Effects of Probiotic Use on Bacterial Translocation in Created Rat Models with Biliary Obstructions

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ABSTRACT

Objective: The aim of this study was to investigate the protective effects of the combined use of probiotic strains on the development of bacterial translocation in addition to liver and intestinal tissue damage due to biliary obstruction in rats.

Materials and Methods: Here, 3 groups each consisting of 10 rats were created: group 1 (sham group), group 2 (obstructive jaundice), and group 3 (obstructive jaundice+probiotic). Groups 1 and 2 were given 1 cc physiological saline solution by oral gavage twice a day; group 3 was given a probiotic solution that included Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium bifidum, Enterococcus faecium, and Bifidobacterium longum microorganisms by oral gavage twice a day.

Results: Markers for liver damage were also found to be significantly improved (p<0.05) in the treatment group (group 3). When compared with groups 2 and 3 in terms of liver histology, damage was found to be significantly more severe in group 2 (p<0.01). With regard to ileal villous depth and ileal inflammation, the pathology was found to be significantly more severe in group 2 than that in group 3 (p<0.05). In blood, spleen, and mesenteric lymph node cultures, group 2 showed a microbiological growth rate of 33.8–58.8%, whereas group 3 showed a microbiological growth rate of 14.3–28.6%. This reduction was evaluated to be statistically significant (p<0.05).

Conclusion: Our study showed that the combined use of a probiotic in bile duct obstructions reduced bacterial translocation and alleviated pathological changes arising in the liver and terminal ileum histology.

Keywords: Bacterial translocation, bile duct obstructions, gram-negative microorganism, combined use of probiotic microorganisms, liver damage

Introduction

Congestion occurring in bile ducts is a life-threatening condition that can be encountered in both children and adults [1]. The interruption of the flow of bile salts into the intestine causes bile accumulation in the liver cells and bile ducts. The absence of bile salts that can inactivate endotoxins in the intestine leads to the development of oxidative damage on the intestinal wall [2]. This condition increases the permeability of the intestinal wall by impairing the structure and functions of the intestinal mucosa [2]. Impaired barrier functions of the intestines decrease the opsonization activity of the humoral immunity and bacteriostatic capacity. Therefore, microorganisms in the intestines pass the mucosal barrier and reach the liver, spleen, and mesenteric lymph nodes in the gastrointestinal tract, thereby disrupting the functions of the immune and reticuloendothelial systems [3-6]. If timely intervention is not exercised, obstructive jaundice can lead to life-threatening clinical pictures due to bacterial translocation [7].

Probiotics, when sufficiently taken, are noninvasive, noncarcinogenic, nonpathogenic, reliable microorganisms that can temporarily colonize the gastrointestinal tract and act against the proliferation of pathogens without impairing the normal flora [8, 9]. Studies have revealed the positive effects of probiotics such as increased intestinal immunity [10], repairing of impaired intestinal mucosal barrier [11], inhibition of translocation of microorganisms [12], elimination of toxins [13], eradication of microbial pathogens [14], and regulation of intestinal functions [15].

The aim of our study is to demonstrate whether multispecies probiotics, which are easily and cheaply available in the market [15], have an improving effect on bacterial translocation caused...
by liver and intestinal damage due to biliary obstruction in rats.

Materials and Methods

Animals
After receiving the approval of the ethics committee (decision number 40595970/168 dated August 2, 2013), 30 male Wistar albino rats between the weights of 170-230 g were accommodated in a room at a suitable temperature (22±2°C) in cages with nesting material, litter, and cardboard tubes under the standard conditions of 12-h light-dark cycles. The rats fasted according to FELASA guidelines. The rats were allowed to freely access standard feed and water whenever they wished. The animals were not given food the day before the operation, but they had free access to water.

