

The Effect of Bifidobacterium Breve A28 and Inulin on Obstructive Jaundice Induced Bacterial Translocation

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ABSTRACT

AIM: Bacterial translocation and septic events is strongly associated. Pro-prebiotics and symbiotics use in the prevention of bacterial translocation has been widely studied. The aim of this study was to investigate the effects of intestinal *Bifidobacterium breve* A28 as a newer probiotic, inulin as a prebiotic and both as a symbiotic on a rat model of obstructive jaundice induced-bacterial translocation.

METHODS: To create a bacterial translocation obstructive jaundice model was used. Four groups, each have 12 rats, designed. Postoperatively 10 days the groups were fed with normal rat food plus pro-pre and symbiotic, and control group was fed only normal rat food. After this period, systemic blood, liver, spleen and mesenteric lymph nodes samples were obtained for microbiological

culture. The Pearson's chi-Square test was used for the statistical analysis.

RESULTS: Probiotic, prebiotic effectively reduced bacterial translocation, separately and as a symbiotic. No significant difference was observed between probiotic and prebiotic groups, and between symbiotic and probiotic groups regarding the rate of bacterial translocation. And there were a significant difference between control and prebiotic group.

CONCLUSIONS: This new mixture of pro and prebiotic on bacterial translocation is promising.

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Key words: Bacterial translocation; Symbiotic; Probiotic; *Bifidobacterium breve* A28; Inulin

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INTRODUCTION

The gut serves a barrier against living microorganisms and antigens within its lumen, not only has functions as simple digestion and excretion. Since Berg and Garlington^[1] described "bacterial translocation" in 1979, there are numerous study widely assessed this theory and verified gut as an immunologic organ. Mainly accepted definition of bacterial translocation (BT) is "passage of viable resident bacteria, inert particles and other macromolecules, such as lipopolysaccharide endotoxin from the gastrointestinal tract via the intestinal mucosal barrier to normally sterile tissues such as mesenteric lymph nodes (MLN's) and other internal organs" and the mechanisms that promote BT are: (a) intestinal bacterial overgrowth;

(b) immunodeficiencies, and (c) increased intestinal permeability^[1].

Probiotics are defined as living microbial supplements, when administered in adequate amounts, affects the host beneficially via modulating the intestinal microbial balance^[2]. They can prevent and treat some gastrointestinal disorders, from diarrhea to carcinogenesis^[3]. Prebiotics are non-digestible supplements that stimulating the growth and/or the effectivity of some probiotics in the colon, when probiotics and prebiotics administered together they defined as symbiotic and showed a synergistic effect^[4,5].

In the present study, we investigated the effects of intestinal Bifidobacterium breve A28 as a probiotic, inulin as a prebiotic and both as a symbiotic on a rat model of obstructive jaundice induced-bacterial translocation. We assessed one probiotic's and a different symbiotic's effect on bacterial translocation and aimed to assess the applicability of newer probiotics to further studies.

MATERIALS AND METHODS

Animals

This experimental study was approved by the animal experimentation ethics committee of Ankara Research and Training Hospital (Ankara, Turkey) and maintained in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals. A total of 48 eight-week-old male Wistar albino rats weighing about 250 g were used. The animals were housed under constant temperature (21°C±2°C) and humidity, with 12-h dark/light cycles and allowed tap water and rat pellets ad libitum before and after the operation.

Experimental design

The animals were randomized into 4 groups, each involving 12 rats. All operations were performed under sterile conditions and all the surgical procedures were performed while rats were anesthetized with ether inhalation, and then 0.2 mL ketamine HCL (Ketalar[®]; Parke-Davis, New Zealand, Australia) was injected into the quadriceps muscle, at a dose of 10 mg/kg. After midline abdominal incision, the common bile duct was identified and mobilized. It was then doubly ligated using 5-0 silk and divided. Then, the abdominal wall was closed with 2-0 silk continuous sutures. Relaparotomy and sample taking was performed on the 10th day.

The rats were divided into four groups: control group were fed with normal rat food, symbiotic group were fed with 1010 CFU/mL B. breve A28 strain (probiotic), 1 mL 5% solution of inulin (prebiotic) and normal rat food, probiotic group were fed with 1010 CFU/mL B. breve A28 strain and normal rat food, and prebiotic group were fed with 1 mL 5% solution of inulin and normal rat food for 10 days postoperatively. Probiotic, prebiotic and symbiotic was given respectively for an 10-day period via temporary orogastric intubation by the trained author. 1 mL liquid mixture containing 1,010 CFU probiotics and/or 1 mL liquid mixture containing 5% solution of inulin was given to certain groups. Ten days following the obstructive jaundice development, the animals had laparotomy under sterile conditions. The samples of systemic blood, liver, spleen and MLN's were obtained under sterile conditions for microbiological culture.

