Full Length Research Paper

Effects of metronidazole and probiotics oligosaccharide on bacterial translocation in protein malnutrition

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The present study aims to evaluate the effects of metronidazole, probiotics oligosaccharide on indigenous microflora and bacterial translocation (BT) in protein malnourished rats. Thirty male Wistar rats were divided into three groups: protein malnourished rats PM (group 1, n = 10) were fed with maize only, protein malnourished rats (group 2, n = 10) were received metronidazole and protein malnourished rats (group 3, n = 10) were received both metronidazole and probiotics-oligosaccharide for fifteen days. Metronidazole (1000 mg/kg/day) was given via an orogastric feeding tube to the second and third groups. Lyophilized probiotics-oligosaccharide (0.5 mg/g body weight/day) was given in two doses via the same route to the third group. All animals were sacrificed after fifteen days of protein malnutrition and cultures of the mesenteric lymph nodes (MLNs), liver, spleen and cecal contents were done. The incidence of bacterial translocation (BT) was 30% (3/10) in protein malnourished group 1, 60% (6/10) in group 2 where protein malnutrition was associated with metronidazole and 25% (2.5/10) in group 3 whose animals were subjected to protein malnutrition associated with metronidazole and probiotics oligosaccharide. A significant increase in the BT incidence was found in group 2 (P < 0.05), while a significant decrease was found in group 3 when compared to group 1. The total bacterial count of cecal flora was significantly low in group 3 than in group 1 (P < 0.01). These results suggest that the incidence of BT in protein malnutrition is increased by using an antibiotic while probiotics-oligosaccharide decreases this incidence in protein malnutrition induced by antibiotic. Thus, we conclude that probiotics-oligosaccharide can effectively protect the intestinal mucosa and prevent BT in protein malnourished infants.

Key words: Bacterial translocation, protein malnutrition, probiotics oligosaccharide, metronidazole and mesenteric lymph nodes (MLNs).

INTRODUCTION

In light of the wide usage of antibiotic drugs in developing countries, it becomes extremely important to determine the effects of malnutrition on the disposition of antibiotics. In the world, severe acute protein malnutrition (PM) affects approximately 13 million children under the age of 5 and it is associated with 1 - 2 million preventable child deaths each year (Collins, 2007). Use of antibiotics promotes the emergence of resistant organisms and multiple-antibiotic resistance has become a major public health issue.
Nowadays, metronidazole (2-methyl-5-nitroimidazole-1-ethanol) is widely used in the treatment of parasitic diseases and mainly against anaerobes, including Bacteroides (Freeman et al., 1997). Metronidazole at the doses used (1 mg/ml drinking water ad libitum) disrupts the intestinal bacterial flora resulting in the destruction of strict anaerobic bacteria and therefore, an overgrowth of enterobacteria in the cecal (Berg, 1981). This allows studying the simultaneous influence of malnutrition and antibiotics. There has been a growing interest in the effects of malnutrition on drug metabolism and pharmacokinetics (Jung and Shah, 1986).

Although malnutrition itself cannot be treated by drug therapy, it can induce situations where drugs are the primary form of treatment. The treatment of infections is the most common and severe drug-related problem associated with protein malnutrition.

In addition, probiotics were used in conjunction with antibiotic therapy to prevent or lessen the severity of antibiotic-associated diarrhea in children (Vanderhoof et al., 1999), although a meta-analysis of this issue showed significant problems in study design in several studies (Cremonini et al., 2001).

A malnourished individual is known to be much more susceptible to infections. In light of the wide usage of antibiotic drugs in developing countries, it becomes extremely important to determine the effects of malnutrition on the disposition of antibiotics (Jung and Shah, 1986).

The gut microflora is an important constituent of the intestinal mucosa barrier and this has led to the concept of probiotics therapy, that is, the application of potentially beneficial microorganisms (Fuller, 1992). Probiotics is a microbial dietary adjuvant that beneficially affects the host physiology by modulating mucosal and systemic immunity, as well as improving nutritional and microbial balance in the intestine tract. They are nonpathogenic microorganisms that, when administered in adequate amounts, confer a health benefit on the host. Probiotics bacteria are noninvasive, yet they need to interact with gastrointestinal (GI) epithelial cells to elicit their immunomodulatory effects. Probiotics have been shown to induce various epithelial cell responses by competing with pathogenic bacteria for host adhesion binding sites, improving epithelial cell barrier function (Myllyluoma et al., 2008).