Experimental Design
Rats were randomly divided into 3 groups, each containing 10 rats, namely, group 1 (sham group), group 2 (obstructive jaundice group), and group 3 (obstructive jaundice+probiotic group). All the surgical procedures were carried out under intramuscular 50 mg/kg Ketalar (ketamine) and 8 mg/kg Rompun (xylazine HCl) anesthesia, which were administered in the supine position through a median incision made under sterile conditions (Figure 1 a-c). Group 1: the main bile duct was released from the surrounding tissues without being tied or incised by laparotomy. Group 2: the main bile duct was tied around tissues without being tied or incised by the main bile duct was released from the sur- face and group 3 (obstructive jaundice+probiotic group). All the surgical procedures were carried out in the supine position through a median incision made under anaesthesia. Which were administered in the supine position through a median incision made under sterile conditions (Figure 1 a-c). Group 1: Group 2: the main bile duct was tied around tissues without being tied or incised by laparotomy. Group 2: the main bile duct was tied around tissues without being tied or incised by laparotomy. Group 3: in addition to the procedure performed in group 2, after intervention, a probiotic solution was administered to the subjects for 7 days. Animals were postoperatively fed orally for 24 h. All the rats were allowed to freely consume a standard diet and water. None of the groups had nutrition restriction. Groups 1 and 2 were given 1 cc physiological saline solution by gavage twice a day every 12 h; group 3 was given the prepared probiotic solution by gavage twice a day every 12 h.

A probiotic in the form of a sachet (NBL Probiotic Gold; Nobel medical, Istanbul, Turkey), containing Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium bifidum, Enterococcus faecium, and Bifidobacterium longum microorganisms, was mixed in water until it was homogeneous and then administered. Each probiotic dose given to the rats was adjusted to be 1 cc:0.125×10^10 CFU for 500 mg/kg of body weight [14, 15].

At the end of the 7th day, relaparotomy was performed through the same incision line under anaesthesia and sterile conditions. Samples were taken from the blood, mesenteric lymph nodes, and spleen tissues for microbiological examinations. The blood sample was taken from the portal vein for biochemical examination. Tissue samples were taken from the terminal ileum and liver for histopathological examination. All the rats were sacrificed under anaesthesia.

Biochemical Analysis
The blood samples were taken into biochemical try tubes with gel. After centrifugation for 5 min at 6500 rpm, the sera were taken and the parameters of alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), and total bilirubin were studied in a biochemical autoanalyzer (Abbott, ARCHITECT c8000; Illinois, USA) using ultraviolet spectrophotometric, colorimetric, and enzymatic methods.

Pathological Analysis
Liver bile duct proliferation was rated using the scoring system of Cheen-Shen et al. [16, 17]. Accordingly, the presence of pathological findings in less than 50% of the portal area was accepted as grade 1. The presence of pathological findings in more than 50% of the portal area was accepted as grade 2. The presence of bridging in the portal area was accepted as grade 3. Hepatocyte degeneration, microabscesses, and capsular inflammation were graded by means of a semiquantitative evaluation [18]. Grading was labeled as follows. Grade 0: no pathology; grade 1: mild pathology; grade 2: moderate pathology; grade 3: severe pathology.

In the evaluation and classification of terminal ileum damage, scoring devised by Ay et al. was used [19]: epithelial damage overlying the villi and reduction in the villous depth, inflammation in the ileum lamina propria and serosa, and hemorrhage in the ileum were evaluated. For all these specified parameters, a semiquantitative grading system was used: 0 means normal; 1 means mild; 2 means moderate; and 3 means severe.

Microbiological Analysis
Mesenteric lymph node and spleen samples along with the blood samples taken from a portal vein of the animals in all the groups were brought to the culture laboratory, paying attention to maintaining aseptic conditions. Mesenteric lymph nodes and splenic tissues were separated into small pieces by a sterile scalpel under aseptic conditions. Subsequently, the samples were weighed and placed in 2 mL liquid thioglycolate medium (Oxoid, UK) and then homogenized. Following homogenization, 10μL

