Dear author This is very interesting paper about fecal microbiota transplantation (FMT) for hepatic encephalopathy (HE). According to your paper, EMT is good effect for intestinal barrier function and especially tight junction protein (claudin-1, claudin-6 and Occludin) . Thereafter, EMT is protective role in treating HE. I ask some questions. 1. Please comment me the reason why in Fig 6 blood samples were collected from portal vein and tail vein. Please comment the significance of portal blood and peripheral blood level in ammonia, cytokine. 2. Please comment the reason why the ammonia level in EMT is similar to control. 3. According to your data, intestinal barrier function and tight junction are important factor to ammonia level. Please comment me the etiology between ammonia level and intestinal barrier function.

Dear Professor:

Thank you for your comments. I hope my reply will satisfy you.

For question 1: Elevated blood ammonia has been considered the main cause of hepatic encephalopathy until now. However, in clinical practice, blood ammonia is often found normal in patients with hepatic encephalopathy, and the method for reducing blood ammonia is not effective in this disease. Therefore, there must be other factors beyond the blood ammonia that are involved in the course of hepatic encephalopathy. The current mainstream view is that hepatic encephalopathy is the result of the interaction between blood ammonia and the systemic inflammatory response [1]. There are other theories that cannot be confirmed one by one in this article. Therefore, we chose blood ammonia and some inflammatory factors as the focus for this study.

The original purpose of studying the portal vein and venous blood samples was simply to understand what would happen to the blood ammonia of the portal vein after passing through the liver; the inflammatory factors were then examined together to make the results more convincing. In our results, we found that the blood ammonia and inflammatory factors of venous blood were significantly decreased after passing through the liver. It appears that the venous blood has a filtering effect after passing through the liver. However, one exception was the high level of IL-6 in the venous blood of VSL# 3 but not of FMT rats. Several studies have shown that IL-6 is an independent risk factor for hepatic encephalopathy or even a predictor[2-4]; this result is different from our expectation that IL-6 would be reduced after it goes through the liver in VSL#

3. This unexpected result gives us reason to speculate that FMT may be better than VSL# 3. However, it is not clear why this occurred. Previous scholars have predicted that probiotics alone may not be beneficial[5].

For question 2: We again confirmed the results of whether the venous blood or the blood ammonia were different in the control group, and there were significant differences.

For Question 3: The main source of blood ammonia is intestinal bacteria, and the relationship between intestinal flora and hepatic encephalopathy can be explained by three components: 1. Intestinal bacteria; 2. Intestinal permeability; and 3. The TLR immune response of the liver.

The first aspect is an extremely complex topic, so we did not think about it in the experimental design; we cannot say more, but this will be our next direction. There was a report that patients with hepatic encephalopathy had stool bacteria consistent with donors after FMT[6]. During the experiment, FMT rats were observed to have diarrhea that then disappeared, while VSL# 3 rats did not. Therefore, we thought that during the FMT process, the intestinal flora of the FMT rats had partially changed or even changed completely, and then the rats adapted to this change, which may affect the production of ammonia in the gut.

The second aspect, under normal circumstances, the intestinal wall barrier by the mucus layer, the intestinal epithelium microvilli and intestinal epithelial cells that can secrete some defensive factors and the close connection between cells enables many harmful substances to be destroyed before entering the portal venous system [7]. In the state of portal hypertension, the permeability of the stomach, small intestine and colon is increased [8], and bacteria and bacterial products will enter the portal system. Therefore, we believe that in this process, non-ionized NH_3 also enters the portal vein. Our results suggest that after FMT, the intestinal permeability decreases and is accompanied by a decrease in blood ammonia.

In the third aspect, the hepatic TLR immune response is mainly related to the bacterial lipopolysaccharide that enters the portal system. Therefore, this part should be less associated with blood ammonia and more closely related to inflammatory factors.

Reference

1.Butterworth RF.The liver-brain axis in liver failure:neuroinflammation and encephalopathy[J].Nat Rev Gastroenterol Hepatol,2013,10(9):522-528.

2.Luo M, Li L, Yang EN et al.Correlation between interleukin-6 and ammonia in patients with overt hepatic encephalopathy due to cirrhosis[J].Clin Res Hepatol Gastroenterol. 2013;37(4):384-90.

3.Li W, Li N, Wang R et al.Interferon gamma, interleukin-6, and -17a levels were correlated with minimal hepatic encephalopathy in HBV patients[J].Hepatol Int. 2015,9(2):218-23.

4.Chia-Fen Tsai, Chi-Jen Chu Yi-Hsiang Huang et al.Detecting Minimal Hepatic Encephalopathy in an Endemic Country for Hepatitis B: The Role of Psychometrics and Serum IL-6[J] .PLOS ONE .2015;10(6):e0128437.

5.Lata J,Jurankova J,Kopacova M,et al.Probiotics in hepatology[J].World J Gastroenterol,2011,17(24):2890-2896.

6.Dina Kao, Brandi Roach, Heekuk Park et al. Fecal Microbiota Transplantation in the Management of Hepatic Encephalopathy. Hepatology. 2016;63(1):339-40.

7.Seo YS,Shah VH.The role of gut-liver axis in the pathogenesis of liver cirrhosis and portal hypertension [J].Clin Mol Hepatol,2012,18(4):337-346.

8.Norman K,Pirlich1 M,Schulzke JD,et al.Increased intestinal permeability in malnourished patients with liver cirrhosis[J].Eur J Clin Nutr,2012,66(10):1116-1119.