Probiotics and prebiotics can be used to either prevent or reduce the severity of microbe-induced gut inflammation. The best known prebiotics are fructo-oligosaccharides, sugars of plant origin. It has interesting properties such as stimulating the growth of probiotics and renewal of cells in the intestinal mucosa.

PROBIONAT® is a product of both a probiotics and Fructo-oligosaccharide (FOS); it is advisable for its regulation of the intestinal flora, especially during an antibiotic in case of diarrhea or intestinal parasites. For all the positive effects of probiotics, three conditions can be met, a cocktail of the best micro-organisms in sufficient quantities and especially live.

Protein malnutrition disrupts the normal ecology of the microflora affecting strictly anaerobes (Tannock and Savage, 1974; Poxton et al., 1997), impairs host immune response and antibacterial defenses (Reynolds et al., 1992; Chandra, 1993), enhances the susceptibility to infection and leads to mucosal atrophy (Reynolds et al., 1996). Malnutrition is a common problem for critical ill patients and nutritional support is mandatory.

The effect of probiotics oligosaccharide in combination with antibiotics on protein malnutrition has not been proposed until now as an alternative to the use of prophylactic antibiotics. Antibiotic prophylaxis is intended to prevent the potential of bacterial contamination as a situation risk. It is unknown if modification of the intestinal flora with such a multispecies probiotics mixture with fructo oligosaccharide reduces bacterial overgrowth and bacterial translocation from the gut and, consequently, alters the course of disease. Therefore, the present study was as an attempt to assess if modification of intestinal flora by a specifically designed, multispecies probiotics mixture with fructo oligosaccharide changes disease course using a well-established rat model of protein malnutrition.

MATERIALS AND METHODS

Animals and diets

Thirty male Wistar rats weighing 60 - 70 g and aged 28 days were used. All animals were obtained with approval from the Animal Research Center of Bab Ezzour University, Algeria. The rats were housed in stainless-steel cages in an animal room maintained at 22 ± 2°C on a 12-h light cycle. After three days of acclimatization, they had free access to water ad libitum and conventionally, pellet food (UAR, Villemoisson-sur-Orge, France). The conventionally diet is containing proteins, fat, carbohydrate, vitamin and minerals (Table 1).

We propose here a new experimental model of malnutrition, based on exclusive use of maize. This diet, similar to that consumed by the severely malnourished children in poor countries, is severely deficient in essential acid amine and vitamins (Ribeiro et al., 1998).

During fifteen days of experimental feeding period, animals were divided into three groups of 10 rats each. A protein malnourished rats (group 1, n = 10) were fed only with maize, a protein malnourished rats (group 2, n = 10) were received an antibiotic (Metronidazole) and a protein malnourished animals of group 3 (n = 10) received both Metronidazole and Probiotics Oligosaccharide.

The malnourished rats of the three groups were fed with maize (diet poly-deficient in essential amino acids) with 10 g/kg of rat/day (EPE Groupe Avicole de l'Ouest, Mostaganem, Algeria); the composition of
Table 1. Standard diet for rats (UAR, Villemoisson-sur-Orge, France).

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Cereals and starchy product</th>
<th>Co-products of the cereals transformation</th>
<th>Oil cakes and other nitrogenized products of vegetable origin</th>
<th>Nitrogenized product</th>
<th>Mineral substances</th>
<th>Oils and grease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analytical Components</td>
<td>Gross products</td>
<td>23%</td>
<td>0.43%</td>
<td>4%</td>
<td>2%</td>
<td>5.5%</td>
</tr>
<tr>
<td></td>
<td>Rough fat content</td>
<td>4%</td>
<td>2%</td>
<td>2%</td>
<td></td>
<td>2%</td>
</tr>
<tr>
<td></td>
<td>Crude fiber</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moisture</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rough ashes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Insoluble ashes in HCl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Copper</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>30 mg/kg</td>
</tr>
<tr>
<td>Vitamins</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12,000 UI/kg</td>
</tr>
<tr>
<td></td>
<td>D3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3,000 UI/kg</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>30 mg/kg</td>
</tr>
</tbody>
</table>

Caloric value = 2900 kcal/kg. Ration day laborer = 18 – 25 g.

Table 2. Composition of maize (EPE Groupe Avicole de l’Ouest, Mostaganem, Algeria).

