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Influence of the "Biostim LBS" Probiotic on Morphological Liver Changes in Experimentally Induced Hepatotoxicity

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The effect of chronic treatment with probiotic Biostim LBS containing the original *Lactobacillus bulgaricus* on morphological hepatic changes in an experimental model of carbon tetrachloride (CCl_4) induced hepatotoxicity was studied. Male white Wistar rats were treated with Biostim LBS in doses of 800 and 1600 mg/kg once daily for 30 consecutive days. CCl_4 was administered during the two last days in a dose of 0,2 mL/kg. On the 31th day material from the liver was taken for histological examination. The application of Biostim LBS favorably influences upon the necrotic changes in the liver induced by the hepatotoxic agent CCl_4.

Key words: Biostim LBS, carbon tetrachloride, liver morphology, hepatoprotection.

Introduction

Probiotics are immunomodulatory bacteria in the gastrointestinal tract that protect their host [4, 6]. The probiotic Biostim LBS (Biomilk) combines live cells of *Lactobacillus bulgaricus*, milk proteins, fats, carbohydrates, natural vitamins, minerals and pectin. The milk products containing lactobacilli proved to exert favourable effects in liver and biliary tract diseases of toxic, bacterial and viral nature [3, 5, 9].

The purpose of this study is to establish the effect of chronic treatment with the probiotic Biostim LBS in different dosages on the morphological liver changes in an experimental model of carbon tetrachloride (CCl_4) induced hepatotoxicity.

Materials and Methods

Male Wistar rats weighing at an average of 250±10 g were used to examine the influence of Biostim LBS. Biostim LBS was administered dissolved ex tempore in distilled water in doses of 800 and 1600 mg/kg bw rat, per sondam, once daily for 30 consecu-

tive days. CCl_4 was applied two days long per sondam in a dose of 0,2 mL/kg bw as 10% solution in sunflower oil.

According to the kind of treatment, the animals were divided into 6 groups with 7 animals each: I group — Biostim LBS 800 mg/kg; II group — Biostim LBS 1600 mg/kg; III group — CCl_4; IV group — Biostim LBS 800 mg/kg and CCl_4; V group — Biostim LBS 1600 mg/kg and CCl_4; VI group — untreated controls.

The animals from groups four and five were initially treated for 30 days with Biostim similarly to those of groups one and two as during the last two days of experiment they were given CCl_4. The animals were anaesthesized and decapitated 24 hours after the last CCl_4 administration.

Material from the liver was taken for histopathological examination, fixed in 5% neutral formaline and Carnoy's solution. Five paraffin sections were stained with hematoxylin eosin (HE), impregnated with silver, after Gomori for reticular fibres' proof and with PAS reaction under and without control of alpha-amylase after McManus for glycogen proof.

Results

Morphological liver changes in animals treated with the probiotic Biostim LBS in doses of 800 mg/kg and 1600 mg/kg (groups I and II)

The examination of the liver revealed a completely preserved histological structure of the organ and no differences in comparison with the controls (Fig. 1).

Morphological liver changes in animals treated with CCl_4 (group III)

There were large areas of coagulation necrosis affecting the hepatocytes from the central and intermediary parts of the hepatic lobules. At numerous places, the ne-

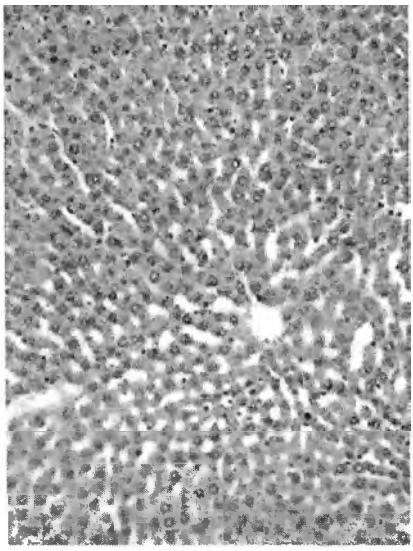

Fig. 1. Liver of an animal treated with Biostim LBS in doses of 800 mg/kg and 1600 mg/kg. × 160

