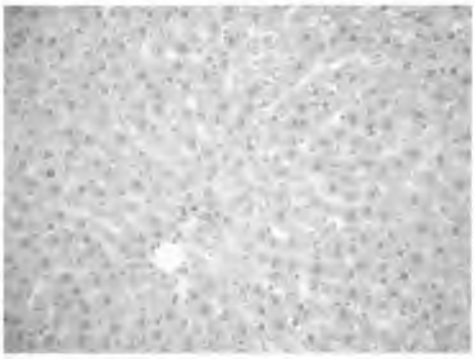
Fig. 2. Experimental group I – CUMS. Slight dilatation of sinus lumen, delicate thickenning of the external connective tissue capsule and reduced amount of glycogen within hepatocyte cytoplasm are visible. The upper panel presents the H+E staining, the middle panel presents staining with Masson's technique and the lower panel presents the PAS method. Magn. 200x

possessed distinct nucleoli. The hepatocyte cytoplasm in H+E staining showed stainability similar to the control. The sinus lumen was slightly dilated. The endothelial cells and Browicz-Kupffer cells were similar to the control group. Staining by the Masson's technique showed the amount of connective tissue in the liver parenchyma similar to that of the control group and slightly thickened external connective tissue capsule. Staining by the PAS method showed a reduced amount of glycogen within hepatocyte cytoplasm.

THE EXPERIMENTAL GROUP IIA – ZINC ASPARTATE (FIG. 3)

The general architectonics of the lobules was preserved. The hepatocytes nuclei were regular in shape. In one of six individuals the hepatocyte cytoplasm showed increased transparence in H+E staining. Irregular, no colouring areas, especially around the nucleus, were visible in the hepatocyte cytoplasm in this case. In other individuals hepatocyte cytoplasm stainability was preserved and an increased number of hepatocytes with intensely acidophilic homogenous cytoplasm was observed, especially in the vicinity of blood vessels. More numerous and enlarged Browicz-Kupffer cells were found intended to the sinus lumen. The sinus lumen was slightly enlarged. A slightly increased amount of connective tissue was observed in slides stained with Masson's technique. Connective tissue fibers

were found mainly in the vicinity of sinusoids. A thickenning of the external connective tissue capsule was noticed. The PAS method revealed a decreased amount of glycogen within hepatocyte cytoplasm.



3A. H+E staining

3B. Masson's staining

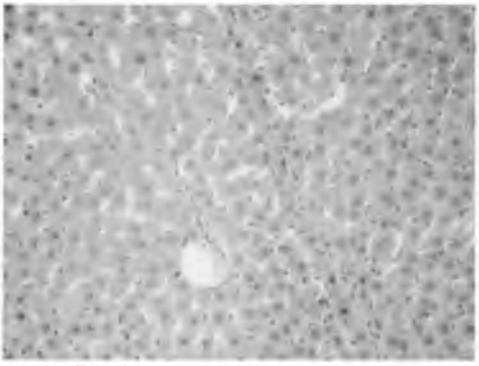
3C. PAS method

Fig. 3. Experimental group IIA – Zinc aspartate. No colouring areas around the nuclei in hepatocytes, more numerous Browicz-Kupffer cells, slight dilatation of the sinus lumen, slight thickenning of the external connective tissue capsule and reduced amount of glycogen within hepatocyte cytoplasm are visible. The upper panel presents the H+E staining, the middle panel presents staining with Masson's technique and the lower panel presents the PAS method. Magn. 200x

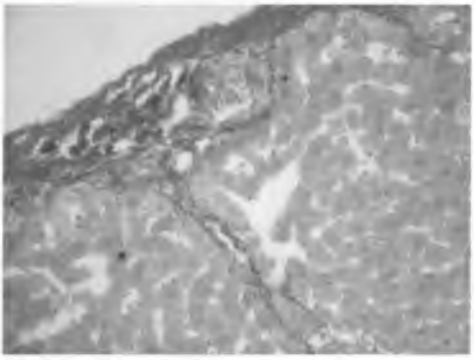
THE EXPERIMENTAL GROUP IIB – CUMS+ZINC ASPARTATE (FIG. 4)