Figure 1. a-c. (a) Determination of the main bile duct and making a suture through the main bile duct. (b) Bile duct after dissection. (c) After the main bile duct has been cut.
of the homogenized samples was inoculated in blood agar (Oxoid, UK) and eosin methyl-
ene blue (EMB) agar (Oxoid, UK) in order to
investigate the presence of both aerobic and
anaerobic Gram-positive and Gram-negative
bacterial growth, respectively. The culture plates
were incubated for 48 h at 37°C. The samples
were incubated in both aerobic and anaerobic
environments. In order to create an anaerobic
environment, cultured media were placed in a
GasPak jar and an oxygen-free environment
was provided. The blood samples were inoculated in
a BACT/ALERT (bioMérieux, France) hemo-
culture device in aerobic and anaerobic culture
bottles. After being incubated in commercially
available culture bottles, the blood samples
were incubated for 7 days at 37°C. At the end of
the incubation, subcultures were obtained
from growth-identified bottles in blood agar
and EMB agar and incubated for 48 h at 37°C;
subsequently, bacterial identification procedures
were initiated. Conventional identification meth-
ods, as well as the automated system (VITEK2,
bioMérieux), as and when needed, were used
for the identification of microorganisms in the
cultures detected to have growth.

Statistical Analysis
The data were evaluated with the Statistical
Package for Social Sciences for Windows (SPSS
Inc., Chicago, IL, USA) version 18. Chi-squared
test was used for the comparison of qualita-
tive data. Independent two-group quantitative
data were assessed by independent samples t
test in the case of normal distribution and by
Mann-Whitney U-test in the absence of normal
distribution. The quantitative data of more than
2 independent groups were assessed by one-
way ANOVA in the case of normal distribution and
by the Kruskal-Wallis test in the absence of
normal distribution. If there is statistical signifi-
cance between the groups in the evaluation of
quantitative data, post hoc analyses were per-
formed using Least Significant Differences (LSD)
test for normally distributed data.

Results
Biochemical Results
ALP, ALT, and AST values were significantly high-
er in group 2 as compared to group 1 (p=0.001
for ALP and ALT; p=0.002 for AST). A statisti-
cally significant decrease was found in group 3
when compared to group 2 (p=0.04 for ALP;
p=0.032 for ALT; p=0.026 for AST). The total
bilirubin and direct bilirubin values were signifi-
cantly higher in group 2 as compared to

group 1 (p=0.013 for total bilirubin; p=0.01 for
direct bilirubin), but no statistically significant
difference was found between groups 2 and 3
(p=0.083 for total bilirubin; p=0.084 for direct
bilirubin). There was no statistically significant
difference between the groups in the compar-
ison of GGT values (p>0.05) (Table 1).

Histopathological Findings
Addressing all the studied parameters, no pathological finding was observed in both the
small intestine and liver in group 1. Histological
examination of the liver revealed no pathology
in group 1 with regard to bile duct proliferation,
hepatic degeneration, microabscess presence,
and capsular inflammation. When compared with
groups 2 and 3, the pathology was found to be
significantly severe in group 2 with regard to
all the parameters (p<0.01 for all the param-
eters). With regard to the ileum villous depth, the
pathology was found to be significantly more se-
vere in group 2 than that in group 3 (p=0.003).
With regard to ileum inflammation, the pathol-
ogy was found to be significantly higher in group
2 than that in group 3 (p=0.001). There was no
significant difference between group 2 and

group 3 (p=0.07) with regard to ileum hemor-
hage (Figure 2 a–j).

Microbiological Findings
It was found that Escherichia coliwas the most
commonly isolated bacteria in the microbiologi-
cal study, followed by Staphylococcus spp. and
Klebsiella pneumonia. Percentage values were
calculated for each different tissue by the ratio
of the number of samples with bacteriological
growth to the total number of cultures. Signifi-
cant growth was found in the blood cultures of

group 2 as compared to those of groups 1 and
3. There was no significant difference between
group 1 and group 3 (1 and 2: p=0.001;1 and
3: p>0.05;2 and 3: p=0.01). There was signifi-
cant growth in the spleen cultures of group 2
as compared to those of groups 1 and 3. There
was no significant difference between group 1
and group 3 (1 and 2: p<0.001;1 and 3: p>0.05;
and 3: p<0.05). In the mesenteric lymph node
cultures, there was significant growth in group 2
as compared to those of groups 1 and 3. There
was no significant difference between groups 1
and 3 (1 and 2: p<0.001;1 and 3: p>0.05) (Table
2).

