Institute of Experimental Morphology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 13
Sofia • 2008

Influence of Probiotics on Histopathological Liver Alterations in Experimental Hypercholesterolemia

M. Georgieva, E. Softova, M. Gabrovska**, P. Borisova, N. Manolov****

*Department of Preclinical and Clinical Pharmacology and Biochemistry,
Prof. Paraskev Stoyanov Medical University of Varna
* Department of General and Clinical Pathology, Prof. Paraskev Stoyanov Medical University of Varna
**Department of Anatomy, Histology and Embryology,
Prof. Paraskev Stoyanov Medical University of Varna
***Clinic of Vascular Surgery, St. Anna University Hospital of Varna,
Prof. Paraskev Stoyanov Medical University of Varna*

The effect of chronic treatment with probiotic Biostim LBS containing the original *Lactobacillus bulgaricus* on liver morphology in hyperlipidemia was studied. Biostim LBS (800 mg/kg bw and 1600 mg/kg bw), Biostim LBS-1 (1600 mg/kg bw) and cholesterol (0,7 mL/100 g) were administered once daily for 30 consecutive days in male Wistar rats. Liver specimens were taken on the 31st day for histological analysis. Steatogenic diet induced fatty degeneration in the hepatocytes that underwent involution after Biostim LBS treatment in a dose of 800 mg/kg bw without complete normalization. The application of Biostim LBS and Biostim LBS-1 in a dose of 1600 mg/kg bw completely restored the hepatocytes. These probiotics possess regenerative and protective properties concerning lipid degeneration. There is no reliable statistical difference between the effects of both probiotics.

Key words: Biostim LBS, cholesterol, fatty degeneration, hepatocytes.

Introduction

There is evidence that Lactobacilli, including *Lactobacillus bulgaricus*, exert favourable effects concerning the prevention and treatment of various gastrointestinal diseases, including colon cancer. They reduce the risk of cardiovascular diseases, possess an immunomodulatory, antiallergic and X-ray protective action at absent teratogenic effect [1, 4, 6, 7, 11].

Probiotic Biostim LBS is a low-lactose, moderately fatty and dry milk product while probiotic Biostim LBS-1 is a low-lactose, fat-free and dry milk product. Both probiotics are obtained through lactic-acid fermentation from ecologically pure cow milk. They contain live cells of *Lactobacillus bulgaricus*, milk proteins, fats, carbohydrates, natural vitamins, minerals, and pectin. They do not contain, however, preservatives and genetically manipulated microorganisms at all.

The purpose of the present study is to establish the effect of a chronic treatment with the probiotic Biostim LBS containing the original *Lactobacillus bulgaricus* on the morphological liver alterations in experimentally induced hyperlipidemia.

Materials and Methods

The study covered 42 white male Wistar rats weighing initially 250±10 g bw. They were maintained under conditions of room temperature and free access to standard food and water.

According to the treatment module, the animals were divided into 8 groups of 6 animals each as the substances were introduced per sondam.

Group I — Biostim LBS 800 mg/kg bw; Group II — Biostim LBS 1600 mg/kg bw; Group III — Biostim LBS-1 1600 mg/kg bw; Group IV — cholesterol 0,7 ml/100 g; Group V — Biostim LBS 800 mg/kg bw and cholesterol; Group VI — Biostim LBS 1600 mg/kg bw and cholesterol; Group VII — Biostim LBS-1 1600 mg/kg bw and cholesterol; Group VIII — pure controls, not treated at all.

Two types of Biostim LBS were used. The animals were given Biostim LBS (800 mg/kg bw and 1600 mg/kg bw) and Biostim LBS-1 (1600 mg/kg bw) once daily for 30 consecutive days. These doses were selected to match the recommended doses in humans. The control animals were given physiological saline. Both probiotics were solved ex tempore in distilled water. Cholesterol was administered into the stomach by a soft tube in a dose of 0,7 ml/100 g (1,5% cholesterol dissolved in sunflower oil) for 30 consecutive days.

On the 31st day, material was taken from rat liver and processed after a standard paraffin method. Some livers were cut by cryostat to prove the fats. Eight-mkm thick sections were stained with hematoxylin-eosin, oil-red (for fats), PAS reaction with Schiff's reagent for glycogen and Gomori for reticular fibres.

Results

The application of an steatogenic diet resulted in a fatty degeneration in the hepatocytes from central and intermediary areas of the hepatic lobules (Fig. 1, 2). These alteration underwent an involution to a great extent after the application of Biostim LBS in a dose of 800 mg/kg bw (Fig. 3) without any complete restoration. However, the administration of both Biostim LBS and of Biostim LBS-1 (Fig. 4) in a dose of 1600 mg/kg bw led to a complete fading away of the changes as the appearance of the hepatocytes could not be distinguished from that of the controls (Fig. 5).