The picture was essentially different from the picture in the control group and experimental groups I and IIA. The most important morphological changes included thickenning of the external connective tissue capsule and increase of the connective tissue amount in the liver parenchyma. External connective tissue capsule surrounding the liver was distinctly thickened. The general architectonics of the parenchyma was not well preserved. The arrangement of hepatocytes was distorted, especially in places with large amount of connective tissue. Hepatocyte cytoplasm stainability was preserved. Their nuclei were round in shape and variable in size. Focal necrosis of hepatocytes was observed. Inflammatory infiltrations were observed around damaged hepatocytes and around blood vessels. In the parenchyma thick connective tissue septa with large blood vessels were visible. The sinusoidal lumen was significantly enlarged and filled with red blood cells. Browicz-Kupffer cells were enlarged and much more numerous. In slides stained with Masson's technique a very thick external connective

tissue capsule was visible. Also, the amount of connective tissue in the liver parenchyma was significantly increased and thick connective tissue septa were observed. Black metal deposits were present in connective tissue septa and capsule. The PAS method revealed a decreased amount of glycogen within hepatocyte cytoplasm.



4A. H+E staining



4B. Masson's staining

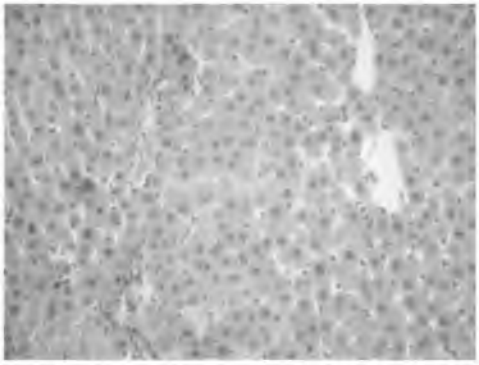
4C. PAS method

Fig. 4. Experimental group IIB – CUMS + Zinc aspartate. CUMS intesifies liver damage induced by zinc aspartate. Distorted arrangement of hepatocytes, thick external connective tissue capsule with black metal deposits, connective tissue septa in the liver parenchyma, more numerous Browicz-Kupffer cells and significantly enlarged sinuses are visible. The upper panel presents the H+E staining, the middle panel presents staining with Masson's technique and the lower panel presents the PAS method. Magn. 200x

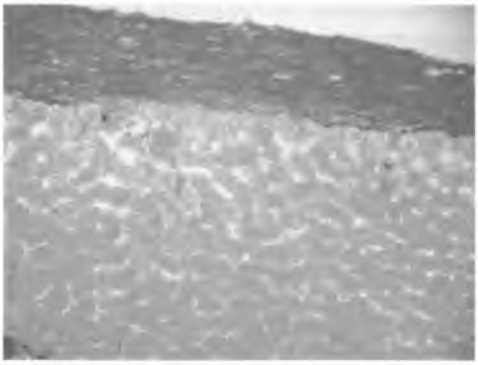
THE EXPERIMENTAL GROUP IIIA – ZINC SULPHATE (FIG. 5)

Distinct morphological changes were observed in this experimental group. External connective tissue capsule surrounding the liver was significantly thickened. The general architectonics of the liver parenchyma was not preserved. Numerous connective tissue septa were observed in the parenchyma. The arrangement of hepatocytes was distorted. Increased number of binuclear hepatocytes was found. Numerous hepatocytes, especially in the vicinity of connective tissue septae, possessed dark nuclei. Hepatocyte nuclei were variable in size. An increased number of hepatocytes showed picnotic nuclei. In many hepatocytes, intensely acidophilic, homogenous cytoplasm was observed. The sinusoids were significantly enlarged, irregular in shape and filled with red blood cells. Browicz-Kupffer cells were