<table>
<thead>
<tr>
<th>Humidity</th>
<th>Protein</th>
<th>Grease</th>
<th>Ashes</th>
<th>Fiber</th>
<th>Carbonhydrate</th>
<th>Calories</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.2</td>
<td>8.4</td>
<td>4.5</td>
<td>1.1</td>
<td>1.3</td>
<td>73.9</td>
<td>370</td>
</tr>
</tbody>
</table>

Values are expressed in % per gram.

Maize was given (Table 2). Food intake was measured daily at 17 h. Induction of malnutrition after 15 days with this diet was published earlier (Dock et al., 2003; Dock-Nascimento et al., 2007). Metronidazole (Hikma Pharmaceuticals, Jordan) was given via orogastric feeding tube to the rats of groups 2 and 3 for 15 days with the dose of 1 g/kg of animal/day. A complex of lyophilized probiotics (Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium lactis, Bifidobacterium longum, Bifidobacterium bifidum, Streptococcus thermophilus) and Fructo-Oligosaccharides (PROBIONAT® Safetynat Limited Epps Building Bridge Road, France) in the form of a capsule contained about 10⁹ germs in lyophilisate of 390 mg. It was given as three doses per day via orogastric feeding tube (1 mg/g of body per day) to the rats of group 3 (10 mg/10 ml). On the day 15 of this treatment, the animals were sacrificed by cervical dislocation and a 0.5 ml blood sample was collected from the inferior vena cava for blood cultures.

Using sterile procedures, the chest and abdominal cavities were reflected with sterile forceps and the exposed viscera were swabbed with a sterile, cotton-topped applicator stick, which was then placed in a tube of brain-heart infusion. The tube was incubated aerobically at 37°C for 24 h to test the bacterial contamination of the viscera.

The MLNs, spleen and liver were removed and all organs were weighed separately. The MLN complex was placed in a sterile grinding tube and homogenized with 9 volume of brain–heart infusion using sterile ground-glass stoppers (Heimo et al., 2001). To determine the bacterial concentrations of homogenates, each organ was diluted in decimal steps up to 1.10⁵ in a sterile solution. After manual grinding, 1 ml of the homogenate was transferred into a tube containing 9 ml of physiologic serum; from this dilution 100 µl aliquots were plated on DRIGALSKI agar plates for enterobacteria culture (Sanofi, Diagnostic Pasteur; France). Spleen and liver was analyzed in the same way as the MLNs. All agar plates for aerobic culture were incubated at 37°C under aerobic conditions for 1 day and then interpreted. The gram-negative enteric was identified using the API 20 E system (Analytab Products, Plainview, New York).

Quantitative culture results were determined by the number of Colony Forming Units per gram of tissue, calculated from the dilutions of organ homogenate and positive tissue cultures. We did not study obligate anaerobic because these organisms are rare members of the intestinal flora of rodents early in life (Raibaud P., 1988) and because they have a low tendency to translocate to extra intestinal sites (Stefen EK et al., 1988; Stefent EK et al., 1983). The
Table 3. Incidence and sites of bacterial translocation (BT) in each group after 15 days of treatment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Incidence of BT</th>
<th>MLN</th>
<th>Spleen</th>
<th>Liver</th>
<th>Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. PM</td>
<td>3/10 (30%)</td>
<td>2/10 (20%)</td>
<td>1/10 (10%)</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
</tr>
<tr>
<td>2. PM + antibiotic</td>
<td>6/10 (60%)*</td>
<td>4/10 (40%) ‡</td>
<td>2/10 (20%)</td>
<td>3/10 (30%) ¶</td>
<td>0/10 (0%)</td>
</tr>
<tr>
<td>3. PM + antibiotic + Probiotics-Oligosaccharide</td>
<td>2.5/10 (25%) †</td>
<td>3/10 (30%) †</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
</tr>
</tbody>
</table>

PM, protein-malnourished group; BT, bacterial translocation; MLN, mesenteric lymph nodes.

*P < 0.01 compared to group 1, †P < 0.05 compared to groups 1 and 2, ‡P < 0.05 compared to group 1 and ¶P < 0.05 compared to groups 1 and 3.

Mesenteric Lymph Nodes (MLNs), spleen, liver and cecal contents were removed and homogenized for quantitative cultures. The colony-forming units (CFU) of bacteria per gram of tissue were estimated after 24 – 48 h.

Statistical analysis

Data were expressed as means ± standard error of the mean. The differences between the different groups were evaluated by chi square analysis with the Yates correction. Continuous data are expressed as mean ± SEM and analyzed with analysis of variance (ANOVA) and the Student unpaired t test. A P-value < 0.05 was considered significant.