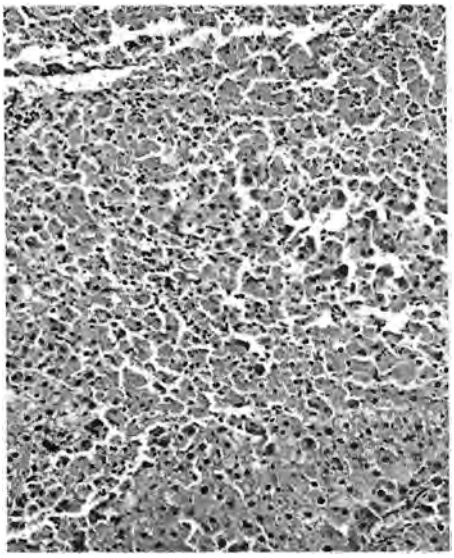

Fig. 2. Liver of an animal treated with CCl_4. Large areas of coagulation necrosis beginning from the central parts of the lobules and tending to merging of the necrotic zones. Lymphocytes and single leukocytes around necrotic hepatocytes. HE, × 200

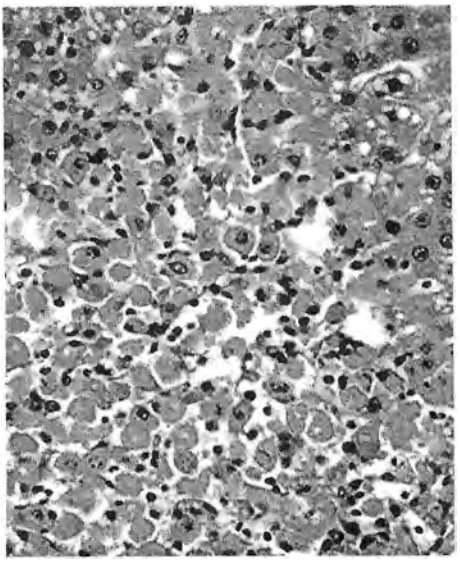

Fig. 3. Liver of an animal treated with CCl_4. Kunselmann's bodies in foci of necrosis. In the periphery there are preserved hepatocytes with hydropic and fatty degeneration. HE, × 400

crotic zones merged and thus the so-called 'bridge necroses' were formed (Fig. 2). In some lobures there were hepatocytes with still preserved cellular membrane but with swallen or picnotic nuclei along with absence of glycogen in the cytoplasm. At this place, amidst the necrotic masses, "free Kunselmann's bodies" could be observed (Fig. 3). Reticular fibres were destroyed.

Morphological liver changes in animals treated with the probiotic Biostim LBS in a dose of 800 mg/kg bw and CCL_4 (group IV)

Only in one animal of this group there was a coagulation necrosis in the central parts of the hepatic lobules and of a smaller size of the affected regions. In all the rest animals there was no coagulation necrosis at all. The alterations being considerably less expressed in comparison with those of the animals treated with CCL_4 only were characterized with a hydropic degeneration manifested to a different extent but in some lobules only — of balloon degeneration (Fig. 4). There were hepatocytes with microvesicular steatosis in the cytoplasm, too.

Morphological liver changes in animals treated with the probiotic Biostim LBS in a dose of 1600 mg/kg bw and CCL_4 (group V)

The application of the preparation in this dosage exerted a manifested hepatoprotective effect in CCL_4 treated animals. On the one hand, there was a considerable reduction of the surface of affection in the hepatic lobules. On the other hand, the comparison with the animals pre-treated with the probiotic Biostim LBS in a dose of 800 mg/kg impressed through the less outlined lesion of the liver parenchyma. It was

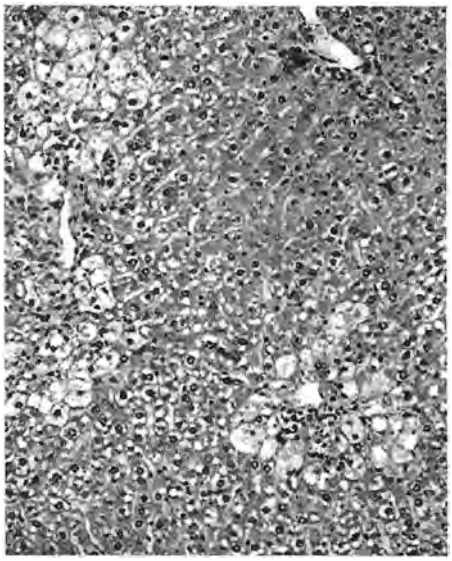

Fig. 4. Liver of an animal treated with Biostim LBS (800 mg/kg bw) and CCl_4. In the hepatocytes of some lobules there is hydropic up to balloon degeneration. There is no coagulation necrosis. bafatty droplets. HE, × 200