Discussion

The lipid lowering effect of the sour milk (yoghourt) is related to the increased content of lactic-acid bacteria in the gastrointestinal tract. These bacteria ferment the carbohydrates from the food that are difficult to digest. Such a fermentation results in an increased production of short-chain fatty acids that reduce the concentration of circulating cholesterol either through inhibition of liver cholesterol synthesis, or through cholesterol redistribution from plasma to the liver. Besides the increased bacterial activity in the intestines leads to enhanced bile acid deconjugation. These deconjugated bile acids cannot be well absorbed by the gastrointestinal mucosa and

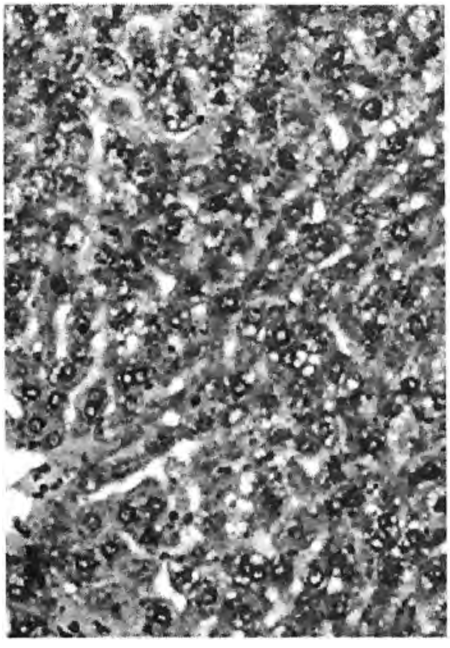

Fig. 1. Liver of an animal on steatogenic diet. Small and merging fatty droplets in the hepatocytes of the hepatic lobule. HE, × 250

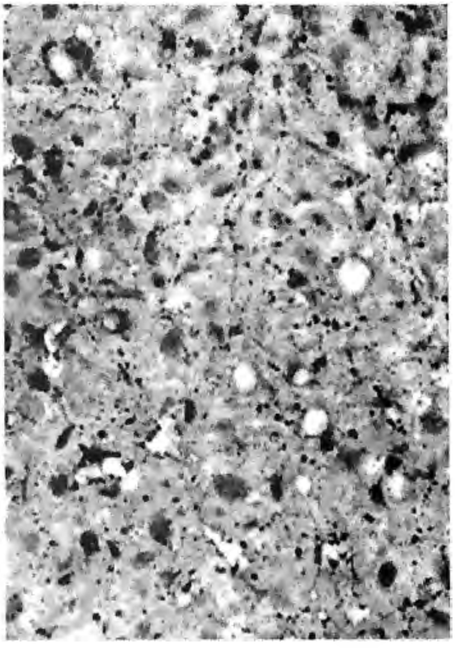

Fig. 2. Liver of an animal on steatogenic diet. Staining with oil-red for fats. × 400

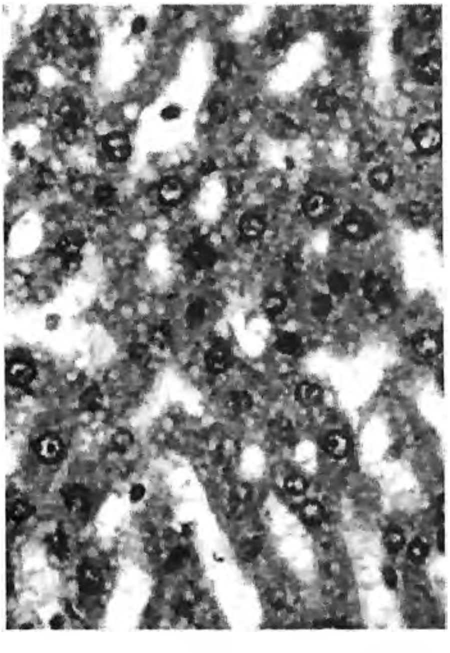

Fig. 3. Liver of an animal on steatogenic diet given Biostim LBS (800 mg/kg bw). In some hepatocytes there are scattered fatty droplets. HE, × 400

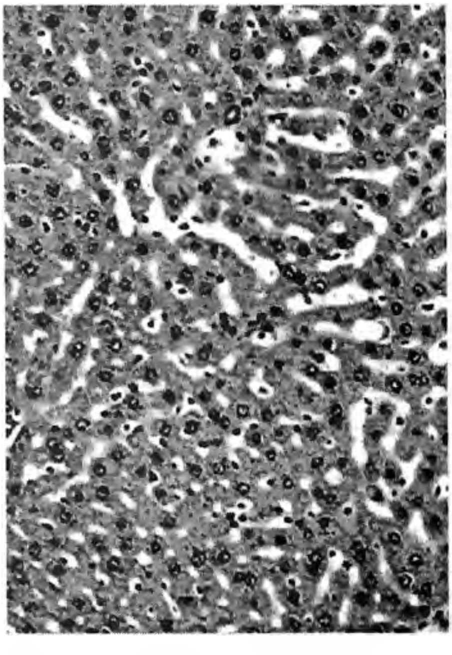

Fig. 4. Liver of an animal on steatogenic diet given Biostim LBS and Biostim LBS-1 (1600 mg/kg bw). Complete restoration of the changes after probiotic application. In the cytoplasm of the hepatocytes there are no fatty droplets at all. HE, × 250