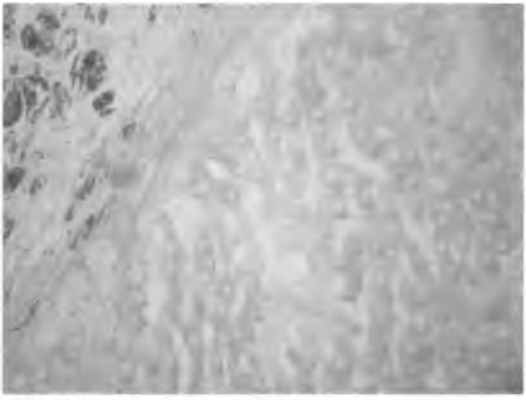
enlarged and much more numerous. Inflammatory infiltrations were observed in connective tissue septae and focally around blood vessels. In slides stained with Masson's technique very thick external connective tissue capsule was visible. Blue connective tissue septa were observed in the liver parenchyma. Black metal deposits were found in connective tissue septa and capsule. The PAS method revealed decreased amount of glycogen within hepatocyte cytoplasm.



5A. H+E staining



5B. Masson's staining



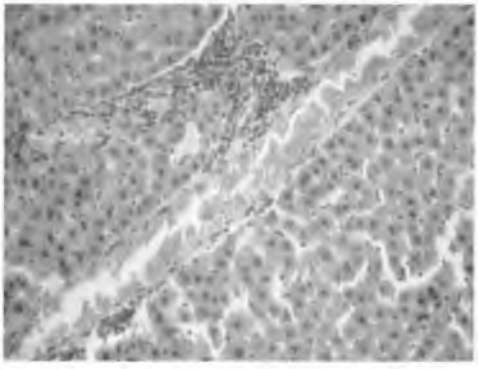
5C. PAS method

Fig. 5. Experimental group IIIA – Zinc sulphate. Distorted arrangement of hepatocytes, thick external connective tissue capsule, intensely acidophilic hepatocyte cytoplasm, reduced amount of glycogen within hepatocyte cytoplasm, inflamatory infiltrations, more numerous Browicz-Kupffer cells and enlarged sinuses are visible. The upper panel presents the H+E staining, the middle panel presents staining with Masson's technique and the lower panel presents the PAS method. Magn. 200x

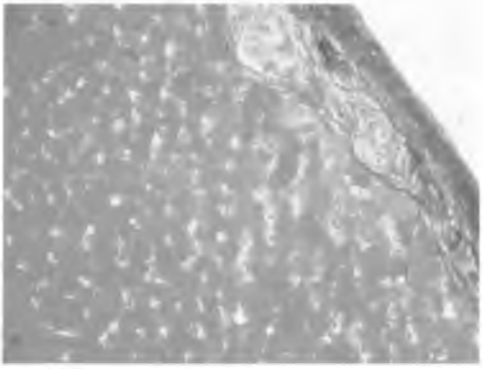
THE EXPERIMENTAL GROUP IIIB – CUMS+ZINC SULPHATE (FIG. 6)

Far reaching morphological changes were observed in this experimental group. External connective tissue capsule surrounding the liver was significantly thickened. The general architectonics of the liver parenchyma was completely distorted. Numerous connective tissue septa were observed in the liver parenchyma. The arrangement of hepatocytes was irregular. An increased number of binuclear hepatocytes was found. Hepatocyte nuclei were variable in size. Increased number of hepatocytes with picnotic nuclei was observed especially in the vicinity of connective tissue septae. In many hepatocytes, intensely acidophilic,

homogenous cytoplasm was observed. The sinusoids were significantly enlarged, irregular in shape and filled with red blood cells. Browicz-Kupffer cells were enlarged and much more numerous. Inflammatory infiltrations were observed in connective tissue septa and focally around blood vessels and in the liver parenchyma. In slides stained with Masson's technique very thick external connective tissue capsule and blue, thick connective tissue septa were observed. Black metal deposits were present in connective tissue septa and capsule. The PAS method revealed decreased amount of glycogen within hepatocyte cytoplasm.



