Iranian Journal of Diabetes and Lipid Disorders; 2010; Vol 9, pp 1-7

Reduction in serum lipid parameters by incorporation of a native strain of Lactobacillus Plantarum A7 in Mice

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Abstract

Background: Probiotics has recently been considered as an alternative biological method for chemical agents in reduction of plasma cholesterol. However, the results of human and animal studies showed various results and sometimes strain dependency and host specify of probiotic strains have explained as the possible reasons for such variations. Therefore, it seems necessary to find new probiotic agents among the native strains through in-vivo experiments.

Methods: *Lactobacillus* bacteria isolated from fecal samples of infants and *Lactobacillus plantarum A7* was selected due to its potent resistance to biliary secretions for evaluation of its effects on cholesterol reduction in mice. Sixteen male rats were fed with a high cholesterol regimen for 14 days (pre-intervention period), and their serum blood samples analyzed for cholesterol, triglyceride, LDL and HDL. Then they randomly divided into two groups (control and treated groups). Treated group received 10^8 CFU/ml of *Lactobacillus plantarum* A7 with the diet formulation which was used in pre-intervention period for 14 days; while, the control group continued to receive the same formula and did not receive any bacteria (intervention period). Serum samples were analyzed for lipid parameters. Blood, spleen and liver samples of both groups were evaluated for translocation.

Results: In pre-intervention period, mean values of cholesterol, triglyceride, HDL and LDL in treated group were 101.3, 105.1, 40.9 and 35.6 mg/dl, respectively and no significance difference was observed between treated and control groups. During intervention period, the mean values of cholesterol, triglyceride, HDL and LDL in treated group were 92, 97.7, 46 and 30.4 mg/dl, respectively and total cholesterol, triglyceride and LDL reduced significantly in treated group but for HDL this difference was not significant. All the translocation tests were negative for either the strain tested.

Conclusion: *L. plantarum* A7 is effective in lowering serum lipid levels in rats. Furthermore, daily usage of 10^8 cells of this strain could be considered safe, regarding its translocation from intestinal lumen to other organs, although more studies on safety must be done in the future.

Keywords: Probiotics, *Lactobacillus plantarum*, Cholesterol

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Introduction

Annually there are many death reports due to coronary heart disease (CHD) associated with hyperlipidemia (1). In Iran, as many countries, CHD associated with hyperlipidemia are considered as the main cause of death. Animal and human studies indicated that reduction of cholesterol or low density lipoproteins (LDL) in plasma could lead to lowering the risk of CHD (2). Although chemical pharmacologic agents are prescribed to regulate the cholesterol level, they could have adverse effects which impose more financial burden on patients (3). Recently, some probiotic strains have been reported to play a role in reduction of such blood lipids (4, 5). Probiotics are defied as living micro-organisms which upon ingestion in certain numbers exert health benefits on the host beyond inherent basic nutrition. Several mechanisms are suggested for cholesterol reducing activity of these bacteria including secretion of bile salt hydrolase enzyme (6), and incorporation of cholesterol into the cytoplasmic membranes of the bacteria as they proliferate in the intestinal tract (7) and assimilation of cholesterol. Yaunghoon and colleagues categorized the factors responsible for the reduction of cholesterol by probiotic bacteria (8); However, There are wide variations in the results of animal and human studies which investigated the cholesterol lowering effects of specific strains of lactic acid bacteria (9, 10).

Some strains of lactobacilli belonging to the certain species are considered as commercial probiotics with specific effects and their pharmaceutical preparations, now are offered in the market. Researches on the cholesterol lowering effects of aforementioned probiotics have not been conclusive and wide variations have been obtained among the accumulated data. Sometime, strain dependency in this trait has been accounted for such variations (11). Also, it has been suggested that with high probability, the influence of probiotics is depending on "at least" partly on the indigenous lactobacilli that are present in the GI tract of the host (12). Since, the diversity of these lactoflora varies between individuals depending on the genetic background, physiological and environmental factors, variation in the results of human studies might have been due to unspecificity of the host. Therefore, it is necessary to find the new probiotic strains among the native strains and presentation of any strain with serum lipid lowering effect needs to be *in vivo* examined, regardless of its species.

Recently, a *Lactobacillus plantarum* strain has been isolated from fecal flora of Iranian healthy infant; it was selected among 52 other lactobacilli isolates and characterized as a probiotic potential strain (13-15). Present study aimed to assess the effects of this strain on rat blood lipid profile; meanwhile the safety aspects of dietary intake of this strain by rats were investigated.

Methods

Bacterial strain and culture media

L. plantarum A7 was provided from the microbial collection of food microbiology laboratory of industrial university of Isfahan. This strain is a human origin strain which was isolated from fecal flora of 19 months healthy Iranian infant (13, 14). It was identified using biochemical and molecular methods and characterized as potential probiotic strain (14, 15). The selection of L. A7 for the present study was due to the high bile resistance of this strain among several *Lactobacillus* spp (15).

