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, seperately 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


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;
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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 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 rat pellets ad libitum before and after the operation. Experimental design

The results of the control group showed that 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%)].

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 cosine 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.[12,13]. 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[14]. These factors also facilitate bacterial overgrowth, and both promote bacterial and endotoxin translocation to MLN's, portal circulation and the liver and the spleen[8]. A lot of conditions cause intestinal bacterial overgrowth and promote BT in the animal models; including oral antibiotics, endotox shock, protein malnutrition, parenteral alimentation, bowel obstruction, and bile duct ligation[15]. 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[6,11,15]. In the literature, there are many publications showing a relationship between obstructive jaundice and BT[7,11,14,17]. 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[16]. 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[21]. 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 sepsemia in hospitalized patients, with *E. coli* being the most prominent[9,20].

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+ 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.](image)

| Table 1 The outcomes, BT rates, positive culture sites and cultured microorganisms types of rats in the study groups. |
|---|---|---|---|---|---|---|---|---|
| Group | 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 *E. coli*; Enterobacter cloacae and *Enterococcus spp.* Also observed |
| Symbiotic group | 8.3 - 1/12 | 12/12 | 1 rat (+) | 1 rat (+) | 1 rat (+) | 1 rat (+) | *Staphylococcus aureus* |
| Probiotic group | 27.2 - 3/11 | 11/12 | 1 rat (+) | 1 rat (+) | 1 rat (+) | 1 rat (+) | *Staphylococcus aureus* |
| Prebiotic group | 45.4 - 5/11 | 11/12 | 1 rat (+) | 2 rats (+) | 1 rat (+) | 1 rat (+) | *Proteus spp.* |
| | | | | | | | |

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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, probiotic 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[10,20]. 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[16,28,31]. 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; pre and symbiotic effect on inflammation or infection. Among these topics. Up to date there are not much study designed to assess pre-

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