6A. H+E staining



6B. Masson's staining

6C. PAS method

Fig. 6. Experimental group IIIB – CUMS + Zinc sulphate. CUMS intesifies liver damage induced by zinc sulphate. Thick external connective tissue capsule with black metal deposits, thick connective tissue septa in the liver parenchyma, distorted arrangement of hepatocytes, focal hepatocyte necrosis with inflamatory infiltrations, activated Browicz-Kupffer cells and significantly enlarged sinuses are visible. The upper panel presents the H+E staining, the middle panel presents staining with Masson's technique and the lower panel presents the PAS method. Magn. 200x

QUANTITATIVE ANALYSIS

The mean values of the thickness of the liver external connective tissue capsula (mm) in respective groups were: 0.72±0.85in the control group; 3.27±4.49 in the I experimental group; 8.23±8.72 in the IIA experimental group; 48.33±36.93 in the IIB experimental group; 107.45±67.92 in the IIIA experimental group; 119.54±72.68 in the IIIB experimental group (Fig. 7). The mean values of the

surface area of sinusoidal capillaries in the subcapsular region of the liver (mm²) in respective groups were: 44.50±43.16 in the control group; 90.10±84.71 in the I experimental group; 153.62±186.94 in the IIA experimental group; 348.38±280.63 in the IIB experimental group; 340.81±380.45 in the IIIA experimental group; 390.16±329.41 in the IIIB experimental group (Fig. 8).

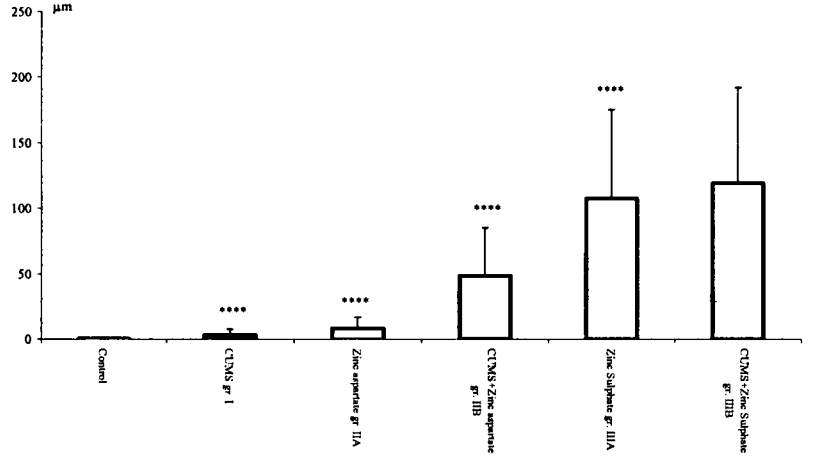


Fig. 7. Thickness of the liver external connective tissue capsule (mm). Animals were subjected to CUMS for 16 weeks. Zinc aspartate was administered at a dose of 15mg/kg/24h (elemental zinc dose of 2.7mg/kg/24h) and zinc sulphate at a dose of 15mg/kg/24h (elemental zinc dose of 3.0mg/kg/24h) for 16 weeks. ****, P<0.0001 CUMS vs Control, Zinc aspartate vs Control, CUMS+Zinc aspartate vs Zinc aspartate, Zinc sulphate vs Control