RESULTS

The incidence of bacterial translocation was 30% (3/10) in protein-malnourished group 1 (PM), 60% (6/10) in rats of group 2 (where PM was associated with metronidazole) and 25% (2.5/10) in group 3 (where protein malnutrition was associated with metronidazole and probiotics oligosaccharide) (Table 3). The incidence of BT in group 3 was not significantly different from that of group 1 (P > 0.05). The same result was obtained when comparing group 2 to group 1 (P > 0.05). The difference in the incidence of BT between groups 2 and 3 was significant (P < 0.05), 60% (6/10) in group 2 and 25% (2.5/10), in group 3.

Bacterial translocation was detected only in the MLNs (3/10, 30%) of the rats of group 3 which were treated with probiotics oligosaccharide. However, it was detected in the MLNs (4/10, 40%), spleen (2/10, 20%) and liver (3/10, 30%) of group 2. The incidence of BT was significantly high in the MLNs of group 2 than that of group 1 (P < 0.05). Regarding the liver, the same result was obtained in group 2 comparing to groups 1 and 3 (P < 0.05). The difference in the incidence of BT in the MLNs was not significantly different between groups 2 and 3 (P > 0.05), or between groups 1 and 3 (P > 0.05). These data indicated that BT did not spread beyond the MLNs in protein malnourished rats treated with probiotics.

Table 4. Number of translocating bacteria in terms of FCU per gram of mesenteric lymph nodes in each group after 15 days of treatment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± standard error of mean × 10³ colony-forming units per gram tissue of MLNs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>57 ± 6</td>
</tr>
<tr>
<td>Group 2</td>
<td>160 ± 23</td>
</tr>
<tr>
<td>Group 3</td>
<td>11 ± 3</td>
</tr>
</tbody>
</table>

Table 4. Number of translocating bacteria in terms of FCU per gram of mesenteric lymph nodes in each group after 15 days of treatment.

Mean ± standard error of mean × 10³ colony-forming units per gram tissue of MLNs.

P = 0.05.

The results of quantitative MLNs cultures are shown in Table 4. Although the number of translocating bacteria per gram of MLNs was decreased by the administration of probiotics oligosaccharide, there was a significant high difference between groups 2 and 1 (P < 0.001) and between groups 3 and 1 (P < 0.05). In addition, the difference between groups 2 and 3 was significantly high (P < 0.001). There was a significant difference between groups 1 and 2 (400 ± 95 UFC/g Vs 160 ± 43 UFC/g, P < 0.05) as shown in Table 5. The total bacterial counts, Gram-negative and Gram-positive, of cecal flora were significantly low in group 1 than that in group 3 (400 ± 95 UFC/g versus 36 ± 4 UFC/g, P < 0.001). 42% of the total bacterial count was Gram-negative in group 1, 51% in group 2 and 13% in group 3. Metronidazole caused a slight overgrowth of Gram-negative bacteria in group 2. Otherwise, this decrease in the Gram-negative population of cecal flora was significant (P < 0.01). No microorganisms were isolated from the blood samples.

DISCUSSION

These results suggest that protein-malnutrition induced translocation of enterobacteria. Metronidazole acted specifically on the strict anaerobic bacteria by reducing their numbers in cecal flora which explains the high rates of overgrowth and translocation of enteric bacteria in the oligosaccharide (group 3).
Table 5. Quantitative results of Gram-negative, Gram-positive and total bacteria in cecal cultures for each group after 15 days of treatment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Gram-negative</th>
<th>Gram-positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. PM</td>
<td>280 ± 70 (42%)</td>
<td>120 ± 35 (28%)</td>
<td>400 ± 95</td>
</tr>
<tr>
<td>2. PM + antibiotic</td>
<td>95 ± 25* (51%)</td>
<td>65 ± 28* (35%)</td>
<td>160 ± 43*</td>
</tr>
<tr>
<td>3. PM + antibiotic + Probiotics-Oligosaccharide</td>
<td>15 ± 3** (13%)</td>
<td>21 ± 3** (65%)</td>
<td>36 ± 4**</td>
</tr>
</tbody>
</table>

Mean ± SEM x 10^5 colony-forming units per gram cecal content.

*P < 0.05 and **P < 0.01 compared to group 1.

malnourished group received metronidazole.

