Int J Clin Exp Med 2015;8(6):9996-10000
www.ijcem.com /ISSN:1940-5901/IJCEM0008521

Original Article

Probiotic and lactulose: influence on gastrointestinal flora and pH value in minimal hepatic encephalopathy rats

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Received March 27, 2015; Accepted June 4, 2015; Epub June 15, 2015; Published June 30, 2015

Abstract: Aim: The present study was conducted to investigate the influence on gastrointestinal flora, counts of bifidobacteria and Enterobacterceae in colon and pH value of gastrointestinal after lactulose and probiotic treatment on rat experimental minimal hepatic encephalopathy (MHE) induced by thioactamide (TAA). Methods: MHE was induced by intraperitoneal injection of TAA. 48 male MHE models were then randomly divided into 4 groups: control group (n = 12); MHE group (n = 12) received tap water ad libitum only; lactulose group (n = 12) and probiotics group (n = 12) gavaged respectively with 8 ml/kg of lactulose and 1.5 g/kg of probiotic preparation Golden Bifid (highly concentrated combination probiotic) dissolved in 2 ml of normal saline, once a day for 8 days. The latency of Brainstem auditory evoked potentials (BAEP) I was used as objective index of MHE. Counts of gastrointestinal flora, counts of bifidobacteria and Enterobacterceae in colon and pH value of gastrointestinal were examined respectively. Results: Compared to MHE group, counts of gastrointestinal flora has greatly decreased, ratio of bifidobacteria and Enterobacterceae has greatly increased, pH value of colon has greatly descended ($P < 0.05$). However, there was no significant difference between lactulose group and probiotic group ($P > 0.05$). Both lactulose and probiotics can effectively prevent bacteria translocation and overgrowth, intensify CR, improved value of B/E, and acidify intestinal, decreased pH value of colon. Conclusion: Probiotic compound Golden Bifid is 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.

Keywords: Minimal hepatic encephalopathy, lactulose, probiotics, PH value, gastrointestinal flora

Introduction

Hepatic encephalopathy (HE) is a complex neuropsychiatric syndrome which has been defined as "a disturbance in central nervous system function because of hepatic insufficiency" [1]. The spectrum of HE ranges from minimal cerebral functional deficits, which can only be found by sensitive psychometric tests, to coma with signs of decerebration. 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 [2].

Exact mechanism of HE/MHE still remains unknown. Gut-derived nitrogenous substances which derived from disorder of intestinal flora are universally acknowledged to play a major role [3]. Lactulose is the most frequently utilized agent in the treatment of MHE. On the other hand, probiotic also has been widely used. Based on the MHE model of rats established in 2004, the present study was conducted to further investigate the influence of gastrointestinal flora, counts of bifidobacteria and Enterobacterceae in colon and PH value of gastrointestinal after lactulose and probiotic treatment on rat experimental MHE induced by thioactamide (TAA).

Materials and methods

Model

A total of 48 male Sprague-Dawley rats (Experimental Animals Center of Sun Yat-Sen

University, SPF grade) weighing 220 to 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 hours for two consecutive days. Rats were fed with regular chow and water ad libitum in cages placed in a room with 12-hour light-dark cycle and constant humidity and temperature (25°C).

MHE models were then randomly divided into 4 groups: Normal group (n = 12); TAA group (n = 12) received tap water ad libitum only; lactulose group (n = 12) and probiotics group (n = 12) gavaged respectively with 8 ml/kg of lactulose (duphalac®, Solvay Pharmaceuticals B.V.) and 1.5 g/kg of Golden Bifid which consisted of Bifidobacterium longum, L.bulgaricus and Str. thermophilus (highly concentrated combination probiotic, provided by Shuangqi pharmaceutical Co., Inner Mongolia, China) dissolved in 2 ml of normal saline, once a day for 8 days (from 5th day before the experiment to 3rd day of the experiment).

Diagnosis

The behavioral manifestations of hepatic encephalopathy in rats that received Intraperitoneal injection of TAA evolve 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. If TAA-treated rats had one of the above manifestations, it could be diagnosed as overt HE. Otherwise, Brainstem auditory evoked potentials (BAEP) 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 objective index of MHE, and the average value of the latency of BAEP I in healthy rats ± 1.96 standard deviation (1.45 ms) was regarded as normal value. MHE was diagnosed if the test scores of the latency of BAEP I of rats was above 1.45 ms. the incidence of MHE and HE was recorded.

Counts of flora colony

After all rats were cut the belly open by etherization, the whole stomach, 5 cm length of near portion jejunum and distal segment ileum were obtained under bacteria free condition and then were rinsed with 10 ml saline respectively. Rinse solution use to cultivate colony (0.1 ml rinse solution is put into bacteria free flat plate and cultivate with normal nutrient agar. Total colony will be counted after cultivate 24 hour in incubator at 37°C).