The supply of Prebiotics and Probiotics

Bifidobacterium breve A28 strain was obtained from the Biotechnology Laboratory of Gazi University (Faculty of Sciences, Department of Biology, Ankara, Turkey). B. breve A28 strain was isolated from the stool of a 28-day-old neonate. For the selection of the probiotic that would be used in the study, 30 Bifidobacterium spp. isolates obtained from breast milk and baby stool were investigated

in the Biotechnology Laboratory of Gazi University. Bifidobacterium breve A28 strain was incubated at 37°C for 4 days in MRS broth (Merck KGaA, Darmstadt, Germany) medium containing cysteine under anaerobic conditions. At the end of the incubation period, cell pellets were obtained by centrifugation at 5,000 rpm for 15 minutes. These pellets were washed twice with physiologic serum solution, and resuspended in 2 mL of physiologic serum solution. This bacteria suspension was given via temporary orogastric intubation to the rats in symbiotic and probiotic groups for 10 days at a dose of 1 mL containing 1,010 CFU for each rat. All animals totally consumed given suspension.

Inulin (from chicory, Sigma, St. Louis, MO, USA), which stimulates the growth of Bifidobacteriae, was used as a prebiotic. A 5% solution of inulin was prepared and given to the rats via temporary orogastric intubation in groups 3 and 4 for 10 days at a dose of 1 mL, for each rat.

Microbiologic Evaluation

Tissue samples obtained from the MLN's, liver and spleen were placed in sterile tubes containing 2 mL thioglycolate medium (Oxoid, Cambridge, UK). After homogenization under aseptic conditions, tissue samples were weighed and inoculated onto 100 µL blood agar (Oxoid) and Levin eosine methylene blue (EMB) agar via quantitative methods. Bacterial growth was evaluated at 24 and 48 hours in the plaques incubated under aerobic conditions at 37°C. All bacterial colonies were counted, and the identification of the bacteria was performed via standard conventional methods. One mL of blood sample was obtained from each rat and inoculated into aerobic pediatric blood culture media. Blood cultures were incubated for 5 days in a BACTEC 9240 (Becton Dickinson, Franklin Lakes, NJ, USA) blood culture system. Positive blood culture samples were inoculated onto blood agar and Levin EMB agar, and incubated at 37°C under aerobic conditions. Growth was evaluated at 24 and 48 hours. The identification of the bacteria was performed via standard conventional methods. Bacterial growth was evaluated as colony forming units (CFU's) per gram. Results, obtained as CFUs, were calculated according to log 10 base.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) 11.0 software (SPSS Inc., Chicago, IL, USA). The Pearson's chi-Square test was used for the statistical analysis. $p < 0.05$ was considered statistically significant.

RESULTS

Results of the control group

BT developed in the blood, MLN's, spleen, and liver cultures of all 9 rats that survived in the control group [9/9 (100%)]. While the dominant microorganism was *E. coli*, growth of *Enterobacter cloacae* and *Enterococcus* spp. was also observed.

Results of the symbiotic group

The growth of *Staphylococcus aureus* was observed in the liver culture of only 1 of the 12 rats in symbiotic group [1/12 (8.3%)].

Results of the probiotic group

Staphylococcus aureus was isolated from MLN's, spleen, and liver cultures of 1 of the 11 rats and from the spleen and liver cultures of another rat. In addition, *Proteus* spp. was isolated from the blood, spleen, and liver cultures of one rat [3/11 (27.2%)].

Results of the prebiotic group

Proteus spp. growth was observed in both blood and liver cultures of 2 of the 11 rats, in blood culture of 1 rat, in the spleen culture of one rat, and in the liver culture of another rat [5/11; (45.4%)].

Overall bacterial translocation rate and survival of the animals

Overall bacterial translocation rate in the control group was 100%, in the prebiotic group 45.4%, in the probiotic group 27.2% and in the symbiotic group 8.3%. Accordingly, all of the animals in the symbiotic group was survived. In the probiotic and prebiotic groups, 1 rat died on the 3rd day. In relation to the bacterial translocation rates, during the observation period, 3 rats were more jaundiced than the other rats and they died 4th day after bile duct ligation (Figure 1).

BT rates and place of culture growth were presented in table 1. Bacterial translocation rates were different between the groups ($p < 0.05$). No significant difference was observed between probiotic and prebiotic groups, and between symbiotic and probiotic groups regarding the rate of bacterial translocation. And there were a significant difference between control and prebiotic group.