In this context, the current observation is that the PM and the PM particularly associated with metronidazole results in overgrowth cecal bacteria. This bacterial overgrowth is most marked in the Gram negative. These levels of intestinal bacterial population help to explain the collapse of the strictly anaerobic bacterial flora by the use of metronidazole and the consequent apparition of bacterial translocation in the GLM, spleen and liver.

In previous studies, we found that protein malnutrition and malnutrition associated with metronidazole promote bacterial overgrowth in cecal and translocation of enterobacteria to MLNs (Benakriche et al., 2008). Berg and Garglinton (1979) demonstrated that penicillin, clindamycin, or metronidazole given orally to specific pathogen-free mice for 4 days decreased the cecal population of indigenous bacteria, especially anaerobic bacteria. A decreasing anaerobic population allows the cecal overgrowth of indigenous, Gram-negative facultative anaerobic enteric bacilli and enhances BT to the MLNs. Berg et al. (1988) demonstrated that a combination of antibiotics and immunosuppressive drugs promotes the systemic spread of translocating bacteria, resulting in lethal sepsis. Generally, metronidazole is considered as a safe drug because of its relative high therapeutic index and limited duration of treatment. Deitch et al. (1985) found that the cecal overgrowth of enteric bacilli caused an enhanced BT after the administration of Metronidazole (500 Units/ml) in PM rats, but the mean of enteric cecal population was significantly decreased by the administration of high dose of penicillin (1500 Units/ml). All of these studies demonstrate that oral antibiotics given in a dose dependent manner may inhibit the growth of anaerobic bacteria and allow intestinal overgrowth of facultative anaerobic Gram-negative bacilli. Therefore, the administration of oral antibiotics may enhance BT to the MLNs and other organs after PM, which impairs both humoral and cellular immunity in addition to the damage of local barriers. In the present study, we found that oral antibiotic treatment enhances BT and promotes the spread of translocating bacteria to the liver in PM rats. However, antibiotic treatment is usually required in the treatment of the inflammatory victims. Bacterial overgrowth in the small intestine has been documented in children with severe malnutrition and might contribute directly to ineffective solubilisation, digestion and absorption of lipid (Murphy et al., 2002).

Despite knowledge in the area of overgrowth and intestinal bacterial translocation, probiotics and prebiotics remain a hot topic. They are the subject of several studies which have shown in mice and rats that some probiotics like S. boulardii, B. longum, P. acnes, L. helveticus, L. rhamnosus and oligosaccharide have a protective effect against bacterial translocation. In addition, it has been shown that fructo-oligosaccharide not only have a protective effect against bacterial translocation but also stimulates the proliferation of Bifidobacteria and lactobacilli.

Recent studies have shown that probiotics, which have been used in the treatment of intestinal disorders, antibiotic-associated diarrhea and Clostridium difficile colitis, are effective for maintaining...
intestinal equilibrium and reducing BT. Probiotics promote an increasing anaerobic population of gastrointestinal tract flora (Duffy, 2000; Mattar et al., 2001) but anaerobic bacterial overgrowth may not be important for BT because anaerobic bacteria rarely translocate to the MLNs. On the other hand, anaerobic bacterial overgrowth have many beneficial effects such as: strengthening gut mucosal barrier function, balancing microbial ecology, adhering to the intestinal mucosa, impeding invasive pathogens, metabolizing dietary proteins and enzymes by intestinal microflora and promoting resilience of the epithelium to gut mucosal permeability. Collins and Gibson (1999) reported that small-bowel colonization by Escherichia coli K1A and BT was decreased by the administration of the probiotics oligosaccharide and suggested that probiotics oligosaccharide may be used for the treatment of BT and sepsis.

It has been recently shown that impaired gut barrier and mucosal immune function by malnutrition can be reversed by L. casei used as an oral adjuvant of renutrition diet. The clinical significance of these findings will be important, as well as improving mucosal immunity, may also induce protection against enteropathogens (Gauffin et al., 2004). Probiotics can protect the intestine by competing with pathogens for attachment, strengthening tight junctions between enterocytes and enhancing the mucosal immune response to pathogens (Lei and Walker, 2001).

Hirofumi et al. (1999) demonstrated that two kinds of probiotics derived from different bacterial genera enhanced epithelial cell proliferation of the gut without altering the gross population levels of cecal microflora. In humans in vivo, lack of protective effect of probiotics (L. plantarum 299V) on bacterial translocation to lymph nodes was confirmed by the same team and same methodology for combining oligosaccharide with L. acidophilus, B. lactis Bb12, S. thermophilus and L. bulgaricus (Anderson et al., 2004).