Cultivation of bifidobacteria and Enterobacterceae

After all rats were cut the belly open by etherization, 0.5 g contents of colon were obtained and diluted to 10-6 according to 10 times dilution with diluent and then will be cultivated in selective bifidobacteria (BS medium, Qingdao Hope-Bio Technology Co., Ltd, China) and Enterobacterceae culture media (MacCon Key Agar, OXOID, England) by instill. Number of bifidobacteria will be counted after cultivated 72 hours in phobic oxygen incubator at 37°C and Enterobacterceae will be counted after cultivated 24-48 hours in normal incubator at 37°C.

Detection of pH value

PH value of stomach, jejunum, ileum and colon were detected by mobile PH detector directly for all rats at the end of the experiment.

Statistical analysis

All values were expressed as the mean ± SD. One-way ANOVA was used to check the differences among them. When P was less than 0.05, the difference was considered statistically significant. Software SPSS10.0 was used in all statistical analysis.

Results

Effects on gastrointestinal flora

Results of flora cultivation with rinse solution show the total number of colony in stomach, jejunum, ileum of TAA group has significantly increased compared to control group and it implicates that plenty of intestinal bacteria shift to upper part and overgrew ($P < 0.05$). While there is no significant difference about colony count among lactulose group, probiotics group and control group. There is significant difference about colony count between two treatment groups and TAA group. It proved that both lactulose and probiotics can effectively prevent bacteria translocation and overgrowth so as to

Table 1. Counts of gastrointestinal colony, bifidobacteria and Enterobacterceae; B/E value in colon

Groups	Stomach colony	Jejunum colony	Ileum colony	Bifidobacteria	Enterobacterceae	B/E value
MHE group	2.2×103	2.5×105	6.5×105	7.61 ± 0.13	8.40 ± 0.12	0.91 ± 0.03
lactulose	1.1×102[a]	1.1×103[a]	2.3×104[a]	8.23 ± 0.27	7.81 ± 0.17	1.05 ± 0.05[a]
Golden Bifid	2.3×102[a]	6.5×102[a]	1.0×103[a]	8.37 ± 0.25	7.68 ± 0.35	1.09 ± 0.07[a]
Normal group	4.6×102	1.2×103	1.3×104	8.81 ± 0.14	7.49 ± 0.12	1.18 ± 0.02[a]

[a]$P < 0.05$ vs MHE group.

Table 2. PH value of stomach, jejunum, ileum, colon

Groups/PH value	Stomach	Jejunum	Ileum	Colon
MHE group	4.76 ± 1.61	6.08 ± 0.13	6.82 ± 0.18	6.80 ± 0.41
Lactulose	3.38 ± 0.49	5.60 ± 0.46	6.02 ± 0.44	5.34 ± 0.72[a]
Golden Bifid	3.46 ± 0.56	5.46 ± 0.60	6.50 ± 0.42	5.98 ± 0.40[a]
Normal	4.90 ± 1.16	6.22 ± 0.41	6.84 ± 0.34	7.16 ± 0.43

[a]$P < 0.05$ vs MHE group.

reduce production and absorption of toxin generated from intestine (**Table 1**).

Effects on bifidobacteria and Enterobacterceae in colon

The ability that intestinal obligate anaerobe inhibit latent pathogenic bacterium overgrow and prevent it adhere to enterocyte calls colonization resistance (CR) and CR is expressed as B/E (ratio of bifidobacteria and Enterobacterceae). Value of B/E > 1 shows that CR is normal and if ≤ 1 implicates that CR has descended. Result of bifidobacteria and Enterobacterceae cultivation in colon shows the number of bifidobacteria, ratio of B/E has significantly decreased and B/E < 1 while number of Enterobacterceae has significantly increased in TAA group compared to control group ($P < 0.05$). There is no significant difference about ratio of B/E among lactulose group, probiotics group and control group. There is significant difference about ratio of B/E between two treatment groups and TAA group. It proved that both lactulose and probiotics can effectively intensify CR, improve value of B/E, prevents bacterial translocation and overgrowth (**Table 1**).

Effects on pH value

Compared to control group and TAA group, value of PH in lactulose group and probiotics group has greatly descended ($P < 0.05$), but there is no significant difference between lactulose group and probiotics group. Results show that both lactulose and probiotics can effectively acidify intestinal, decrease PH value of colon under 6.0 so that can decrease absorption of ammonia and promote growth of healthy bacteria (**Table 2**).

