THE HYPOCHOLESTEROLEMIC EFFECT OF PROBIOTICS IN THE HYPERLIPIDEMIC HAMSTER

CAMELIA STANCU[a], FLOAREA SERBANCEA[b], GABRIELA BOTEZ[a] and ANCA SIMA[a]

[a]Institute of Cellular Biology and Pathology "Nicolae Simionescu", Lipoproteins and Atherosclerosis Department, 8, B.P Hasdeu Street, PO-Box 35-14, Bucharest 050568, Romania, e-mail: camelia.stancu@icbp.ro
[b]Institute for Food Chemistry, 1, Gârlei Street, Bucharest, Romania, e-mail: floriserbancea@yahoo.com

Received November 13, 2008

Hypercholesterolemia is a major risk factor for the development of atherosclerosis; a number of pharmacological and non-pharmacological (including dietary) approaches being employed to reduce it. Up until now, the trials to prove the hypocholesterolemic effect of probiotics have not been convincing. There are only few data suggesting that the supplementation of the diet with functional food products containing probiotic bacteria lower LDL-cholesterol (LDL-C) concentration in patients with moderately elevated cholesterol concentration in plasma. The aim of the present study was to evaluate *in vivo* the anti-atherosclerotic effect of a probiotic compound, containing the *Lactobacillus plantarum* bacteria, using an animal model of diet induced atherosclerosis, the hyperlipidemic hamster. Results show that this probiotic compound administration to hyperlipidemic hamsters induces: a significant decrease of the total serum cholesterol, a significant increase of the serum antioxidant potential, expressed by the paraoxonase 1 activity, a decrease of LDL-C, triglycerides, and glucose levels, and a small increase of HDL-C concentration in serum. In conclusion, our data support the administration of probiotic lactic acid bacteria to decrease serum cholesterol and increase the antioxidant potential in hypercholesterolemic subjects.

Key words: Probiotics; Hypercholesterolemia; Hyperlipidemic hamster; PON1.

INTRODUCTION

Atherosclerosis is a malady affecting the cardiovascular system and generating coronary heart disease, which constitutes the major cause of death in many countries[1]. It has been recognized that elevated serum cholesterol is an important risk factor associated with atherosclerosis[2]. Along the time, numerous drugs that lower serum cholesterol have been developed to treat hypercholesterolemic subjects, the best example being the statins[3]. However, the undesirable side effects of these compounds were observed and have caused concerns about their long term therapeutic use[3]. For this reason, a number of non-pharmacological approaches (including dietary ones) resulting in serum cholesterol reduction were tested.

Present knowledge concerning probiotics and their action is derived from many years of tradition in the consumption of fermented milk products, the existing data on the strains of lactic bacteria, and their positive, harmless action for the benefit of health.

The first who have used the term probiotics were Lilly and Stillwell in 1965[4] when referring to substances produced by protozoa, which in turn stimulate the growth of other organisms. The microorganisms constituting the intestinal flora reside in the various segments of human intestinal tract, and may affect the host homeostasis[5]. Probiotics are devoid of side effects and do not cause accumulation of toxic substances in the body. They are administered for therapeutic, prophylactic and nutritional purposes both to humans

and animals. Interest for probiotics increased due to their potential benefits in treating an abundance of diseases such as neoplasms, atherosclerosis, diabetes, hypertension or HIV infection.

Data from the literature are based on a single animal model used for the evaluation of the hypocholesterolemic effect of lactic bacteria and also for the determination of the minimal necessary dose of the microorganism, a critical step in the development of a safe probiotic product[6]. There are reports about the anti-inflammatory effect of probiotics: the primary targets for probiotic-mediated suppression of human TNFα and MCP-1 transcription being the activation of c-Jun and AP-1[7].

