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• *BRIEF REPORTS* •

# Comparison of probiotics and lactulose in the treatment of minimal hepatic encephalopathy in rats

Lin Jia, Mei-Hua Zhang

Lin Jia, Department of Digestive Diseases, Affiliated First People's Municipal Hospital, Guangzhou Medical College, Guangzhou 510180, Guangdong Province, China
Mei-Hua Zhang, Department of Digestive Diseases, Affiliated First People's Municipal Hospital, Guangzhou Medical College, Guangzhou 510180, Guangdong Province, China
Correspondence to: Dr. Lin Jia, Department of Digestive Diseases, First People's Municipal Hospital of Guangzhou, 1 Panfu Road, Guangzhou 510180, Guangdong Province, China. gzjialin@hotmail.com
Telephone: +86-20-81628877 Fax: +86-20-81628809
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## Abstract

**AIM:** To compare the efficacy of probiotic preparation Golden Bifid and lactulose on rat experimental model of minimal hepatic encephalopathy (MHE) induced by thioactamide (TAA).

**METHODS:** MHE was induced by intraperitoneal injection of TAA (200 mg/kg) every 24 h for two consecutive days. Thirty-six male MHE models were then randomly divided into 3 groups: TAA group ($n$ = 12) received tap water *ad libitum* only; lactulose group ($n$ = 12) and probiotics group ($n$ = 12) were gavaged, respectively with 8 mL/kg of lactulose and 1.5 g/kg of probiotic preparation Golden Bifid (highly concentrated combination of probiotic) dissolved in 2 mL of normal saline, once a day for 8 d (from the 5th d before the experiment to the 3rd d of the experiment). The latency of brainstem auditory evoked potentials (BAEP) I was used as an objective index of MHE. The incidence of MHE, the level of serum endotoxin, ammonia, liver function and histological grade of hepatic injury of rats were examined individually.

**RESULTS:** There were no overt HE and rat deaths in 3 groups. The incidence of MHE, the levels of blood ammonia and endotoxin in TAA group, which were 83.3% (10/12), 168.33±15.44 mg/dL and 0.36±0.04 EU/mL, respectively, were significantly higher than those in lactulose group, which were 33.3% (4/12), 110.25±7.39 mg/dL and 0.19±0.02 EU/mL, and probiotics group, which were 33.3% (4/12), 108.58±10.24 mg/dL and 0.13±0.03 EU/mL respectively ($P$ <0.001). It showed that either probiotics or lactulose could significantly lower the level of hyperammonemia and hyper-endotoxemia, lighten centrolobular necrotic areas as well as inflammatory reaction in the liver of rats, normalize the latency of BAEP, and decrease the incidence of MHE. However, no significant differences were observed between these two groups ($P$ >0.05).

**CONCLUSION:** Probiotic compound Golden Bifid is at least as useful as lactulose for the prevention and treatment of MHE. Probiotic therapy may be a safe, natural, well-tolerated therapy appropriate for the long-term treatment of MHE.



**Key words:** Minimal hepatic encephalopathy; Probiotics; Lactulose

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## INTRODUCTION

Hepatic encephalopathy (HE) is a common and serious complication of liver diseases, ranging from minimal disease (grade 0) to hepatic coma (grade IV)[1,2]. Minimal hepatic encephalopathy (MHE) is a term that describes patients with chronic liver disease who have no clinical symptoms of brain dysfunction, but perform substantially worse on psychometric tests compared to healthy controls[3-5]. Although the exact pathogenesis of HE still remains unknown, the products of gut flora metabolism, such as ammonia, endotoxin, and benzodiazepine-like substances have been universally recognized as critical in recent years[6-10].

Present treatment strategies, including lactulose and poorly absorbable antibiotics, may not be the optimal therapy for all HE patients due to their side effects and patients' poor compliance with therapy. Being viable bacteria given orally to improve health, probiotics were found to have multiple mechanisms of action to disrupt the pathogenesis of HE, and probiotic therapy was supposed to be the ideal strategy for HE[6,7].