L. plantarum A7 was inoculated in 200 ml deMan-Rogosa-Sharpe (MRS) broth, the cultured media incubated media (Merck) at 37° C in aerobic conditions for after 16-20 hours to reach at an OD of 8-8.5. Culture media was centrifuged and the bacterial pellet was diluted in skimmed milk to prepare bacterial population of 1×10^8 CFU/ml, and then dispensed into microtubes (1.5 ml/tube). The microtubes were stored at 4° C to be used later. The content of each microtube was used as a unit of bacterial daily dose via oral gavage to each rat. Preparation of skimmed milk containing bacteria was performed weekly. The bacterial viability and its concentration

were checked using MRS agar plating during the storage period. The results of a preliminary study indicated that the bacteria in skimmed milk were best preserved at 4° C during a time period of one week.

Animals

Sixteen male Wistar rats weighing about 85 grams were used in this experiment. The rats were housed two per cage. After an acclimatization period of one week, all animals were fed with skim milk containing 10% w/v cream as a high cholesterol regimen for a period of 14 days. An initial blood sample was collected from each animal. Then, the rats were semi-randomly divided into 2 experiment groups of 8 in each. One group considered as treated group and the other as the control. For another period of 14 days all the rats were fed with the same high cholesterol regimen, but only treated group also received a daily dose of 1×10^8 CFU/ml of *Lactobacillus plantarum* via oral gavage. Then, another blood sample was collected from each rat. Sterile capillary tubes were used to collect 1 ml blood through retro-orbital sampling from each rat under careful general anesthesia using diethyl ether. Blood samples were centrifuged for five minutes at 4000 RPM to provide isolated serum samples. All serum samples were analyzed to measure total cholesterol, triglycerides, LDL and HDL.

Bacterial translocation

To evaluate safely use of the bacteria, their translocation in blood, liver and spleen were studied. At the end of the intervention period and subsequently after the second blood sample collection, the tested rats were deeply anesthetized and their spleens and livers were dissected under aseptic condition. Fifteen micro liter of each blood sample was cultured on MRS agar and brain heart infusion agar (BHI). Cultured plates containing MRS were incubated anaerobically (5%Co2, gaspack A, Merk) and the plates containing BHI were incubated aerobically. The organ samples separated from each animal, one gram tissue of each tested organ was homogenized in peptone water. One hundred micro liter of the homogenized samples was cultured in MRS and BHI agar media then incubated for 48 hours in both anaerobic and aerobic conditions, respectively. Presence of any bacteria on agar plates considered as positive. The results were presented as the number of rats translocation occurred per total number of the rats (the incidence of translocation).

Statistical analysis

During the study, a couple of rats died, so the data obtained from the experiments produce a pair of unequivalent blocks, for further analysis. The results are reported as the means of each measured lipid parameter ±SD. If it was required, T-test and Mann-Whitney were performed to compare lipid levels between the two treatment groups. To compare lipid levels before and after intervention, Paired t-test and Wilcoxoh test, if need, were performed. The data for bacterial translocation differences were analyzed using Chi-test.

Results

The results of the mean values of measured lipid parameters at the end of pre-intervention period have been presented in Table 1. At this stage, according to the statistical analysis, no significant difference was observed between the treated and control groups (P= 0.87). Table 2 has represented the results of the mean values of measured lipid parameters at the end of intervention period. Comparing lipid profile in treated and control groups revealed cholesterol, triglyceride and LDL levels were significantly reduced in treated group (P=0.02, P<0.001 and P=0.01, respectively) but no significant difference was observed for HDL (P= 0.19).

Table 1- Lipid profile in rats before intervention with L. plantarum.

Groups	Cholesterol(mg/dl)	Triglycerides(mg/dl)	HDL(mg/dl)	LDL(mg/dl)
Treated Group	101.3 (10.6)	105.1 (2.3)	40.9 (8.3)	35.6 (6.5)
Control Group	102.4 (14.6)	102.4 (16.9)	38.7 (7.2)	33.4 (7)
Total	101.9 (12.3)	103.8 (11.7)	39.8 (7.5)	34.5 (6.6)

Data presented as Mean(SD), In comparison P-values were not significant (P>0.05),
N= Eight male Wistar rats in both control and treated groups.

Table 2- Lipid profile in rats after intervention with L. plantarum

Group	Cholesterol(mg/dl) *	Triglycerides(mg/dl) *	HDL(mg/dl)	LDL(mg/dl) *
Treated Group	92 (8)	97.7 (10)	46 (9.3)	30.4 (7.1)
Control Group	106.3 (13.5)	146.6 (8.6)	39.1 (9.1)	42.4 (8.7)
Total	99 (13.1)	122 (26.9)	42.6 (9.5)	36.4 (9.8)

Data presented as Mean(SD), *In comparison P-values were significant (P<0.05),
N= Eight male Wistar rats in both control and treated groups.

Table 3- Bacterial translocation to blood, spleen and liver as incidence (number of rats where translocation was observed to total number of rats.)