DISCUSSION

The absence of bile in the gastrointestinal tract causes translocation of bacteria and endotoxin. After bile duct obstruction, the development of BT is explained with increased intestinal permeability and bacterial overgrowth^[6]. The underlying mechanism of increased intestinal

permeability in obstructive jaundice is not clearly identified, but absence of anti-oxidant and anti-infective agents such as bile salts, bile pigments and phospholipids in the intestinal mucosa are thought to be key factors for its occurrence^[7]. These factors also facilitate bacterial overgrowth, and both promote bacterial and endotoxin translocation to MLN's, portal circulation and the liver and the spleen^[7]. A lot of conditions cause intestinal bacterial overgrowth and promote BT in the animal models; including oral antibiotics, endotoxic shock, protein malnutrition, parenteral alimentation, bowel obstruction, and bile duct ligation^[8]. Some authors have shown that the immune system is impaired during obstructive jaundice, and the host's ability to defeat the translocating microorganisms is deteriorated^[9,10]. Also increased oxidative stress in obstructive jaundice can cause intestinal mucosal injury and stimulates intestinal endotoxin translocation^[11]. The risk of developing sepsis and related conditions during obstructive jaundice increases with infected bile, however it can also be developed with sterile bile^[12,13]. This detail may be the clue for explaining why one third of bacteriemic patients dying of sepsis with no septic focus is identified^[14]. Clinical and experimental studies showed that the intestines are the major source of bacteria responsible in the pathogenesis of obstructive jaundice induced-sepsis^[6,13,15]. In the literature, there are many publications showing a relationship between obstructive jaundice and BT^[6,7,13,16,17]. Today, BT thought to be responsible for sepsis in numerous surgical conditions, including elective abdominal surgery, intestinal obstruction, colorectal cancer, shock, pancreatitis, patients receiving long term parenteral nutrition, malnourished and obstructive jaundiced patients^[18]. MacFie *et al.*^[19] showed an association between BT and increased incidence of septic events, but BT was not associated with mortality. The mechanism of BT was not clearly explained but Berg and Garlington's theory still maintaining its validity with expanding molecular details added^[1]. Although, the bacterial migration route has not been clarified, most translocated bacterial types from the gastrointestinal tract are Enterobacteriaceae, which are the bacterial types that most commonly cause septicemia in hospitalized patients, with *E. coli* being the most prominent^[16,20].

Previous studies suggest that lactic acid bacteria such as *L. acidophilus* and *B. bifidum* stimulate the immune system and cause protective effect against BT^[21,22]. Bifidobacterium produces glutamine from NH_4^+ and glutamate^[23]. Karatepe *et al.*^[24] suggested that glutamine is thought to be effective in reducing BT and oxidative damage in obstructive jaundice. Fukushima *et al.*^[25] showed an association between *B. bifidum* and local IgA secretion in the gut mucosa. Caplan *et al.*^[26] suggested that Bifidobacteria prevents increased permeability in the gut, positively affect microvillus environment and therefore protect the host. Because of its valuable effect on preventing BT, a Bifidobacterium bacteria selected in this study. Among 30 Bifidobacterium spp., *B. breve* A28 strain was selected because of its superior characteristics as a probiotic, such as resistance against acid

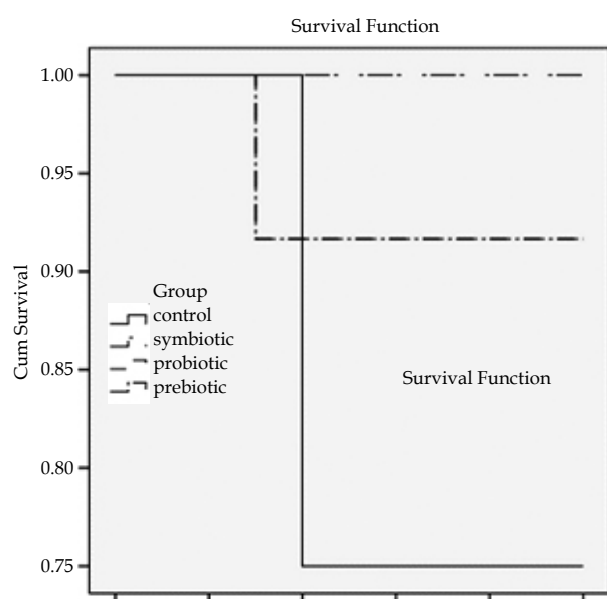


Figure 1 The survival tables of rats in the study groups.

Table 1 The outcomes, BT rates, positive culture sites and cultured microorganisms types of rats in the study groups.

