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Original Article

Inhibitory Effects of Lactulose on Enteric Bacterial Dynamics and Gut Permeability in Bile-Duct Ligated Rats

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ABSTRACT

Background and Aim: The prebiotic potential of lactulose is well known, but its effect to inhibit enteric bacterial dynamics and gut permeability has not yet been investigated in experimental studies. The present study aims to assess the effects of lactulose on the enteric bacterial dynamics gut permeability in a model of bile duct ligation in Wistar rats. **Methods:** Wistar rats were assigned into 3 groups, the Sham-operated control, bile duct ligated (BDL) and BDL+ Lac group on the 28th day of bile duct ligation. Animals of BDL + Lac groups were treated with lactulose (1.0 g/kg), administrated by gavage, once a day for 7 days. Bacterial translocation into the organs and bacterial overgrowth as the indicators of the enteric bacterial dynamics and the levels of DAO and D-lactate as the biochemical makers of the gut permeability were measured in each group. **Results:** The bacterial colony forming units (CFU) in spleen and liver of BDL+Lac group was significantly decreased compared with BDL model group ($P<0.01$) and significant decrease of bacterial CFU was seen in the intestinal lavage in BDL+Lac group compared to BDL group ($P<0.01$). On the other hand, Lactulose revealed significant inhibitory effect as evidence by decreasing plasma DAO activity and D-lactate levels *(*$P<0.01$) in this model. **Conclusion:** These results indicate that lactulose can inhibit the enteric bacterial overgrowth, bacterial translocation (BT) and increasing gut permeability by opening the mucosa tight junction in ductal obstruction and obviate even systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS) induced by obstructive jaundice.

Key words: Bile duct ligation, Lactulose, Enteric bacterial overgrowth, Gut permeability.

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INTRODUCTION

Cholestasis represents the consequence of impaired bile formation and generally classified as extra- and intrahepatic disorders.[1] Cholestasis is a reduction in bile flow that leads to the intrahepatic accumulation of bile acids and other toxic compounds with progression of liver pathology, including hepatocellular injury and fibrosis.[2] The failure of bile salts excretion in cholestasis leads to retention of hydrophobic bile salts within the hepatocytes causing apoptosis and necrosis.[3] Moreover, abnormal flux of bile acids and bilirubin in the liver leads to retention and accumulation of toxic hydrophobic bile salts within hepatocytes,[4] causing inflammatory reactions, hepatocyte death, and periductular fibrosis.[5] Cholestatic patients are prone to septic complications after major surgery due to an increased susceptibility to endotoxin and hypotension.[6] Furthermore, patients with obstructive jaundice are susceptible to systemic inflammatory response syndrome (SIRS), even sepsis and multiple organ dysfunction syndrome (MODS) perioperatively. On the order hand, research has shown that BDL rat exhibits cholestasis, increased systemic oxidative stress, increased circulatory and multiple organ damage.[7] Lactulose is a synthetic disaccharide and has up to now been mainly used as a medicinal drug.[8] Lactulose as a medicinal drug is registered in over 100 countries. Indications are hepatic encephalopathy and constipation, and in some countries, the treatment of salmonella carrier.[9] Moreover, it is used as a prebiotic in food applications. However, it is unknown whether Lactulose can protect the enteric bacterial overgrowth, BT and inhibit the intestinal permeability in biliary obstruction. We investigated the inhibitory effect of Lactulose on the enteric bacterial dynamics and intestinal permeability in cholestatic rats induced by bile duct ligation.

MATERIALS AND METHODS

Animals

Male Wistar rats (260g) were provided by Laboratory Animal Centre of Sinuiju University of Medical Sciences and adapted in a lab environment before experiments for a week. 21 rats are randomly chosen and during the experiment, feed and water were available to rats at any time. The temperature was maintained at 20±2°C and the humidity was 55%. The study was approved by the Ethics Committee for

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Bile-Duct Ligation

The animals were submitted to a bile-duct ligation surgery as previously described.[10] Sham-animals were subjected to the same surgical procedure, except the bile duct was not ligated.