Probiotic compound Golden Bifid is a highly concentrated combination of probiotic containing bifidobacteria, lactobacilli and a mixture of *Streptococcus thermophilus* strains [6]. Based on the MHE model of rats established in 2004[11], the present study was conducted to further compare the efficacy of Golden Bifid and lactulose on rat experimental MHE induced by thioactamide (TAA).

## MATERIALS AND METHODS

### *Induction of MHE model and its grouping*

A total of 36 male Sprague-Dawley rats (Experimental

Animal Center of Sun Yat-Sen University) weighing 220-250 g were used. MHE was induced by intraperitoneal injection of TAA (200 mg/kg in normal saline, purity >99%, Shanghai Central Chemical Factory) every 24 h for two consecutive days[11]. Rats were fed with regular chow and water *ad libitum* in cages placed in a room with a 12-h light/dark cycle and constant humidity and temperature (25 ℃).

MHE models were then randomly divided into 3 groups: TAA group ($n$ = 12) received tap water *ad libitum* only; lactulose group ($n$ = 12) and probiotic group ($n$ = 12) were gavaged respectively with 8 mL/kg of lactulose (Duphalac®, Solvay Pharmaceuticals B.V.) and 1.5 g/kg of Golden Bifid (highly concentrated combination of probiotic, provided by Shuangqi Pharmaceutical Co., Inner Mongolia, China) dissolved in 2 mL of normal saline, once a day for 8 d (from the 5th d before the experiment to the 3rd d of the experiment).

At the 3rd d of the experiment, brainstem auditory evoked potentials (BAEP), the serum level of endotoxin and ammonia, and liver function tests were carried for a quantitative evaluation of the efficacy of the Golden Bifid and lactulose treatment.

### Diagnosis of HE and MHE in rats

The behavioral manifestations of hepatic encephalopathy in the rats that received intraperitoneal injection of TAA evolved through four stages: (1) lethargy; (2) mild ataxia; (3) lack of spontaneous movement, loss of righting reflex, but positive response to tail pinch, and (4) coma, no response to tail pinch[12]. If TAA-treated rats showed one of the manifestations, it could be diagnosed as overt HE. Otherwise, evoked potentials of rats should be tested to confirm the diagnosis of MHE.

In our previous studies, the latency of BAEP of healthy rats was used as an objective index of MHE, and the average value of the latency of BAEP I in healthy rats ±1.96 SD (1.45 ms) was regarded as normal value. MHE was diagnosed if the test score of the latency of BAEP I of rats was above 1.45 ms. Incidence of MHE and HE was recorded[11].

### Analysis of serum endotoxin, ammonia and liver function tests

All the blood samples for the endotoxin determination were stored in endotoxin-free tubes. The serum was pretreated with perchloric acid for removal of the possible inhibitors to limulus amebocyte lysate (LAL). Serum level of endotoxin was determined by LAL test with LAL kits (purchased from Yihua Medical Technology Co., Shanghai, China).

Liver function tests, including total bilirubin (TB), albumin, serum glutamic-oxalacetic transaminase (SGOT) and glutamic-pyruvic transaminase (SGPT) were measured using a biochemical autoanalyzer (Hitachi Co., Tokyo, Japan). Tail vein ammonia was measured using a blood ammonia detector (Model AA-4120, Kyoto DaiiChi Kagaku Company, Japan).

### Hepatic histopathology of rats

Liver tissue samples from the right major liver lobe were obtained, fixed in formalin and embedded in paraffin. Five-micron thin tissue sections were obtained and stained with hematoxylin and eosin for microscopic examination. Histological grade of hepatic injury was determined by a semi-quantitative method based on the criteria described below: Grade 0: normal liver, grade 1: edema in the liver cell, grade 2: changes as balloon in liver cell, grade 3: necrosis as dots in the liver cell, inflammation cells in the portal vein section, grade 4: necrosis as small pieces.

Two pathologists who had no knowledge of the sample sources and each other's assessment examined the stained slides independently.

### Statistical analysis

All the values were expressed as the mean±SD. One-way ANOVA was used to check the differences among them. $\chi^2$ test was used to check the differences of the incidence of MHE among the groups. When $P$ was less than 0.05, the difference was considered statistically significant. Software SPSS10.0 was used in all statistical analyses.