Discussion

Hepatic encephalopathy is a common and serious complication of chronic liver disease and can be clinically overt or less apparent. Pathogenesis of HE is considered to be multifactorial and remains unclear. It was always explained as such doctrines like hyperammonemia, unbalance of amino acid, false neurotransmitter and GABA/BZ et al in the past time. Recently, reports suggest that disorder of intestinal flora and bacterial translocation which will greatly add up production and absorption of intestinal toxin have close relevance with the genesis and development of hepatic encephalopathy [4]. There exist different degree disorders of intestinal flora in patients with chronic hepatic disease. Such beneficial bacteria as bifidobacteria has greatly decreased while Urease-producing bacteria as Enterobacterceae overgrew Cirrhotics harbor more gut urease-active bacteria than controls Urease-producing bacteria are mostly gram negative Enterobacterceae and they are the source of gut-derived toxin. This disorder will induce attenuation of intestinal colonization resistance, promote Urease-producing bacteria translocation and delayed gastrointestinal transit time [5]. As results, production and absorption of toxin generated from intestinal will significantly increase, but because of poor function, liver cannot metabolism these toxin completely in time so that will induce toxin retention. Liver function subject further injury and will promote occurrence and development of hepatic encephalopathy in the end.

Lactulose is a non-absorbable synthetic disaccharide and it neither metabolized nor absorbed in small intestines. It has multiple effects on gut flora and there are several potential mechanisms of its action [6]. It's obvious laxity effect may reduce production and absorption of ammonia. However, there are Additional mechanisms of lactulose for HE: 1) Decreasing ammonia production and increasing assimilation of nitrogenous products by bacteria. 2) Acidifying the colon contents and lowing value of luminal PH resulting decrease of ammonia absorption from the gut [7]. 3) Lactulose may function as a prebiotic in the treatment of HE so can effectively modulate intestinal flora. It significantly increases concentrations of bifidobacteria and lactobacilli; lactulose also can effectively inhibit Urease-producing pathogenic bacteria like Enterobacterceae [8]. The modulation of beneficial bacteria and Urease-producing bacteria finally can intensify intestinal colonization resistance, reduce bacterial translocation and overgrowth. As for so many advantages, lactulose has been look on as a classical drug for HE/MHE treatment.

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. Therefore, lactulose may not be optimal therapy for all HE patients due to side effects, and cost, and relatively poor compliance with therapy, particularly for the long-term treatment of MHE [9]. Clearly, safe, well-tolerated alternatives are needed. Since probiotics are a safe, natural, well-tolerated therapy appropriate for long-term use, probiotic therapy is supposed to be ideal strategies for H&E, and has been gradually accepted worldwide in recent years [10].

Previous 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 faecium SF68. All articles on the effect of probiotics on HE have demonstrated efficacy and lack of adverse effects [11-17]. 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, was at least as useful as lactulose for long-term treatment of chronic grade 1-2 HE. It had no adverse effects, and in contrast to lactulose, treatment can be interrupted for 2 weeks without losing the beneficial effects [11]. However, these above studies were limited to therapy with single probiotic products and treatment of overt HE. Therefore, Solga further proposed 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 [10].

There is no useful MHE model in previous study, the model we established with TAA can induce MHE, is similar to fulminant hepatic failure (FHF) model. However, based on the dose-effect relationship of modeling in our previous research, high dose of TAA actually could induce HE, but low dose (200 mg/kg) which was used in this study was appropriate for MHE inducing. Thus based on establishment of MHE model induced by TAA, we conduct the experimental study to explore the influence on gastrointestinal flora, counts of bifidobacteria and Enterobacterceae in colon and pH value of gastrointestinal after lactulose and probiotic treatment on rat experimental MHE induced by thioactamide (TAA). The results suggest that through lactulose and probiotic treatment, compared to TAA group, counts of gastrointestinal bacteria has greatly descended, ratio of bifidobacteria and Enterobacterceae has greatly increased, PH value of colon has greatly descended ($P < 0.05$). And there is no significant difference between lactulose group and probiotic group ($P > 0.05$). Both lactulose and probiotics can effectively prevent bacteria translocation and overgrowth, intensify CR, improve value of B/E, acidify intestinal, decrease PH value of colon so as to reduce production and absorption of toxin generated from intestinal.

As noted above, probiotic compound Golden Bifid showed excellent effects on prevent bacteria translocation and overgrowth, intensify CR, improve value of B/E, acidify intestinal, decrease PH value of colon which was as effective as lactulose in the prevention and treatment of MHE. It agrees with the Loguercio' conclusions on chronic HE and confirms the Solga's

hypothesis for the first time. The Probiotic therapy is a safe, effective, and well-tolerated strategy for him, especially appropriate for long-term treatment of MHE [18].

Disclosure of conflict of interest

None.

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