Effect of *Lactobacillus rhamnosus* on Intestinal Mucosal Barrier Function in Mice with Post-Infectious Irritable Bowel Syndrome

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To explore the effect of *Lactobacillus rhamnosus* on intestinal mucosal barrier function in post-infectious irritable bowel syndrome mice. Fifty-four 8 w old Specific-pathogen free male Sprague Dawley mice were randomly divided into the control group (n=18), model group (n=18), and experimental group (n=18). The post-infectious irritable bowel syndrome model was established by the *Trichinella spiralis* infection. The experimental group was treated with *Lactobacillus rhamnosus* (1.0×10^7 CFU/kg, 0.5 ml), twice a day for 1 w, while the control group and model group were given the same dose of normal saline for 1 w. The changes in body weight, fecal water content, intestinal motility, and D-lactic acid levels of mice in each group were compared. The body weight of mice in the model group and the experimental group was lower than that in the control group, while that in the experimental group was higher than that in the model group (p<0.05). The fecal water content of mice in the model group and the experimental group was higher than that in the control group, while that in the experimental group was lower than that in the model group (p<0.05). The Bristol score of the model group and the experimental group was higher than that of the control group, and the Bristol score of the experimental group was lower than that of the model group (p<0.05). The level of D-lactic acid in the model group and the experimental group was higher than that in the control group, while that in the experimental group was lower than that in the model group (p<0.05). *Lactobacillus rhamnosus* can improve the intestinal motility and intestinal mucosal barrier function in post-infectious irritable bowel syndrome mice.

Key words: *Lactobacillus rhamnoides*, infection, irritable bowel syndrome, intestine mucosal barrier function

Irritable bowel syndrome (IBS) was a functional gastrointestinal disease whose main symptoms were the abdominal discomfort, abdominal pain with abnormal stool characteristics and changes in defecation habits. Its specific pathogenesis was complex, and it was generally considered that its pathogenesis was related to a variety of influencing factors[1,2]. Post-infectious irritable bowel syndrome (PI-IBS) was one of the important subtypes of IBS. About 7%-30% of IBS patients have a history of acute intestinal infection[3,4]. In recent years, it has been found that intestinal mucosal bacteria and immune activation play an important role in mediating the pathogenesis of PI-IBS[5]. *Lactobacillus rhamnosus* (*L. rhamnosus*) belongs to the genus *Bacillus lactis* and was first isolated from the intestines of healthy people. In recent years, much attention has been paid to the application of *L. rhamnosus* in the regulation of intestinal flora[6]. Therefore, this study aims to explore the effect of *L. rhamnosus* on the intestinal mucosal barrier function in PI-IBS mice.

**MATERIALS AND METHODS**

**Experimental animals and drugs:**

Fifty-four 8 w old Specific-pathogen free (SPF) male Sprague Dawley (SD) mice (20±2 g) were purchased...
from Beijing Vital River Laboratory Animal Technology Co. Ltd and adaptive breeding for 3 d (60 % humidity, 25°C). *L. Rhamnosus* was purchased from Shandong Onalai Biotechnology Co., Ltd.

**Reagents and instruments:**

The main reagents including sodium chloride injection and chloral hydrate (CR double-Crane Co. Ltd), and *Trichinella spiralis* cysts (Lanzhou Veterinary research institute). The main instruments including BX40 optical microscope (OLYMPUS Co., Ltd), HM-315 histotome (Leica RM201), ESJ200-4 electronic balance (Longteng Electronic Co., Ltd), and centrifuge 5417 (Eppendorf Co., Ltd).

**Grouping:**

Fifty-four 8 w old SPF male SD mice were randomly divided into the control group (n=18), model group (n=18), and experimental group (n=18).

**Model Preparation:**

The PI-IBS model was established by the *Trichinella spiralis* infection. The muscle larvae in SD rats infected with *Trichinella spiralis* was isolated by 1.5 % pepsin artificial digestion and suspended in normal saline. The mice were fed with 0.2 ml saline containing 300 larvae.

The criteria of successful modeling: at the 8th w after infection, the abdominal withdrawal reflex and colonic transit function test were obviously abnormal, and there was no obvious abnormality in intestinal histopathology.

**Administration method:**

The experimental group was treated with *L. rhamnosus* (1.0×10⁷ CFU/kg, 0.5 ml), twice a day for 1 w, while the control group and model group were given the same dose of normal saline for 1 w.

**Detection indicators:**

Detection of changes in the body weight of mice in each group. The changes in fecal water content of mice in each group were detected and the feces of mice were collected every 2 h for 8 h continuously. The dry and wet weight of feces was weighed and the water content in the feces of mice was calculated. The changes in intestinal motility of mice in each group were detected and evaluated by Bristoli score. The scores of normal feces, soft or unformed feces and water feces were 1, 2 and 3 scores respectively. Detection of D-lactic acid levels changes of intestinal mucosal barrier in mice of each group by spectrophotometry.

**Statistical analysis:**

SPSS22.0 was used for statistical analysis. T-test was used to analyze the measurement data between the two groups, F-test was used to analyze the measurement data between groups, and x² test was used to analyze the counting data. p<0.05 indicates that the difference is statistically significant.