Remarkably, FOS dose-dependently increased salmonella numbers in cecal contents and mucosa and caused a major increase in infection-induced diarrhea. In addition, FOS enhanced translocation of salmonella. Thus, in contrast to most expectations, FOS dose-dependently impairs the resistance to salmonella infection in rats (Sandrak et al., 2003).

L. fermentum KLD has previously been used as both a prophylactic and therapeutic agent in the treatment of gastrointestinal disturbances and is an interesting candidate probiotic strain (Marteau et al., 2001).

Zaouche et al. (2000) investigated the effects of S. boulardii on bacterial overgrowth and translocation in rats with resected small intestine and found that it did not modify bacterial overgrowth and translocation, but it enhanced the functional adaptation of the remaining intestinal segments. Another study by Berg et al. (1993) investigating the effects of S. boulardii on the translocation of C. albicans in antibiotic-decontaminated specific pathogen free mice revealed that it decreased the incidence of C. albicans translocating to the MLNs, liver, kidneys and also the number of translocating C. albicans per gram MLNs, spleen, and kidneys.

The present study, which was conducted to evaluate the effects of probiotics oligosaccharide on BT in protein malnutrition associated with metronidazole treatment, showed that probiotics oligosaccharide significantly decreased the population of Gram-negative bacteria in cecal flora. Although we could not examine the anaerobic flora, these data correlate with that of other reports in which probiotics increase the anaerobic bacterial count and decrease the Gram-negative facultative anaerobic and aerobic bacteria count in the GI tract (Mattar et al., 2001; Ishibashi and Yamazaki, 2001). Anaerobic bacteria are generally decreased by antibiotic treatment and this ecological imbalance allows Gram negative bacteria to proliferate and enhances BT (Berg, 1981). The results of our study suggest that probiotics oligosaccharide may counteract this undesirable side effect of antibiotics. Interestingly, the incidence of BT in the rats treated with probiotics oligosaccharide (group 3) was not significantly higher than that of the group 1, but was significantly higher than that of the rats given the antibiotic alone (group 2). Although there was no significant difference in the incidence of BT and the number of translocating bacteria per gram of MLN between groups 2 and 3, we found that probiotics oligosaccharide decreased the incidence of BT from that which occurred in group 1 and also decreased the number of translocating bacteria. Furthermore, the administration of probiotics oligosaccharide ensured that BT was limited to the MLNs, whereas it extended to the liver in rats not given probiotics oligosaccharide. Selection of strains was based on their in vitro antibacterial and immunomodulatory properties. L. acidophilus, L. rhamnosus, B. lactis, B. longum, S. thermophilus and fructo-oligosaccharides were selected for their ability to suppress the growth of Gram-negative bacteria and their antimicrobial effects. The lower incidence of BT in the rats given oral probiotics oligosaccharide may not only related to improved intestinal ecological balance, but also to the effects of probiotics oligosaccharide on host immune responses, including stimulation of the
production and secretion of intestinal s-IgA, enhancing both phagocytic activity and the maturation of enterocytes.

Protein malnutrition is known from biopsy studies to be associated with villous atrophy, decreased villous-crypt ratio and increased cellularity of the lamina propria. Although severe mucosal injury occurs only in a proportion of malnourished children, it is more common in kwashiorkor; possibly due to the effect of protein depletion on mucosal recovery (Viteri et al., 1973; Brunser et al., 1968; Bhan, 1996), atrophy of intestinal villi facilitates the breakdown of the mucosal barrier and thus, translocation of bacteria from endogenous flora. Abnormal intestinal permeability in kwashiorkor correlates with disease severity and improves only slowly with nutritional rehabilitation (Brewster et al., 1997).

On the other hand, abnormal intestinal permeability is a feature of bacterial translocation in malnourished adult patients with multi-organ failure secondary to trauma, sepsis or burns (Deitch, 1990).

In conclusion, probiotics oligosaccharide may protect the balance of GI tract flora by inhibiting the growth of Gram-negative bacteria and assisting the growth of anaerobic bacteria. On the other hand, it may enhance the host immune responses. These effects of probiotics oligosaccharide may lower the incidence of BT and the number of translocating bacteria in protein malnourished victims being treated with metronidazole. Thus, probiotics oligosaccharide may be effective in preventing BT to the MLNs and other organs in protein malnourished victims requiring antibiotic therapy.

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