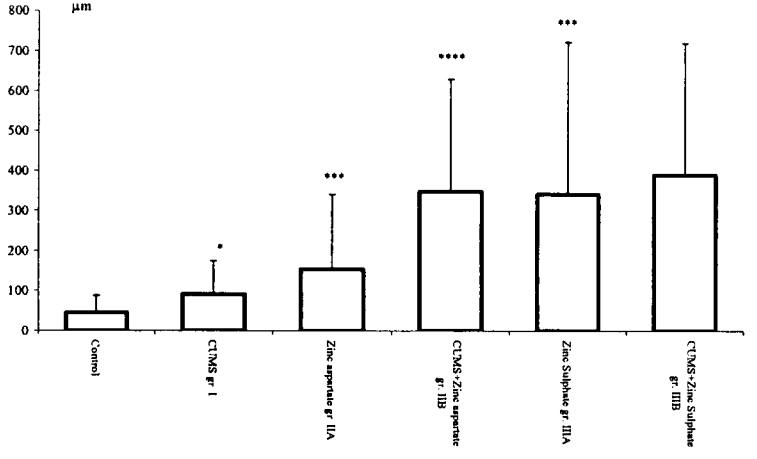


Fig. 8. Surface area of sinusoidal capillaries in the subcapsular region of the liver (mm²). Animals were subjected to CUMS for 16 weeks. Zinc aspartate was administered at a dose of 15mg/kg/24h (elemental zinc dose of 2.7mg/kg/24h) and zinc sulphate at a dose of 15mg/kg/24h (elemental zinc dose of 3.0mg/kg/24h) for 16 weeks. *, P<0.05 CUMS vs Control; ***, P<0.001 Zinc aspartate vs Control, Zinc sulphate vs Control; ****, P<0.0001 CUMS+Zinc aspartate vs Zinc aspartate

DISCUSSION

The morphological changes revealed under the light microscope in the animals subjected to chronic unpredictable mild stress (CUMS) for 16 weeks were characterized by a slight dilatation of the sinusoidal lumen and delicate thickenning of the external connective tissue capsule. Staining by the PAS method has shown reduced amount of glycogen within hepatocyte cytoplasm. Dilatation of the sinusoidal lumen observed after CUMS may be the result of alterations in hepatic blood flow which is influenced by sympathomimetic activity. Earlier animal and human studies have demonstrated an initial reduction and subsequent increase in hepatic blood flow, which coincided with an observed increase and subsequent return to normal in serum catecholamine concentrations during stress (17). Reduced amount of glycogen observed in PAS staining method may be the result of glycogenolysis induced by glucocorticosteroids secreted by suprarenal cortex in response to stress.

Histological changes observed after chronic (16 weeks) administration of zinc aspartate were characterized by slight thickenning of the external connective tissue capsule with delicate fibrosis in the vicinity of sinusoidal capillaries, slight dilatation of the liver sinusoids and activation of Browicz-Kupffer cells. These changes indicate increased hepatic fibrogenesis induced by zinc administration. Significantly stronger fibrogenesis and hepatocyte damage were observed after administration of zinc sulphate. Very thick external connective tissue capsule surrounding the liver and thick connective tissue septa in the liver parenchyma with accumulated metal particles were visible in the group receiving zinc sulphate. Evident fibrosis in the vicinity of sinusoids, focal inflammatory infiltrations in the liver parenchyma and much more numerous activated Browicz-Kupffer cells were also observed. These changes were accompanied by morphological damage of hepatocytes. It should be underlied that the doses of elemental zinc used in our experiment (from 2.7 to 3.0 mg/kg/24h) were twenty folds higher than estimated safe amount of zinc in human. The recommended daily allowance of zinc for adult man is 15mg and the estimated safe amount of zinc is 0.15mg/kg (1).