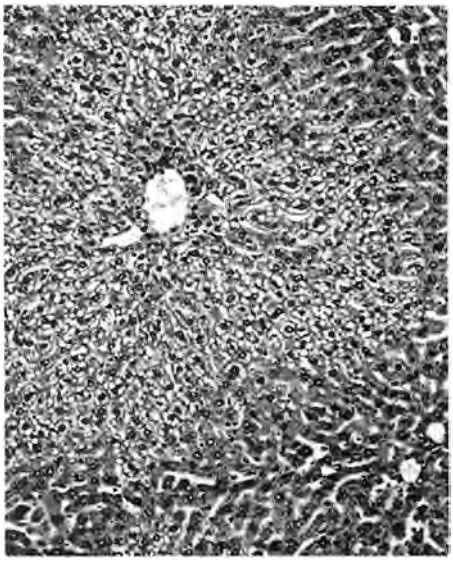

Fig. 5. Liver of an animal treated with Biostim LBS (1600 mg/kg bw) and CCl_4. Hydropic degeneration in the hepatocytes from the central and, partially, from the intermediary area of the lobule. In the periphery there are hepatocytes with small-droplet fatty degeneration. HE, × 200

manifested by the development of a vacuolar, partially, up to hydropic degeneration of the hepatocytes from the central and, partially, from the intermediary areas of the lobules (Fig. 5). Around these parts, at places, hepatocytes with small-droplet fatty degeneration could be observed. Necrotic changes and balloon degeneration in the hepatocytes that were observed in other groups were missing. The amount of the glycogen granules was comparatively regularly distributed in the cytoplasm of the cells. The kind of the reticular fibres did not differ from that of the control, untreated animals.

Discussion

In toxicology, acute poisoning with CCL_4 are a concrete example of a dramatic disturbance of the balance between the free radical peroxidation of the lipids and the antioxidant capacity of the organism [2,10].

The biotransformation of CCL_4 in the liver generates free radicals. The formed free radicals CCL_3^+ act on the organism in two main directions: first, they immediately damage the different enzymatic systems and first of all the oxygenases CYP 450 and second, that deserves the greatest attention the free radicals CCL_3^+ unlock the processes of free radical peroxidatioon of unsaturated faty acids. New radicals RO_2^+ and hydrogen peroxides ROON formed from the aforementioned fatty acids lead to structural and functional changes in different biological membranes. Next follows a displacement of the oxidative links, disturbace of the integrity of the cellular membranes, exudation of intracellular proteolytic enzymes and apoptosis [2, 10].

The probiotic Biostim LBS containing *L. bulgaricus* protects the liver from the damaging action of CCL_4. This is, most probably, a result from the inhibition of lipid peroxidation and stimulation of the cellular antioxidant system [1, 7, 8].

There are literature data about the antioxidant effect of probiotics containing lactobacilli. The authors establish that lactic acid bacteria clean the eractive oxygen radicals, possess helating capacity for metal ions, realize enzyme inhibition and possess a reduction activity. Milk proteins containing in the lactic acid products enhance the concentrations of glutathione in the liver. The glutathione is important for the detoxication of endogenic and exogenic carcinogens and free radicals as well as it regulated the immune function [11, 12].

Conclusion

The data from the present investigation demonstrate that the probiotic Biostim LBS administered in definite dosages exterts a hepatoprotective effect in acute hepatic toxicity in rats induced by CCL_4. Preliminary treatment with this probiotic leads to reduction of the severity of the degenerative and necrotic changes in the liver caused by CCL_4 and of the size of the area of the necrotic alterations as well. Our data confirm the clinical significance of the chronic application of Biostim LBS in hepatic diseases such as viral hepatitis, in ethanol-induced liver damage and in experimental hepatotoxicity as well.

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