## RESULTS

### Probiotics effects on the incidence of MHE

There were no overt HE and rat deaths in 3 groups. The incidence of MHE in TAA group was 83.3% (10/12), if 1.45 ms was regarded as the normal value of latency of BAEP I of rats (Table 1). Administration of lactulose and probiotics could significantly normalize the latency of BAEP, and decrease the incidence of MHE (33.3% and 33.3% respectively, $P$<0.05), however, there was no difference in the incidence of MHE between lactulose group and probiotics group ($P$>0.05).

### Probiotic effects on endotoxin, ammonia and liver function tests

The levels of blood ammonia and endotoxin in TAA group were 168.33±15.44 μg/dL and 0.36±0.04 EU/mL respectively, which were significantly higher than those in lactulose group, which were 62.25±7.63 μg/dL and 0.07±0.02 EU/mL, and probiotics group, which were 62.25±7.63 μg/dL and 0.07±0.02 EU/mL, respectively ($P$<0.05). It revealed that lactulose and probiotics could significantly improve the ammonemia and endotoxemia in MHE models, as presented in Table 1.

**Table 1 Incidence of MHE, serum level of endotoxin and ammonia in three groups**

| Group | Latency of BAEP I (ms) | Incidence of MHE (%) | Level of endotoxin (EU/mL) | Level of ammonia (μg/dL) |
|---|---|---|---|---|
| TAA | 1.52±0.07 | 83.3 (10/12) | 0.36±0.04 | 168.33±15.44 |
| Lactulose | 1.43±0.04[a] | 33.3 (4/12)[a] | 0.13±0.02[a] | 110.25±7.39[a] |
| Probiotics | 1.42±0.09[a] | 33.3 (4/12)[a] | 0.13±0.03[a] | 108.58±10.24[a] |

[a]$P$<0.05 *vs* TAA group.

Serum ALT, AST, albumin and TB concentrations in lactulose and probiotic groups showed slight improvement compared with those in TAA group ($P$> 0.05) (Table 2).

However, there were no significant differences between lactulose group and probiotic group ($P>0.05$).

**Table 2** Serum content of ALT, AST, albumin and TB in three groups

| Group | ALT (IU/L) | AST (IU/L) | Albumin (g/L) | TB (μmol/L) |
|---|---|---|---|---|
| TAA | 137.00±20.55 | 430.75±60.25 | 31.27±1.26 | 14.97±2.76 |
| Lactulose | 132.58±18.95 | 421.17±60.00 | 31.95±0.96 | 12.23±1.07 |
| Probiotics | 128.00±16.21 | 409.67±63.02 | 32.23±1.07 | 13.76±2.18 |

### *Probiotic effects on hepatic histopathology of rats*

The control livers showed a normal lobular architecture with central veins and radiating hepatic cords. Two consecutive intraperitoneal injections of TAA (200 mg/kg) at 48 h time intervals caused severe pathological damages such as inflammation, dot necrosis or patchy necrosis. Administration of lactulose and probiotics could significantly lighten centrolobular necrotic areas as well as inflammatory reaction in rats subjected to TAA ($P<0.05$). Semi-quantitative hepatic injury staging scores are shown in Table 3.

**Table 3** Hepatic histopathology of rats in three groups

| Group | Grade 0 | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
|---|---|---|---|---|---|
| TAA | 0 | 0 | 0 | 10 | 2 |
| Lactulose | 1 | 6 | 3 | 1[a] | 0 |
| Probiotics | 1 | 7 | 2 | 2[a] | 0 |

[a]$P<0.05$ *vs* TAA group.

## DISCUSSION

According to a consensus statement about HE in 1998, the study of HE has been greatly hindered by the lack of properly designed therapeutic trials[1,3]. The exact pathogenesis of HE remains uncertain, but is almost certainly multi-factorial. Gut-derived nitrogenous substances are universally acknowledged to play a major role. Specifically, many nitrogenous products of gut flora metabolism including ammonia, endotoxin, benzodiazepine-like substances, or mercaptans are implicated in the genesis of HE[6-10]. Consequently, treatment of HE generally aims to reduce the production and absorption of intestinal toxins such as modifying the quantity and type of protein intake, reducing the intestinal transit time, and the proteolytic flora and increasing the saccharolytic flora[6,7,13-16].