There is no information concerning the antioxidant effect of probiotics administration, the oxidative stress being a risk factor in atherogenesis. The paraoxonase 1 (PON1) is an enzyme associated with high density lipoproteins (HDL) particles and is considered the most important anti-oxidant protein in serum[8]. HDL are synthesized in the intestine, the site where probiotics exert their action. There are studies evidencing that the intestine is capable of producing and releasing PON1 in the circulation[9].

Although the advertising for probiotics tries to convince people about their potential benefits, knowledge about their mechanism of action remains scarce: how do probiotics work, which strains are more active, what can be expected to be achieved and what dosage is required for their efficacy.

The aim of our study was to evaluate *in vivo* the effects of the probiotic bacteria *Lactobacillus plantarum*, using an animal model of diet induced atherosclerosis, the hyperlipidemic hamster[10]. The results of this study support the idea that probiotics administration has pleiotropic anti-atherosclerotic effects, beside reducing the serum cholesterol, such as: stimulating the anti-atherogenic properties of HDL, their anti-oxidant action, lowering triglycerides and glucose levels.

MATERIALS AND METHODS

Reagents. Enzymatic kits for total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, and glucose determination were purchased from Dialab. The paraoxon and all other reagents used were of analytical grade and were purchased from Sigma-Aldrich Chemie GmbH, Germany. The probiotic compound was prepared by the Institute for Food Chemistry (Bucharest, Romania), and contained a liophylized mix of the Yo-MIX 205 (DANISCO) strain of *Lactobacillus plantarum* and inuline (Frutasun® high fructose syrup Sensus); the *Lactobacillus plantarum* content of this product was 10^9 cfu/g.

Animals. Golden Syrian male hamsters (100–120g weight) were kept in standard conditions, with free access to granulated food and maintained on a 12 hour light-dark cycle. Hyperlipidemia was induced by feeding a lipid rich diet (1% cholesterol and 15% butter) for 6 months. After this time, the animals were divided into two groups: (i) hyperlipidemic hamsters (HH) (n=20), and (ii) hyperlipidemic hamsters gavaged with 30 mg/kg body/day probiotic compound (HPB) containing *Lactobacillus plantarum* bacteria (300µl aqueous suspension/animal) (n=20).

Biochemical assays. Blood was collected from the retro-orbital plexus of hamsters (fasted overnight), before and after 6 weeks of treatment with probiotics. Blood was allowed to clot, and after a 5 minute centrifugation at 2,000xg serum was collected. Serum total cholesterol, triglycerides, low density lipoproteins cholesterol (LDL-C), HDL-C, and glucose were measured with enzymatic kits from Dialab. Paraoxonase 1 (PON1) activity was determined as the capacity of the enzyme to hydrolyse the paraoxon substrate using a method described by Rozenberg *et al.*[11].

Statistical analysis. All the statistical calculations were done using the One Way ANOVA Test, the results are given as means ± SD, and $p < 0.05$ was considered significant.

RESULTS

Serum total, LDL and HDL cholesterol determination

Changes in serum biochemical parameters of the HH following 6 weeks of PB administration revealed different responses of the animals to this treatment. Thus, 50% of the animals from HPB group have responded by significantly decreasing the total cholesterol (TC) level in serum (from 821.29 ± 89.28 mg/dl at week 0 of PB gavage to 624.86 ± 45.71 mg/dl at week 6) and were considered the positively responsive group (R group), while 50% of HPB animals have shown no decrease of TC (from 841.14 ± 102.95 mg/dl to 1249 ± 199 mg/dl) and were considered the negatively responsive group (NR group) (Fig. 1a). The LDL-C levels in animals of R group and NR group presented a similar trend as TC values, but the differences were not statistically significant (Fig. 1b). Thus, the value of LDL-C/TC ratio for R group decreased from 0.31 ± 0.09 at week 0 of PB treatment to 0.18 ± 0.09 at week 6, while for NR group the ratio changed from 0.33 ± 0.07 at week 0 of PB treatment to 0.36 ± 0.07 at week 6. The HDL-C values showed a significant increase in R group, compared with NR group (Fig. 1c). Thus, the HDL-C/TC ratio for R group increased from 0.08 ± 0.02 at week 0 of PB treatment to 0.19 ± 0.05 at week 6, while for NR group the ratio remained unchanged from 0.07 ± 0.01 at week 0 to 0.08 ± 0.03 at week 6.