Mechanisms that underlie zinc-mediated hepatotoxicity are not completely explained. Generally metal hepatotoxicity involves two pathways, one for the initial injury produced by the direct effects of metal excess and the other for the subsequent injury produced by inflammation. Primary injury appears to be caused by the binding of Zn^{2+} to sulfhydryl groups in critical macromolecules (metallothionein, glutathione). This binding causes oxidative stress, the mitochondrial permeabilty transition and mitochondrial dysfunction. Kirk et al. (7) demonstrated that high intracellular free zinc promotes neural death by inhibiting cellular energy production. Inhibition of mitochondrial ATP synthesis is the key effect that leads to cell death. Bay et al. (2) demonstrated that acute zinc administration reduced the hepatic cytochrome P450 and glutathione content and causes ultrastructural changes of hepatocytes in the shape of mitochondrial swollowing. Although zinc excess may injure hepatocytes directly, there are also reasons to believe that hepatocellular injury is produced as the result of ischemia caused by damage to endothelial cells. It was also demonstrated that oxidative stress selectively damages hepatic sinusoidal endothelial cells (4). The results of our study indicate that zinc-induced hepatotoxicity is associated with peliosis hepatis, which is consistent with damage to the sinusoidal endothelium. Secondary injury from excess zinc exposure is thought to occur from the activation of Browicz-Kupffer cells and a cascade of events involving several types of liver cells and a large number of inflammatory and cytotoxic mediators. The activation of Browicz-Kupffer cells is initiated directly or indirectly through injuried endothelial and parenchymal cells. Activated Browicz-Kupffer cells release inflammatory mediators (e.g., cytokines, chemokines, adhesion molecules) that induce a cascade of cellular and humoral responses. Browicz-Kupffer cells play an important role in inflammatory liver diseases leading to fibrosis because they secrete proinflamatory cytokines, IL-1β, IL-6 and TNF-α that

stimulate stellate cells that are primarily responsible for fibrogenesis (2, 11). The results of our research indicate that focal inflammatory infiltrations are important components of zinc-induced hepatotoxicity. These inflammatory infiltrations were observed at the sites of necrosis, especially in the vicinity of blood vessels. Undoubtedly, zinc doses applied in our experiment were hepatotoxic and excess of metal particles accumulated in the liver connective tissue capsule and septa. Zinc deposits in the liver, pancreas and kidney were also described by Khalifa et al. (6) in the case of zinc toxicity in psychiatric patients due to massive coin ingestion. Hepatic necrosis, pancreatitis, renal failure, coagulopathy, leukopenia and hypo-cupremic anemia were found as the result of zinc toxicity.

Our results indicate that chronic stress evidently intensifies zinc-induced liver toxicity. Morphological changes of hepatocytes, hepatic sinusoids and liver fibrosis were significantly stronger in animals subjected to CUMS procedure concomitantly with intraperitoneal administration of zinc. Intensification of liver damage after CUMS regarded both used zinc compounds: zinc aspartate and zinc sulphate. The morphological picture of the liver after concomitant administration of zinc and CUMS procedure indicates liver cirrhosis with inflammation. Comparing the influence of zinc itself and CUMS itself with the concomitant influence of both on the liver morphology it may be concluded that chronic stress intensifies zinc-induced hepatotoxicity leading to liver cirrhosis.

The mechanism of stress-mediated intensification of zinc hepatotoxicity may be connected with increased generation of toxic metabolites and decreased protective mechanism as the result of chronic stress. It has been demonstrated that chronic stress increases free radical generation and lipid peroxidation in the liver and causes cytochrome P450 and glutathione reduction in rats (15). Stress was also found to modulate different enzymatic systems which are vital components of the detoxifying mechanisms of the body (8). Chronic stress impairs oxidative metabolism in hepatocytes (16). Fromenty et al. (5) underlie that mitochondria limit the efficiency of mitochondrial ATP synthesis by uncoupling oxidative phosphorylation in hepatocytes. This increases hepatocyte susceptibility to injury if new oxidative stress is placed on the cell and explains an intensification of liver damage under the influence of two or more damaging factors. But still, little is known about the biochemical mechanisms by which stress affects xenobiotic metabolism and further studies are necessary to investigate its precise role in this field.

CONCLUSIONS

1. 16-week-long chronic unpredictable mild stress (CUMS) causes delicate morphological changes in the rat liver characterized by slight dilatation of the sinusoidal lumen, slight thickening of the external connective tissue capsule and reduced glycogen deposits in hepatocytes.