Presently, lactulose is considered to be the 'standard therapy' for HE due to its efficacy. However, lactulose has an unpleasant taste and causes flatulence, diarrhea, abdominal pain or intestinal malabsorption, which does not contribute to the improvement of patients' quality of life[17-19]. *Pai et al*[20] showed that only 20% patients favored the taste of lactulose, and 30% patients complained of meteorism and flatulence, and 20% patients complained of nausea. A high rate of dropouts (21-31%) was reported in some studies when treated with lactulose at a dose titrated to pass 2-3 semi-soft stools a day[18,19]. Therefore, lactulose may not be the optimal therapy for all HE patients due to side effects, cost, and relatively poor compliance with therapy, particularly for the long-term treatment of MHE. Clearly, safe and well-tolerated alternatives are needed. Since probiotic is a safe, natural, well-tolerated therapy appropriate for long-term use, probiotic therapy is supposed to be the ideal strategy for HE, and has been gradually accepted worldwide in recent years[6].

A number of studies have been performed using several strains of fermentative lactic-producing bacteria in order to modify the composition of gut flora. These trials employed high doses of non-urease-producing bacteria, either *Lactobacillus acidophilus* or *Enterococcus fecium* SF68. The effects of probiotics on HE have been demonstrated in many studies and no adverse effects were reported[7,16,21,22]. In a carefully conducted randomized controlled study, either short-term or long-term administration of SF68 in compensated patients with cirrhosis could enhance tolerance to protein load, lower ammonia levels, and improve neurological symptoms in patients with HE, and was at least as useful as lactulose for long-term treatment of chronic grades 1-2 of HE. It had no adverse effects, and in contrast to lactulose, treatment can be interrupted for 2 wk without losing the beneficial effects[7]. However, these above studies were limited to therapy with single aforementioned probiotic product and treatment of overt HE. Therefore, *Solga*[6] further proposed a hypothesis that probiotic compound may be superior to the single one, and probiotic compound VSL#3, which contains viable, lyophilized bifidobacteria, lactobacilli and a mixture of *Streptococcus thermophilus* strains might be ideally suited to HE[23,24].

Unfortunately, no useful animal models exist for MHE study[6]. We used the latency of BAEP I as an objective index of MHE, and established animal model of MHE induced by injection of small dose of TAA in rats for the first time. The MHE model had a high level of ammonemia and endotoxemia, and its incidence was 83.3%[11]. The efficacy of probiotics and lactulose on rat experimental MHE has not been elucidated before.

In the current study, Golden Bifid served as a representative of probiotic compound VSL#3[23,24]. We used the well-established MHE model to compare the efficacy of Golden Bifid and lactulose, and tried to reason Solga's hypothesis. The results demonstrated that either Golden Bifid or lactulose could significantly lower the level of hyperammonemia and hyper-endotoxemia, lighten centrolobular necrotic areas as well as inflammatory reaction in the liver of rats, normalize the latency of BAEP, and decrease the incidence of MHE. However, there were no significant differences between lactulose group and Golden Bifid group. Probiotics may exhibit efficacy in the treatment of MHE by decreasing total ammonia in the portal blood, as well as in the uptake of other toxins, reducing inflammation and oxidative stress in hepatocytes leading to increased hepatic clearance of ammonia and other toxins[6].

In conclusion, probiotic compound Golden Bifid showed excellent effects in lowering the level of ammonemia and endotoxemia, improving hepatic histopathology of rats, and decreasing the incidence of MHE. It was as effective as lactulose in the prevention and treatment of MHE. Our study agrees with Loguercio's conclusions on chronic HE

and confirms Solga's hypothesis for the first time. The probiotic therapy is a safe, effective, and well-tolerated strategy for HE, especially appropriate for long-term treatment of MHE.

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