35136 Fecal Microbiota Transplantation Prevents Hepatic Encephalopathy in Rats with Carbon Tetrachloride-induced Acute Hepatic Dysfunction This study was conducted to understand the effect of FMT on HE using an in vivo rat Hepatic Encephalopathy model. This is an interesting manuscript with clear rationale and hypothesis. Appropriated methods and techniques have been employed. The authors have discussed their findings. However, a few concerns were noted 1. Some methodology sections are not explained. 2. Please explain whether CCl4 treatment was continued during ethanol feeding. This aspect is unclear 3. The method for FMT should be described. How was it done? Secondly, better explanation is warranted for VSL treatment? The authors state, it was given by intestinal tube. Is that gavaging? 4. Will this publication be printed in color? If not, there should be some patterns on the columns such that a reader can understand in black and white reprints. 5. It appears that VSL was superior to FMT in almost all outcomes. This aspect is not discussed adequately. Why not use VSL? Why do the authors think that FMT is better? 6. The authors have shown improvement in inflammatory markers and intestinal permeability as the mechanism by which FMT prevents HE. The authors should discuss how alterations in microbiota affects ammonia production. Is it through improving liver's function or it that more ammonia producing bacteria are present in their experimental model of HE and FMT is reducing that? 7. Even though the authors have included a certificate of language, there are many sentences that need to be reworded. An example - first two sentences in "Scientific research process" section. The entire section is very poorly written. 8. Histology pictures should be bigger resolution with arrows marking their findings.

Dear Professor:

Thanks for your comments.I hope my reply will satisfy you.

1.Some of the experimental methods involved in this article are not perfect, but all the experimental steps are supported by previous literature.

2.The step of CCl4 treatmentis based on other documents and has been described in the text. Rats were given subcutaneous injection of 5 mL • kg-1 of CCl4 solution (a mixture of CCl4 and peanut oil at ratio 2:3) twice a week and were fed 5% alcohol in drinking water with a normal complete diet for 9 consecutive weeks.

3.The method of FMT has been described in the article,please note the **Preparation of donor fecal material**.This step is also based on other documents.The method of burying the duodenum tube: After successfully anesthetizing the rat, small incisions of 1 cm were cut in the abdomen. Then, the duodenum was pulled out, and a small incision was made; the PE50 tube was inserted into the duodenum. The PE50 tube was placed in the back of the neck and was placed outside of the skin subcutaneously, and the end of the tube was tightly packed with a homemade stopper. If we wanted to inject the bacteria, we opened the homemade plug and injected the bacteria into the PE50 tube with a 1 ml syringe needle. Some brief descriptions are added in the article.

4.Since the number of groups involved in this paper is so large, the experimental results cannot be expressed more clearly in black and white, so we used the same color to distinguish different groups.

5.Our results suggest that VSL seemed to improve hepatic function, blood ammonia, intestinal permeability, related proteins and the TLR protein of hepatic encephalopathy, but in two important indicators, IL - 6 and cognitive function, a clear advantage was not shown.

To date, there is no "gold standard" for the diagnosis of hepatic encephalopathy. In addition to clinical manifestations, cognitive function is commonly used in the international community to determine whether hepatic encephalopathy is present [1]. Our results suggest that FMT for improving cognitive functions may not only be as good as VSL# 3 (no significant differences between the two of them in the results) but also, in some ways, such as in the cruise speed of rats, even be superior to VSL# 3.

In addition, although our results suggest that VSL can reduce IL-1 beta and TNF-alpha in the tail vein, the effect on IL-6 appears to be reversed. Previous studies have confirmed that IL-6 is an independent risk factor for hepatic encephalopathy, which is significantly negatively correlated with the cognitive function score of HE patients and can be used as a predictor of HE [2-4]. This result is different from what we expected, which was that IL-6 would decrease after it goes through the liver in VSL# 3. Previous scholars have predicted that probiotics alone may not be good[5]. There has been no documented evidence that IL-1 beta and TNF-alpha can be independent risk factors or even predictors of hepatic encephalopathy such as IL-6. Therefore, this unexpected result gives us reason to speculate that FMT may be better than VSL# 3.

6.The main source of blood ammonia is intestinal bacteria, and the relationship between intestinal flora and hepatic encephalopathy can be explained by three components: 1. Intestinal bacteria; 2. Intestinal permeability; and 3. The TLR immune response of the liver.

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In the third aspect, the hepatic TLR immune response is mainly related to the bacterial lipopolysaccharide that enters the portal system. Therefore, this part should be less associated with blood ammonia and more closely related to inflammatory factors.The current mainstream view is that hepatic encephalopathy is the result of the interaction between blood ammonia and the systemic inflammatory response [9]. There are other theories that cannot be confirmed one by one in this article.

7.Thank you for correcting the language, we will revise it and provide the certificate of AJE.

8.We will also improve the histological picture.

Reference

1.American Association for the Study of Liver Diseases, European Association for the Study of the Liver. Hepatic encephalopathy in chronic liver disease:2014 practice guideline by the European Association for the Study of the Liver and the American Association for the Study of Liver Diseases[J].J Hepatol,2014,61(3):642-659.

2.Luo M, Li L, Yang EN et al.Correlation between interleukin-6 and ammonia in patients with overt hepatic encephalopathy due to cirrhosis[J].Clin Res Hepatol Gastroenterol. 2013;37(4):384-90.

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7.Seo YS,Shah VH.The role of gut-liver axis in the pathogenesis of liver cirrhosis and portal hypertension [J].Clin Mol Hepatol,2012,18(4):337-346.

8.Norman K,Pirlich1 M,Schulzke JD,et al.Increased intestinal permeability in malnourished patients with liver cirrhosis[J].Eur J Clin Nutr,2012,66(10):1116-1119.

9.Butterworth RF.The liver-brain axis in liver failure:neuroinflammation and encephalopathy[J].Nat Rev Gastroenterol Hepatol,2013,10(9):522-528.