	BT rate (%-n)	Survived (n)	MLN's	Blood	Liver	Spleen	Cultured microorganisms
Control group	100 - 9/9	9/12	9 rats (+)	9 rats (+)	9 rats (+)	9 rats (+)	Dominant <i>E. Coli</i> , <i>Enterobacter cloacae</i> and <i>Enterococcus spp.</i> Also observed
Symbiotic group	8,3 - 1/12	12/12			1 rat (+)		<i>Staphylococcus aureus</i>
Probiotic group	27,2 - 3/11	11/12	1 rat (+)		1 rat (+)	1 rat (+)	<i>Staphylococcus aureus</i>
Prebiotic group	45,4 - 5/11	11/12		1 rat (+)	1 rat (+)	1 rat (+)	<i>Proteus spp.</i>
				2 rats (+)	2 rats (+)		<i>Proteus spp.</i>
				1 rat (+)			<i>Proteus spp.</i>
					1 rat (+)	1 rat (+)	<i>Proteus spp.</i>

and bile, hemagglutination, and exopolysaccharide production, which plays a role in attachment to the Caco-2 epithelial cells, as well as onto epithelial surfaces. Also, prebiotic selection was made according to the previous studies, which demonstrated that inulin selectively stimulate the growth of bifidobacteria in the colon^[4,5,27].

E. coli, *proteus spp.*, *enterobacter cloacae*, *enterococcus spp.*, *proteus spp.* and *staphylococcus aureus* growth was observed in the samples. In agreement with other studies, the dominant microorganism isolated was *E. coli*^[16,28]. MLN's cultures were reported as one of the most reliable ways of monitoring BT in experimental studies^[29,30]. In our study, except for the control group, only one animal in the probiotic group has positive MLN's culture. Otherwise blood, liver and spleen culture was positive in some animals of symbiotic, probiotic and prebiotic groups. Although there are studies evaluating the development of BT within 1-3 weeks of obstructive jaundice, majority of studies have reported that BT occurs in 7 days of obstructive jaundice in rats^[16,28,30,31]. In our study, bacterial growth was observed in the cultures of 1 of 12 rats in the symbiotic group, 3 of 11 rats in the probiotic group, and 5 of 11 rats in the prebiotic group. Ruan *et al*^[32] demonstrated lower rates of BT in hemorrhagic rats treated with probiotics for 7 days compared to rats in the control group (80% to 40%). Mangell *et al*^[33] observed 75% to 100% BT in MLN's and liver culture in intraperitoneal lipopolysaccharide-applied control rats and this rate was reduced to 0% to 12% in the group fed with prebiotics, respectively. In experimental studies using different models, various degrees of reduction have been demonstrated in the BT rates of probiotic-treated animals^[34-36].

Symbiotic use to promote a health benefit is recently recognized topic. Up to date there are not much study designed to assess pre-pro and symbiotic effect on inflammation or infection. Among these studies separately investigating these effects is very rare. Symbiotics; decreased acute pancreatitis induced endotoxemia, reduced pathologic bacterial population in bowel, effective in prophylaxis against opportunistic intestinal infections with antibiotic-resistant pathogens and showed high anti-inflammatory activity at experimental colitis model in rats^[37-41]. Osman *et al*^[39,42] showed decreased bacterial translocation in the liver and MLN's with different symbiotics at different animal model of liver injury and colitis. On the other hand Rishi *et al* showed separate protective effect of prebiotic and probiotic against Salmonella-induced liver damage, but they did not showed synergistic effect as a symbiotic^[43]. Rayes *et al*^[44] overviewed pre-pro and symbiotic effect in surgery. Due to existing randomized controlled trials, they suggested that symbiotics have positive effect in patients with high-risk operations, but symbiotic preparations should be extensively tested before using them in clinical trials. In our study we separately investigated pre-pro and symbiotic effect. As a result, regarding the rate of bacterial translocation, there were no significant difference was observed between probiotic and prebiotic group, no significant difference between symbiotic and probiotic group and there were a significant difference between control and probiotic group. It would be helpful to add the sham operation group to assess surgical stress and anaesthesia effect on BT. It's well known that BT is much more easily induced in rats than humans and by all likelihood experiments in rats may not suitable models for evaluating potentially therapeutic or prophylactic measures aimed for clinical use. There is an obvious need for new clinical trials planned for this purpose.

Our results indicate that probiotic and prebiotic prevented the development of BT and more effective outcomes were achieved with symbiotic. However, further studies with different pro-prebiotics and different animal models are needed to determine genuine effects of the possible new treatment strategy on BT.

CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

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