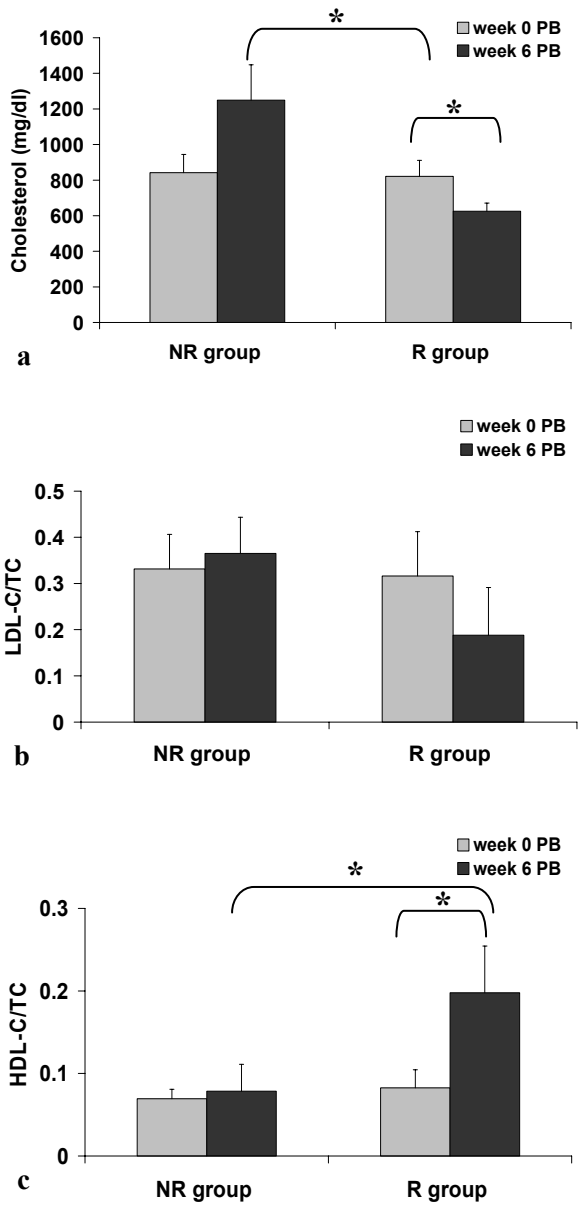


Fig. 1. Histograms representing the measure serum parameters: (a) cholesterol, (b) LDL-C/TC, (c) HDL-C/TC, at week 0 (before PB gavage), and after 6 weeks of probiotics administration (week 6 PB) in the positively (R) and negatively (NR) responsive groups; *p<0.05.

Triglycerides determination

In vivo administration of PB to HH for 6 weeks induced a slight decrease of TG concentration in sera (from 547 ± 182 mg/dl to 488 ± 107 mg/dl) in the R group as compared with NR group, where the TG levels increased from 695 ± 218 mg/dl to 1063 ± 149 mg/dl. The difference between the TG levels in the sera of NR group and R group at 6 weeks of PB treatment was statistically significant (Fig. 2).

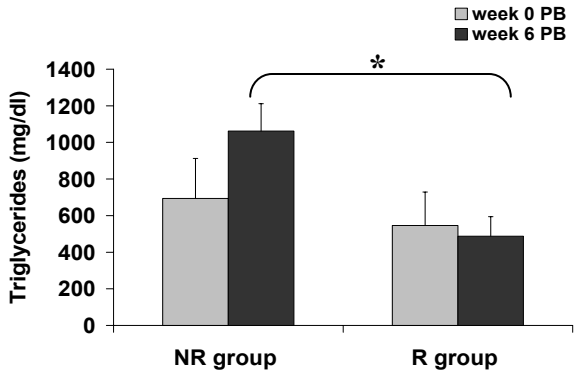


Fig. 2. Histograms presenting the values for serum triglycerides levels, before (week 0 PB), and after 6 weeks of probiotics administration (week 6 PB) in the positively (R) and negatively (NR) responsive groups; *p<0.05.