**RESULTS AND DISCUSSION**

Table 1 showed that the body weight of the model group and the experimental group was lower than that of the control group, while that of the experimental group was higher than that of the model group (fig. 1). Table 2 showed that the fecal water content of the model group and the experimental group was higher than that of the control group while that of the experimental group was lower than that of the model group (fig. 2).

Table 3 showed that the Bristoli score of the model group and the experimental group was higher than that of the control group, and the Bristoli score of the experimental group was lower than that of the model group (fig. 3).

**Table 1: Comparison of body weight in each group (x̅±S)**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>18</td>
<td>18.54±0.64</td>
</tr>
<tr>
<td>Model group</td>
<td>18</td>
<td>15.41±0.45*</td>
</tr>
<tr>
<td>Experimental group</td>
<td>18</td>
<td>16.98±0.71*△</td>
</tr>
</tbody>
</table>

Note: Compared with the control group, *p<0.05; compared with the model group, △p<0.05

**Table 2: Comparison of fecal water content in each group (x̅±S)**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Fecal water content (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>18</td>
<td>47.85±3.24</td>
</tr>
<tr>
<td>Model group</td>
<td>18</td>
<td>58.38±6.57*</td>
</tr>
<tr>
<td>Experimental group</td>
<td>18</td>
<td>51.32±2.98*△</td>
</tr>
</tbody>
</table>

Note: Compared with the control group, *p<0.05; compared with the model group, △p<0.05
group (fig. 3). Table 4 showed that the D-lactic acid levels of the model group and the experimental group was higher than that of the control group, while that of the experimental group was lower than that of the model group (fig. 4).

IBS was a common disorder of gastrointestinal function, but the specific etiology and pathogenesis had not been fully elucidated[7-9]. In recent years, investigations have shown that the incidence of IBS is gradually increasing, and it often occurs in young and middle-aged people, which seriously affects the quality of life and physical and mental health of patients[10]. PI-IBS means that the patient was infected by enteroviruses, parasites or bacteria and develops IBS symptoms after mucosal inflammation gradually fades and pathogens were cleared[11-13]. Therefore, how to prevent and treat PI-IBS is of great significance.

*L. rhamnosus* was first isolated from the intestines of healthy people in the 1980s. Because *L. rhamnosus* can maintain the balance of human intestinal micro ecology, *L. rhamnosus* has become one of the most concerned probiotics in the world in recent years[14]. Pharmacological studies have shown that *L. rhamnosus* has the function of stabilizing and maintaining intestinal mucosal barrier, stimulating the non-specific and specific immune response, antagonizing pathogenic or conditional pathogenic bacteria and fostering normal flora[15-16]. This study showed that the body weight of mice in the model group and the experimental group was lower than that in the control group, while that in the experimental group was higher than that in the model group, indicating that *L. rhamnosus* could add mouse body weight. The fecal water content of the model group and the experimental group was more than that of the control group, while that of the experimental group was less than that of the model group, indicating that *L. rhamnosus* could increase the fecal water content of mice. The Bristoli score of the model group and the experimental group was higher than that of the control group, while that of the experimental group was lower than that of the model group, indicating that *L. rhamnosus* could improve the intestinal motility of mice.
There were different degrees of intestinal mucosal barrier injury in IBS. D-lactic acid was mainly found in the mucosa or the upper villi of mammals, most of which were found in the intestinal mucosal villi, and only a few in the endometrial villi of the viscera[17]. D-lactic acid was a highly active intracellular enzyme. Its activity was closely related to the height of the villi and the synthesis of protein and nucleic acid in mucosal cells. It can be used as an ideal index to reflect the structural and functional integrity of intestinal mucosa. When the intestinal mucosal epithelial cells were necrotic or damaged, D-lactic acid was released into the blood or along with the necrotic and exfoliated intestinal mucosal cells into the intestinal cavity, resulting in an increase in the activity of D-lactic acid in the intestinal cavity and plasma, and a decrease in the activity of D-lactic acid in the intestinal mucosa[18]. This study showed that the level of D-lactic acid in the model group and the experimental group was higher than that in the control group, while that in the experimental group was lower than that in the model group, indicating that *L. rhamnosus* could improve the intestinal mucosal barrier function by reducing the level of D-lactic acid. Because the PI-IBS model mice do not have a complete intestinal mucosal barrier, the high level of D-lactic acid may be the reason for the increase of intestinal mucosal permeability of PI-IBS, or it may be due to the increase of fecal water content in mice. Thus, *L. rhamnosus* can reduce fecal water content, and improve the intestinal mucosal barrier function.

To sum up, *L. rhamnosus* can improve the intestinal motility and intestinal mucosal barrier function in PI-IBS mice, which has important research value.

**Author’s contributions:**

XINLI FENG and D. ZHANG conceived and designed the experiments; Q. DI and S. LOU performed the experiments; YING CHANG and JIE MENG analyzed the data and wrote the paper.

**Acknowledgements:**

This work was supported by The Youth Research Fund Project of Affiliated Hospital of Hebei University (No. 2016Q003), The Baoding Science and Technology Plan Project (No. 2041ZF181). YING CHANG and JIE MENG are contributed equally to this work, XINLI FENG and D. ZHANG are considered co-corresponding in this paper.

**Conflicts of interest**

The authors report no conflicts of interest.

**REFERENCES**