Discussion
In patients with obstructive jaundice, the accumu-
lation of bile salts in hepatocytes and sepsis result-
from bacterial translocation can cause dam-
age to hepatocytes [20]. When compared with
groups 2 and 3 with regard to ALT, ALP and AST
values, the biochemical markers of liver damage
[21] were found to be significantly improved in
the probiotic treatment group (i.e., group 3).
Although GGT elevation in the blood usually
indicates a liver problem, it may be indicative of
other health problems, too. Its elevation along
with other liver enzymes suggests that the GGT
source is the liver. When compared with groups
2 and 3 with regard to GGT and bilirubin values, a
marginal improvement was found, but it was not

Table 1. Levels of ALP, ALT, AST, DBIL, TBIL, and GGT (mean±SD)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>1-2</th>
<th>2-3</th>
<th>1-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALP (U/L)</td>
<td>124±28.1</td>
<td>349±16.5</td>
<td>259±101</td>
<td>0.001</td>
<td>0.004</td>
<td>0.04</td>
</tr>
<tr>
<td>ALT (μ/l)</td>
<td>53.8±9.4</td>
<td>222±110</td>
<td>123.1±71</td>
<td>0.01</td>
<td>0.032</td>
<td>0.122</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>187±24.8</td>
<td>628±314</td>
<td>35±153</td>
<td>0.02</td>
<td>0.026</td>
<td>0.152</td>
</tr>
<tr>
<td>DBIL (μmol/L)</td>
<td>0.1±0.01</td>
<td>5.02±4.8</td>
<td>2.01±1.8</td>
<td>0.01</td>
<td>0.084</td>
<td>0.259</td>
</tr>
<tr>
<td>TBIL (μmol/L)</td>
<td>0.1±0.01</td>
<td>8.43±8.6</td>
<td>3.08±3.1</td>
<td>0.013</td>
<td>0.083</td>
<td>0.318</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>2.17±1.1</td>
<td>6.5±7.2</td>
<td>5.29±4.6</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Blood Culture</td>
<td>8.3%</td>
<td>33.3%</td>
<td>14.3%</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>MLN Culture</td>
<td>1.7%</td>
<td>58.3%</td>
<td>21.4%</td>
<td>&lt;0.001</td>
<td>0.01</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Spleen Culture</td>
<td>3.3%</td>
<td>50%</td>
<td>28.6%</td>
<td>&lt;0.001</td>
<td>0.04</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; ALP: Alkaline Phosphatase; GGT: Gamma-Glutamyl
Transpeptidase; TBIL: Total Bilirubin; DBIL: Direct Bilirubin; MLN: Mesenteric Lymph Node. Continuous variables
are represented as mean±SD, while the others are represented as percentages.

Table 2. Bacterial growth rates in culture samples

<table>
<thead>
<tr>
<th>Culture Type</th>
<th>Group 1 (%)</th>
<th>Group 2 (%)</th>
<th>Group 3 (%)</th>
<th>1 and 2</th>
<th>2 and 3</th>
<th>1 and 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood culture</td>
<td>8.3</td>
<td>33.3</td>
<td>14.3</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Mesenteric lymph node culture</td>
<td>1.67</td>
<td>58.3</td>
<td>21.4</td>
<td>&lt;0.001</td>
<td>0.01</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Spleen culture</td>
<td>3.33</td>
<td>50</td>
<td>28.6</td>
<td>&lt;0.001</td>
<td>0.04</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>
statistically significant. These results are consistent with the results reported in the literature [22].