Glucose determination

In vivo administration of PB to HH for 6 weeks induced a slight decrease of glucose levels in sera of R group hamsters (from 123 ± 46 mg/dl to 88 ± 28 mg/dl) as compared with NR group, where the glucose concentrations slightly increased (from 110 ± 20 mg/dl to 147 ± 24 mg/dl). The difference between glucose levels in the sera of NR group and R group at 6 weeks of PB treatment was statistically significant (Fig. 3).

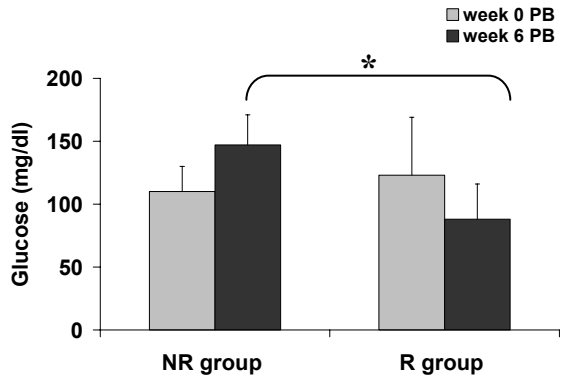
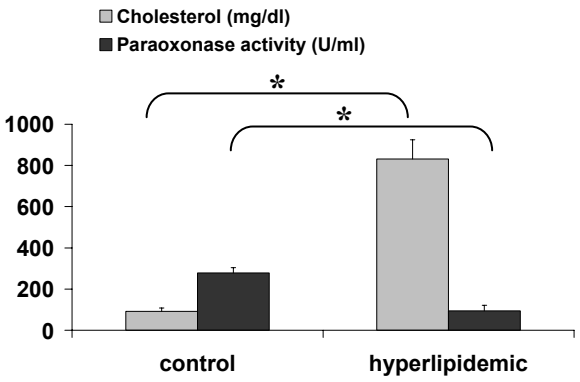


Fig. 3. Histograms presenting the values for sera glucose levels before (week 0 PB) and after 6 weeks of probiotics administration (week 6 PB), in the positively (R) and negatively (NR) responsive groups; *p<0.05.

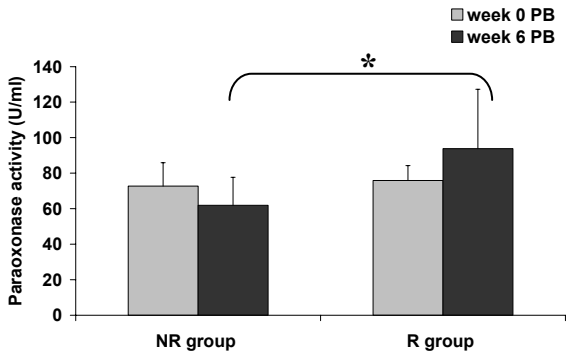
The anti-oxidant effect of PB

The modulation of the anti-oxidant capacity of the serum by the PB treatment was evaluated by measuring the enzymatic activity of PON1. The PON1 activity was negatively correlated with the hyperlipidemic status. In HH with total serum cholesterol levels of 831.21 ± 96.11 mg/dl (8 fold the control value of 92.25 ± 16.13 mg/dl), the

specific PON1 activity was 3 fold decreased compared to control animals (from 278.52 ± 25.16 U/ml to 94.57 ± 27.51 U/ml) (Fig. 4a). *In vivo* administration of PB to HH for 6 weeks induced an increase of PON1 activity in the sera of the R group hamsters (from 76 ± 8.4 U/ml to 93.8 ± 33.4 U/ml), as compared with NR group, where the PON1 activity decreased (from 72.7 ± 13.1 U/ml to 61.8 ± 15.9 U/ml). The difference between PON1 activity in the sera of R group and NR group at 6 weeks of PB treatment was statistically significant (Fig. 4b).