Group	Blood		Liver		Spleen	
	MRS	BHI	MRS	BHI	MRS	BHI
Treated Group	0/7	0/7	1/7	3/7	1/7	1/7
Control Group	0/7	0/7	2/7	3/7	0/7	1/7

N= Eight male Wistar rats in both control and treated groups.

Bacterial translocation

Development of bacterial colonies, (even a single colony) from the cultures of blood, spleen and livers samples was considered as positive and the results were indicated as the number of rats in which the bacteria were detected (bacterial translocation) to the total number of rats (Table 3).

No bacterial colony was found in the blood cultures of both groups (no bacteremia). In spleen and liver difference in incidence of translocation between treated group and control group was not statistically significant. Biochemical analysis of isolated spleen and liver showed that none of the isolates belonged to *Lactobacillus plantarum* A7.

Discussion

High concentrations of the total cholesterol and LDL-cholesterol are highly associated with increased risk of coronary heart disease. Reduction in total cholesterol, triglyceride and LDL-cholesterol in hypercholesterolemic men reduces the incidence of cardiovascular disease. In the present study, introduction of a defined concentration of *Lactobacillus plantarum* A7 reduced total cholesterol, LDL and triglyceride in rats fed with lipid-rich diet but it had no effects on serum HDL-cholesterol.

The finding that daily gavage of *Lactobacillus plantarum* A7 lowers serum total cholesterol concentrations in rats confirms other studies. Taranto *et al.* reported that over a 7 days study *Lactobacillus reuteri* reduced the concentration of total serum cholesterol concentration in mice (16). In another experiments, Nguyen demonstrated that *Lactobacillus plantarum* PHO4 reduced total cholesterol level in hypercholesterolemic mice (17).

Regarding the effect of the tested strain on the concentration of serum triglyceride, it should be noted that triglyceride was the most affected parameter by the daily gavage of *Lactobacillus plantarum* A7. This result is in accordance with other studies which reported the effectiveness of selected strains of lactic acid bacteria; e.g. *L. casei* (18), *L. reuteri* (16, 19) and *Lactobacillus plantarum* PHO4 (17).

The data obtained in this study for the influence of L. A7 on serum LDL and serum

HDL, generally agreed with other findings (20-22). The data from human and animal studies suggest a moderate cholesterol lowering effect of some probiotic strains; however, the potential mechanisms for this claimed effect remained unknown and must to be clarified. Several *in vitro* experiments with lactobacilli (23-25) and bifidobacteria (26-28) provided the evidence that some strains of these bacteria have the potential to assimilate cholesterol in the presence of bile acids.

Gilliland *et al.* have suggested that bile acid resistant *L. acidophilus* which assimilates cholesterol had a hypocholesterolemic effect in pigs (29). The removal of cholesterol from the culture has been attributed to cholesterol incorporation into the bacterial cell membrane. They have claimed that *L. acidophilus* could remove cholesterol from a growth medium only in the presence of bile and under anaerobic conditions. Because these conditions are expected to occur in the intestine, this could enable the organisms to assimilate at least part of the dietary cholesterol. Thus, cholesterol could not be available to be absorbed into the blood circulation. Another mechanism that may contribute to cholesterol-lowering effects is bile salt hydrolase (BSH) enzyme activity of *Lactobacillu*s strains (30). Deconjugated bile salts are less efficiently reabsorbed than their conjugated counterparts, which results in the excretion of larger amounts of free bile acids in feces. Also, free bile salts are less efficient in solubilizing and absorption of lipids in the gut. Therefore, deconjugation of bile salts could lead to a reduction in serum cholesterol either through increasing the demand for cholesterol for de novo synthesis of bile acids to replace the lost in feces or by reducing cholesterol solubility and absorption of cholesterol in the intestinal lumen.

The results of this study indicated that *L. plantarum* A7 has the potential to reduce serum cholesterol, LDL and triglyceride levels. Since *L. plantarum* A7, was isolated from human gut and previously characterized as a high resistant strain to bile components (15), further studies are required to determine its mechanisms for serum lipid lowering effects.

As discussed earlier, daily gavage of 10^8 CFU per ml of bacteria in this study was at least ten times more than daily doses that used in other studies; nonetheless, the assessment of pathogenicity of these bacteria must be carefully recognized before considering any trial in human. One of the best indicators to assess the probiotic toxicity is bacterial translocation from gut to other organs such as blood, spleen and liver; because, it is the first step in pathogenesis of intestinal bacteria (31). In this study, ingestion of high concentration of bacteria in rats (10^8cfu/ml) indicated that there was no bacteremia in either of the control or treatment group. Comparing the number of bacteria isolated from liver and spleen samples in treated and control animals indicated that there were few bacteria in spleen and liver samples which were not related to *Lactobacillus plantarum* A7 treatment. Deitich *et al.* reported that some bacteria may be present in liver and spleen of healthy rats (32). Therefore, it does not seem the high bacterial doses administrated to rats with the probiotic activity produces bacterial translocation to blood, liver and spleen.

Acknowledgements

This study was supported by a grant from Isfahan University of medical sciences as research project number 387129.

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