Lactulose Treatment

Lactulose (Lactulona®, 667 mg of lactulose/mL, Daiichi Sankyo, Brazil) was purchased from Mannyon pharmaceutical company, DPR Korea. On the 28th day post-surgery, the animals were distributed into 3 groups: Sham, BDL and BDL + Lac. Animals of BDL + Lac groups were treated with lactulose (1.0 g/kg), administrated by gavage, once a day for 7 days.

Analysis of Bacterial Translocation

Under sterile conditions, parts of the liver and spleen were removed and weighed. Each organ was homogenized and sonicated in 10 times the volume of sterile PBS, and cultured on fresh blood agar plates (Scientific Biotech Corp.) at 37°C for 24 hours. The bacterial colonies were counted and normalized to colony-forming units per gram of tissue (CFU/g).[11]

Measurement of Luminal Bacterial Counts

The distal small intestine of 10 cm length, with thread ligation at one end, was removed. The intestinal loop was instilled with sterile PBS (0.25 ml) and rocked back and forth 10 times. The intestinal lavage was plated on fresh blood agar overnight, and the number of bacteria was expressed as log colony-forming units per ml (log CFU/ml).

DAO Activity and D-lactate Levels in Plasma

Plasma was harvested from the collected abdominal aortic blood and kept at −20°C. Permeability of the intestinal mucosa was assayed by measuring D-lactate and DAO (diamine oxidase) levels in the plasma. Plasma D-lactate levels were measured by enzymatic spectrophotometric assay as previously described.[12] Plasma DAO activities were also determined by enzymatic spectrophotometry as previously described.[13]

Statistical Analysis of Data

Quantitative data are reported as mean ± standard error of the mean. Statistical differences in basal characteristics between the groups were calculated by one-way analysis of variance and t-test for continuous variables. P<0.01 was considered statistically significant. All statistical analyses were performed using the SPSS 16.0 software.

RESULTS

Table 1 showed that the inhibitory effect of Lactulose on bacterial translocation into spleen and liver tissues in BDL rats. As shown in Table 1, the bacterial CFU in BDL+Lac group was significantly decreased compared with BDL model group (P<0.01).

A significant decrease of bacterial CFU was seen in the intestinal lavage in BDL+Lac group compared to BDL group (P<0.01).

The activity of DAO and levels of D-lactate in plasma significantly increased in BDL group compared with sham group (P<0.01), but the levels of these indices were significantly decreased with BDL group in BDL+Lac group (P<0.01) (Table 2).

Table 1: The effects of Lactulose on bacterial translocation.

	Spleen (CFU/g)	Liver (CFU/g)
Sham	1.2 ± 0.6	19.8 ± 4.3
BDL	1325.1 ± 237.9**	172.2 ± 22.4**
BDL + Lac	679.2 ± 87.2△△	86.9 ± 13.1△△

Each value represents the mean ± SEM of 7 rats per group. **P<0.01 as compared with normal group. △△ P<0.01 as compared with model group. CFU: Colony forming units

Table 2: The effects of Lactulose on biochemical markers of intestinal permeability.

	DAO (U/L)	Lactic acid (μg/L)
Sham	4.3 ± 1.1	475.2 ± 10.3
BDL	15.7 ± 3.7**	721.5± 25.4**
BDL + Lac	679.2 ± 87.2△△	512.7± 19.7△△

Each value represents the mean ± SEM of 7 rats per group. **P<0.01 as compared with the normal group. △△ P<0.01 as compared with the model group. BDL: Bile duct ligated; DAO: Diamine oxidase.