a



b

Fig. 4. The anti-oxidant potential of serum after PB treatment measured as paraoxonase 1 activity: (a) in hyperlipidemic hamsters versus control animals, (b) in the positively (R) and negatively responsive (NR) groups, at week 0 PB, and after 6 weeks of probiotics administration (week 6 PB); *p<0.05.

DISCUSSION

Atherosclerosis is one of the widespread causes of morbidity and mortality in the world, due to the cerebral and coronary arteries diseases it generates, that may lead to stroke or acute myocardial infarction. One of the main risk factors in atherosclerosis initiation and progression is high serum cholesterol concentration, high LDL cholesterol and low HDL cholesterol. Many treatments tried to manage the cardiovascular lipid-related risks, but they frequently were associated with side effects[12]. The investigation of the potential effect of functional food on some of the risk factors of atherosclerosis due to the role of acidophilic bacteria in reducing cholesterol concentration has been suggested[12]. However, many problems remain to be solved, because we do not know exactly how probiotics work, what are their beneficial effects in atherosclerosis, or what is the suitable dosage.

In the present study we used an animal model of diet induced atherosclerosis, the hyperlipidemic hamster, to investigate whether probiotics administration can stop or reverse the pro-atherogenic process. The employed animal model mimics human atherosclerosis and presents many similarities with the human lipid metabolism[10]. Our results demonstrate that PB treatment of HH with very high concentrations of lipids in the serum induces a 2 fold decrease of serum cholesterol in the positively responsive group (R group), having the LDL-C levels slightly decreased and the HDL-C levels increased. The PB administration to HH induced a slight decrease in serum triglycerides and glucose concentrations, showing that PB may have other beneficial effects beside the hypocholesterolemic one. Data from the group of Naruszewicz M. *et al.*[13] have shown that in the serum from patients with moderately elevated cholesterol concentrations the supplementation of the diet with *Lactobacillus plantarum 299v* significantly lowered LDL cholesterol concentration. The mechanism of action may relate to these bacteria being settled in the large intestine, where they are responsible for the fermentation of dietary fiber, the end products being the short-chain fatty acids (acetic, propionic, and butyric acids)[14]. Only acetic and propionic acids are absorbed in the blood, pass into the liver, and enter the metabolic pathways[15]. It has been postulated that only short-chain fatty acids, mainly propionic acid, may improve glucose tolerance and inhibit cholesterol synthesis in the liver, presumably by inhibiting the rise in the serum concentration of free fatty acids and by improving insulin sensitivity[16,17]. There are other studies suggesting that the probiotics reduce serum cholesterol levels due to their capability to compete with cholesterol for intestinal absorption[12].

Our results demonstrate an increase of the anti-oxidant potential of HDL particles following 6 weeks of PB treatment of responsive hyperlipidemic

hamsters, expressed as a 40% increase of the PON1 activity in the R group compared to the NR group. In the literature there are no data about the antioxidant effect of PB administration.

In conclusion, our data show that administration of probiotics to hyperlipidemic animals have pleiotropic anti-atherosclerotic effects: reducing the serum cholesterol, increasing the antioxidant potential of HDL, lowering triglycerides and glucose levels. The present report, together with the data existing in the literature, support the probiotics administration as a safe treatment for atherosclerosis.

ACKNOWLEDGEMENT

This project was supported by a grant from the Romanian Ministry of Education and Research (CEEX #80/2006 PNII41-067/2007). The authors thank Mrs. Ioana Andreescu and Mrs. Safta Nae for their excellent technical assistance, and Dr. Emanuel Dragan for his qualified assistance.