In the literature, several studies have shown that probiotic microorganisms have an anti-inflammatory effect and reduced inflammation [23, 24]. It was observed that the use of *Lactobacillus plantarum* decreased intestinal epithelial apoptosis and oxidative stress, while simultaneously strengthening intercellular adhesion supporting the mucosal integrity; further, the use of glutamine and probiotics reduced bacterial translocation. Improvements were found in all the biochemical, pathological, and microbiological findings in rats that underwent choledoch ligation [25-27]. There was no pathology in group 1 in our study.

In accordance with the literature, a significant difference was found in group 2 as compared to group 3. The highest histopathological changes (hepatocyte degeneration, bile duct proliferation, microabscess foci, and capsule inflammation) were evaluated in the liver samples, while ileal hemorrhage, ileal inflammation, reduction in villous depth, and villous atrophy were evaluated in the ileum samples) were evident in group 2, which also showed the highest bacterial translocation, suggesting that pathology was caused by endotoxemia. In biliary obstruction, endotoxemia may be due to the decreased clearance of portal endotoxins in biliary obstruction or increased endotoxin absorption from the gastrointestinal tract. Moreover, it is believed that the absence of bile salts resulting in the inactivation of endotoxin in the intestine causes the activation of endotoxin in portal circulation, thereby forming the basis for bacterial translocation [26-28].

Figure 2. a-j. In group 2: (a) hepatocyte degeneration in the liver HE×400. (b) Biliary duct proliferation HE×100. (c) Microabscess in the liver HE×100. (d) Inflammation in the liver capsule HE×100. (e) Villous atrophy in the ileum HE×100. (f) Inflammation in the ileum submucosa HE×100. In group 3: (g) proliferation of bile duct in the liver HE×100. (h) Microabscess in the liver HE×200. (i) Villous atrophy in the ileum HE×100. (j) Inflammation in the ileal submucosa HE×100 [2]
There are experimental studies showing that probiotics reduce bacterial translocation [15, 16, 23, 24]. In some studies, probiotics have been observed to stimulate the immunity and increase IgA, providing resistance to pathogens such as viruses, Clostridium, and E. coli [27]. It was observed that cecal colonization was significantly less in the group treated with Lactobacillus acidophilus, Bifidobacterium bifidum, and Lactobacillus bulgaricus in rats with tied bile ducts [28]; further, hepatic encephalopathy was less common due to decreased pH values in the stomach, jejunum, ileum, and colon in rats that were given probiotics [29]. After giving Lactobacillus, rats underwent small intestine resection: the intestinal immunity of the probiotic-administered group was stable [30]. In our study in which we investigated the effects of combined probiotic (Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium bifidum, Enterococcus faecium, and Bifidobacterium longum) microorganisms on rats with bile obstructions, there was significant growth in the microbiological blood, spleen, and mesenteric lymph node cultures of group 2 as compared to those of group 1. In group 3, a reduction was observed in the growth rate and statistical analysis performed showed a significant difference. These results supported the view that the source of bacterial infection in obstructive jaundice is the gastrointestinal system [20, 30]. We assume that the cause of no growth in the cultures of group 1 is the affected intestinal flora due to surgical stress [31].

This study has certain limitations. First, we have limited cases with many stronger outcomes. This was because the ethical committee of animal experiments requested to limit the number of experimental animals to be used in the study. Second, the molecular study could not be done because of cost and lack of funding.

In conclusion, the use of probiotics in rat models with obstructive jaundice resulted in improvement in biochemical parameters, significantly reduced pathology in the liver and terminal ileum, and reduced bacterial translocation in mesenteric lymph node, spleen, and blood cultures. Therefore, cheap and easy to find probiotics [15] have been observed to have a protective effect in obstructive jaundice pathologies that are still associated with high mortality and morbidity.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Hatay Mustafa Kemal University by the decision number 40595970/168 and dated 02.08.2013.

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.


Conflict of Interest: The authors have no conflict of interest to declare.

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