2. Zinc aspartate and zinc sulphate administered at doses of 15mg/kg/24h for 16 weeks induce morphological liver damage.

3. Zinc-induced morphological liver damage includes: external connective tissue capsule thickenning, hepatic fibrosis with metal deposits, focal hepatocyte necrosis with inflammatory infiltrations, activation of Browicz-Kupffer cells and dilatation of liver sinusoids. Hepatotoxicity of zinc sulphate is stronger than zinc aspartate.

4. In stressed animals zinc-induced morphological liver damage was stronger than in unstressed rats treated with the same zinc preparations.

5. It may be concluded that chronic stress intensifies zinc-induced hepatotoxicity leading to liver cirrhosis.

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SUMMARY

The aim of the present study was histological assessment of the liver after chronic stress and long-term administration of zinc. The experiment was carried out on adult male Wistar rats. Animals were subjected to chronic unpredictable mild stress procedure (CUMS) for 16 weeks. Two zinc compounds, zinc aspartate and zinc sulphate were administered ip in a dose of 15mg/kg/24h each once daily for 16-weeks 1h before stress session. The animals were divided into six groups: one control and five

experimental (I-CUMS, IIA-zinc aspartate,IIB-CUMS+zinc aspartate; IIIA-zinc sulphate; IIIB-CUMS+zinc sulphate). Liver slices stained with hematoxylin and eosin, Masson's technique and PAS method were assessed using light microscope. Ouantitative analysis of morphological changes was carried out using a computer analyser of histological pictures. The results indicate that CUMS causes slight morphological changes in the rat liver characterized by dilatation of the sinusoidal lumen, delicate thickenning of the external connective tissue capsule and reduced glycogen deposits in hepatocytes. Chronic administration of zinc leads to hepatotoxic changes (fibrosis with focal necrosis and inflammatory infiltrations). Hepatotoxicity of zinc sulphate is stronger than zinc aspartate. Chronic stress intensifies zinc-induced hepatotoxicity leading to liver cirrhosis.

Wpływ przewlekłego stresu i długotrwałego podawania cynku na morfologię wątroby szczura: badania histologiczne i morfometryczne

Celem pracy była ocena histologiczna wątroby po przewlekłym stresie i długotrwałym podawaniu cynku. Badania wykonano na dorosłych samcach szczurach szczepu Wistar. Zwierzęta poddano procedurze łagodnego, przewlekłego stresu (CUMS) przez okres 16 tygodni. W doświadczeniu zastosowano dwa związki cynku – asparaginian cynku i siarczan cynku w dawce 15mg/kg/24h każdy w ciągu 16 tygodni 1godz. przed bodźcem stresowym. Zwierzęta podzielono na 6 grup: jedną kontrolną i pięć doświadczalnych (I-CUMS, IIA-asparaginian cynku, IIB-CUMS+asparaginian cynku; IIIA-siarczan cynku; IIIB-CUMS+siarczan cynku). Przy pomocy mikroskopu świetlnego oceniano preparaty wątroby barwione hematoksyliną i eozyną, metodą Massona i metodą PAS. Analizę ilościową zmian morfologicznych przeprowadzono przy użyciu komputerowego analizatora obrazów histologicznych. Otrzymane wyniki wskazują na to, że CUMS wywołuje niewielkie zmiany morfologiczne wątroby w postaci poszerzenia naczyń zatokowych wątroby, delikatnego pogrubienia zewnętrznej torebki łącznotkankowej i zmniejszonego gromadzenia glikogenu w hepatocytach. Przewlekłe podawanie cynku prowadzi do zmian hepatotoksycznych (włóknienie, ogniskowa martwica i nacieki zapalne). Hepatotoksyczne działanie siarczanu cynku jest silniejsze niż asparaginianu cynku. Przewlekły stres nasila wywołaną cynkiem hepatotoksyczność, prowadząc do marskości wątroby.