REFERENCES

1. Tognarini D.P.; Sullivan D.R.; Thomas D.W.; Sikaris K., Reducing CVD risk - integration of clinical. and laboratory criteria, *Aust Fam Physician,* **2008**, 37(6):422–7.
2. Hansson G.K.; Robertson A.K.; Söderberg-Nauclér C., Inflammation and atherosclerosis, *Annu Rev Pathol*, **2006,** 1:297–329.
3. Sima A.; Stancu C., Statins: mechanism of action and effects, *J Cell Mol Med*, **2001**, 5(4):378–3874.
4. Lilly D.M.; Stillwell R.H., Probiotics: growth-promoting factors produced by microorganisms, *Science*, **1965,** 147:747–8.
5. Scarpellini E.; Cazzato A.; Lauritano C.; Gabrielli M.; Lupascu A.; Gerardino L.; Abenavoli L.; Petruzzellis C.; Gasbarrini G.; Gasbarrini A., Probiotics: which and when?, *Dig Dis,* **2008,** 26(2):175–82.
6. Taranto M.P.; Perdigón G.; Médici M.; De Valdez G.F., Animal model for in vivo evaluation of cholesterol reduction by lactic Acid bacteria, *Methods Mol Biol,* **2004**, 268:417–22.
7. Lin Y.P.; Thibodeaux C.H.; Peña J.A.; Ferry G.D.; Versalovic J., Probiotic Lactobacillus reuteri suppress proinflammatory cytokines via c-Jun, *Inflamm Bowel Dis,* **2008**.
8. Mackness M.; Mackness B.; Paraoxonase 1 and atherosclerosis: is the gene or the protein more important?, *Free Radic. Biol.Med,* **2004**, 37:1317–1323.
9. Shamir R.; Hartman C.; Karry R.; Pavlotzky E.; Eliakim R.; Lachter J.; Suissa A.; Aviram M., Paraoxonases (PONs) 1, 2, and 3 are expressed in human and mouse gastrointestinal tract and in Caco-2 cell line: Selective secretion of PON1 and PON2, *Free Radical Biology & Medicine*, **2005**, 39: 336–344.
10. Sima A.; Bulla A.; Simionescu N., Experimental obstructive coronary atherosclerosis in the hyperlipidemic hamster, J Submicrosc Cytol Pathol, **1990,** 22:1–16.
11. Rozenberg O.; Shih D.M.; Aviram A., Paraoxonase 1 (PON1) attenuates macrophage oxidative status: studies in PON1 transfected cells and in PON1 transgenic mice, *Atherosclerosis,* **2005,** 181, 9–18.
12. Viljoen A., New approaches in the diagnosis of atherosclerosis and treatment of cardiovascular disease, *Recent Patents Cardiovasc Drug Discov,* **2008,** 3(2):84–91.
13. Bukowska H.; Pieczul-Mróz J.; Jastrzêbska M.; Chelstowski K.; Naruszewicz M., Decrease in fibrinogen and LDL-cholesterol levels upon supplementation of diet with Lactobacillus plantarum in subjects with moderately elevated cholesterol, *Atherosclerosis,* **1997**, 137:437–8.
14. Naruszewicz M.; Johansson M.-L.; Zapolska-Downar D.; Bukowska H., Effect of Lactobacillus plantarum 299v on cardiovascular disease risk factors in smokers, *Am J Clin Nutr,* **2002**, 76:1249–55.
15. Nordgaard I.; Mortensen P.B., Digestive processes in the human colon. *Nutrition,* **1995,** 11:37–45.
16. Venter C.S.; Vorster H.H., Possible metabolic consequences of fermentation in the colon for humans. *Med Hypotheses,* **1989,** 29:161–6.
17. Nishina P.M.; Freedland R.A., Effects of propionate on lipid biosynthesis in isolated rat hepatocytes, *J Nutr*, **1990,** 120:668–72.