DISCUSSION

Researches have shown that multisystemic alteration is the main cause of mortality. Patients with obstructive jaundice are susceptible to SIRS, sepsis and MODS perioperatively. Lactulose considered the most effective and commonly used non-absorbable disaccharide in clinical practice.[14] Lactulose is a completely harmless substance in recommended doses, it does not affect the blood sugar levels in diabetic patients. Moreover, with the growing interest in functional foods, the use of non-digestible oligosaccharides such as lactulose as prebiotic ingredients has increased considerably during recent years and lactulose offers excellent and scientifically tested functional properties and applications for the development of new functional foods. Several studies have reported the effects of Lactulose for the prevention and treatment of some diseases but there has been no research to investigate the inhibitory effects on the enteric bacterial dynamics and gut permeability in obstructive jaundice. Therefore, the present study aimed to investigate the inhibitory effect of Lactulose on the enteric bacterial overgrowth dynamics and gut permeability in bile duct ligated rats. Bile duct ligation model in rats has been used to study the mechanisms of new products in human patients with liver cirrhosis.[15] Our study showed that Lactulose can inhibit the enteric bacterial overgrowth, BT and the gut permeability in BDL rats. In order to demonstrate the efficiency of Lactulose in this model, we administrated Lactulose (1.0 g/kg, orally) for 7 days from the 28th day after bile duct ligation. In some severe diseases, a massive quantity of bacteria and antigens penetrates into extra-intestinal organs, which is termed bacterial translocation (BT). Changes in gut microfloral population have been implicated in the mechanism of BT.

A significant increase in the bacterial CFU was found in the spleen of BDL rats compared to that of sham rats and also the number of viable bacteria in the liver was also higher in BDL rats than in sham controls. Table 1 showed that the bacterial CFU in BDL+Lac group was significantly decreased compared with BDL model group (P<0.01). A 1.4-fold increase of the luminal bacterial counts was seen in the distal small intestine of BDL rats compared to sham controls, suggesting enteric bacterial overgrowth following BDL (Figure 1). But a significant decrease of bacterial colony forming units (CFU) was seen in the intestinal lavage in BDL+Lac group compared to BDL group (P<0.01). Plasma D-lactate level and DAO activity can reflect the presence of intestinal injuries, including intestinal mucosal barrier damage. Therefore the changes in these indices can suggest that the mucosal barrier was damaged and also gut permeability was increased in BDL rats. The activity of DAO and levels of D-lactate significantly increased in BDL group compared with sham group (P<0.01), but the levels of these indices were significantly decreased with BDL group in BDL+Lac group (P<0.01).

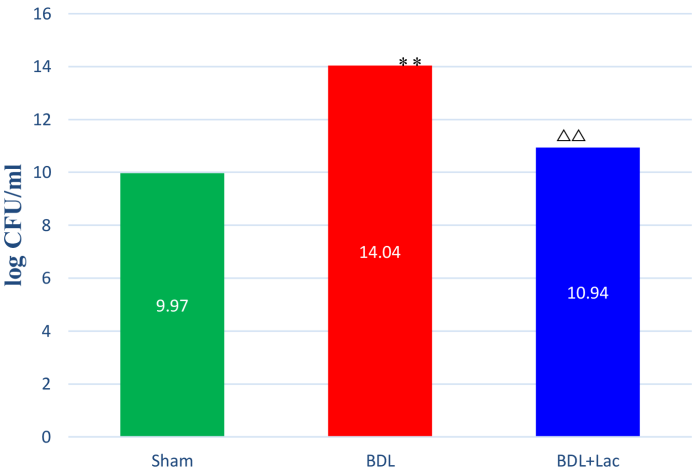

Figure 1: The effects of Lactulose on Bacterial overgrowth in the small intestinal lumen.
Each value represents the mean ± SEM of 7 rats per group. **P<0.01 as compared with normal group. △△P<0.01 as compared with model group.

CONCLUSION

As a limitation to our study, we could not measure endotoxin levels in the blood and compare them with other prebiotics. However, it has recently been shown that Lactulose administration results in the decrease of BT, and bacterial overgrowth in the small intestine and the levels of DAO and D-lactate indicated the gut permeability in the BDL model.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

BDL: Bile Duct Ligation; **SIRS:** Systemic Inflammatory Response Syndrome; **MODS:** Multiple Organ Dysfunction Syndrome; **BT:** Bacterial Translocation; **DAO:** Diamine Oxidase; **CFU:** Colony Forming Units.

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