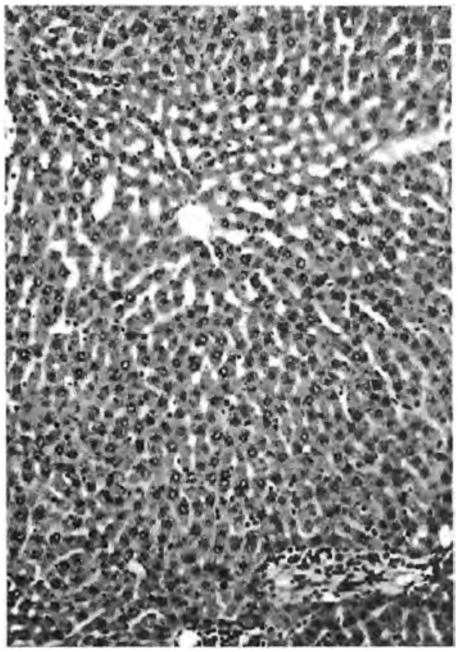

Fig. 5. Liver of a control animal. Preserved histological structure. HE, × 160

are liberated [9]. Therefore, the cholesterol as a bile acid precursor is used to a greater extent for de novo synthesis of the bile acids. Other authors report that the investigations on animals and humans show a moderate hypocholesterolemic action of lactic acid products containing strains of *Lactobacillus* and *Bifidobacteria* [8]. Besides the inhibition of the absorption of exogenous cholesterol by the small intestine either through bile acid deconjugation and influencing upon cholesterol metabolism, or through direct cholesterol assimilation by the bacterial cells has been supposed. The mechanisms for serum lipid lowering have been suggested in other in-vitro and in-vivo investigations. In-vitro experiments by some authors [2, 5, 10] demonstrate that intestinal lactic acid bacteria are capable of assimilate and bind both cholesterol and bile acids. It is known that serum cholesterol values decrease when the probiotics suppress the intestinal bile acid resorption as cholesterol catabolism is stimulated in the liver [3].

Conclusion

The probiotics Biostim LBS and Biostim LBS-1 possess regenerative properties concerning hepatocytes and protect the liver from lipid degeneration caused by the steatogenic diet. There is no reliable statistical difference between the effects of both probiotics.

References

1. Donchev, N., R. Enikova. Antitumor effect of dietary regiments including sour-milk products fermented by original Bulgarian strains.— Biotechnology, **1**, 1992, 43-47.
2. Grundy, S. M. Treatment of hypercholesterolemia by interference with bile acid metabolism. — Arch. Intern. Med., **130**, 1972, 638-648.
3. Hashimoto, H., K. Yamazaki, F. He, M. Kawase, M. Hosod, A. Hosono. Hypocholesterolemic effects of *Lactobacillus casei* subsp. casei TMC 0409 strain observed in the rats fed cholesterol contained diets. — Anim. Sci. J., **72**, 1999, 90-97.
4. Heller, K. J. Probiotic bacteria in fermented foods: product characteristics and starter organisms. — Am. J. Clin. Nutr., 73, 2001, S374-S379.
5. Hosono, A., H. Otani, N. Yasuih, M. Watanuki. Impact of fermented milk on human health: cholesterol-lowering and immunomodulatory properties of fermented milk. — Anim. Sci. J., **73**, 2002, 241.
6. Huis in't Veld, J. H. J., R. Havenaar. Selections criteria and application of probiotic microorganisms in man and animal. — Microecol. Ther., **26**, 1997, 43-58.
7. Ivanova, I., M. Jenova. Preventive role of liophilic dairy products "Bulgaricum" and "Biostim". — Cancer Lett., **114**, 1997, 93-95.
8. Pereira, D. I., G. R. Gibson. Effects of consumption of probiotics and prebiotics on serum lipid levels in humans. — Crit. Rev. Biochem. Mol. Biol., **37**, 2002, 259-281.
9. St-Onge, M. P., E. R. Farnworth, P. J. Jones. Consumption of fermented and non-fermented dairy products: effects on cholesterol concentrations and metabolism. — Am. J. Clin. Nutr., **71**, 2000, 674-681.
10. Xiao, J. Z., S. Kondo, N. Takahashi, K. Miyaji, K. Oshida, A. Hiramatsu, K. Iwatsuki, S. Kokubo, A. Hosono. Effects of milk products fermented by *Bifidobacterium longum* on blood lipids in rats and healthy adult male volunteers. — J. Dairy Sci., **86**, 2003, 2452-2461.
11. Георгиева, М., Н. Александров, А. Белчева. Изследване на пробиотика „Биос-