November 22th, 2022

Andrzej S Tarnawski, DSc, MD, PhD, Professor
Editor-in-Chief,
World Journal of Gastroenterology.

Dear Editors,

Thank you for your letter and for the reviewers' comments concerning our manuscript titled "**Role of gut microbiota in the pathogenesis and therapeutics of minimal hepatic encephalopathy via the gut-liver-brain axis**" (Manuscript ID: 80405). The comments are all valuable and have helped us revise and improve our manuscript, and have also helped us clearly bring out the significance of our study. We have gone through the reviewers' comments carefully and have made appropriate revisions to the manuscript that we hope will meet with your approval. The revised text has been marked in red in the manuscript. Responses to the reviewers' comments and the main revisions to the manuscript are described below:

RESPONSES TO COMMENTS FROM REVIEWER #1:

1. Question:

Dear author, thank you for the submission of your mini-review to our journal. This is an interesting article reviewing the treatment of hepatic encephalopathy from the perspective of the intestinal flora. Since it is a review article, the main points may be difficult to understand, but it is considered to be a useful article for researchers involved in this field. I think this paper can be published as is, except for a few points described below. Minor points: P5, diagnosis methods → diagnostic methods. P7, [Nevertheless, increasing evidence indicates that systemic inflammation impairs integrity and increases the BBB[34].]. This sentence is difficult to understand. P14, line 4 unclear → unclear. Period is

missing.

Response:

Thank you for your valuable comment. The incorrect collocation in Page 5 has been corrected, and the missing punctuation in Page 14 has been added. Meanwhile, the sentence with unclear expression in Page 7 has been revised.

The following revisions have been made to the manuscript:

P5: Because of the lack of uniform criteria for diagnosing MHE, the results of the currently available diagnostic methods for MHE are inconsistent in clinical practice.

P7: Nevertheless, increasing evidence indicates that systemic inflammation impairs the BBB integrity and further increases the BBB permeability[34].

P14: Owing to these differences, uniform criteria for selecting ideal FMT donors are lacking, and the optimal FMT dosing regimen remains unclear.

2. Question:

(1) The title should mention the article type. E.g., review; (2) Remove this structure. "This review provides references for the future research of pathophysiological mechanisms of MHE and the improvement of microbiome therapies for MHE." (3) It is advised to revise the manuscript regarding format. E.g., "there are no specific clinical studies of FMT for MHE treatment". (4) Grammar should be addressed. E.g., punctuation "remains unclear Moreover".

Response:

Thank you for your valuable comment. (1) I think the title of this review comprehensively summarizes the main content of the article, and the article type could appear more appropriately in the journal's catalog; (2) According to your valuable suggestion, the sentence "This review provides references for the future research of pathophysiological mechanisms of MHE and the improvement of microbiome therapies for MHE." has been removed; (3) Based on your valuable comment, the sentence "there are no specific clinical studies of FMT for MHE treatment" has been revised; (4) The missing punctuation has been added.

The following revisions have been made to the manuscript:

(3) The role of FMT in preventing OHE recurrence by modulating gut microbiota dysbiosis has been demonstrated; however, no clinical trial regarding the FMT for MHE treatment has been reported so far.

(4) Owing to these differences, uniform criteria for selecting ideal FMT donors are lacking, and the optimal FMT dosing regimen remains unclear.

3. Question:

The manuscript meets all the criteria in the checklists.

Response:

Thank you for your valuable comment.

4. Question:

It is an interesting well-presented study. Please explain more the potential mechanism involved regarding the action of microbiota, and the potential interaction with the genetic background.

Response:

Thank you for your valuable comment. We have supplemented the relevant mechanisms regarding the small intestinal bacterial overgrowth (SIBO), bacterial translocation, systemic inflammation, and blood–brain barrier (BBB) permeability. Furthermore, we have modified the Figures to illustrate the newly supplemented mechanisms.

The following texts have been added to the manuscript:

Small intestinal bacterial overgrowth (SIBO) is a pathological dysregulation of gut microbiota, characterized by excessive bacteria and/or abnormal bacterial composition in the small intestine. Approximately 48% to 73% of cirrhotic patients have SIBO[21]. Intestinal immune dysfunction, intestinal dysmotility, and decreased bile acid synthesis are implicated in the pathogenesis of SIBO[22]. SIBO is closely associated with the severity of advanced liver cirrhosis, and it has been validated as a significant risk factor for MHE[23,24]. In MHE patients, the gut microbiota dysbiosis resulting from the SIBO has been characterized by lower bacterial diversity, decreased autochthonous beneficial bacteria, and

increased pathogenic Gram-negative bacteria[8,14,25].

Bacterial translocation

In healthy individuals, the characteristic structure and immune system of the intestinal mucosa can prevent bacteria and their byproducts from entering the systemic circulation. In patients with liver cirrhosis, the SIBO decreases the synthesis of secondary bile acids by inhibiting the activation of Farnesoid X receptor and Takeda G protein-coupled receptor, which reduces intestinal immunoglobulin A levels and further compromises the immune function of the intestinal mucosa[33,34]. Moreover, the SIBO induces decreased synthesis of antimicrobial peptide and activates mucosal immune responses, resulting in intestinal inflammation and impaired intestinal epithelium integrity[35]. Furthermore, the SIBO increases the permeability of epithelial intercellular junctions via the down-regulation of tight junction protein expressions[36]. These potential mechanisms induce a "leaky gut" which facilitates the transfer of pathogenic bacteria and their metabolites from the intestinal tract to the circulatory system, resulting in systemic inflammation (Figure 1).

Systemic inflammation

Translocated bacteria and their products, such as pathogen-associated molecular patterns, are transported to the liver through the portal vein. At the molecular level, pathogen-associated molecular patterns are recognized by Toll-like receptors and cytoplasmic nucleotide-binding oligomerization domain-like receptors, and primarily stimulate hepatic Kupffer cells through the activation of MyD88-dependent and NF-κB signaling pathways[37,38]. Innate immune responses in the liver are triggered, resulting in liver damage, with the release of damage-associated molecular patterns and the production of proinflammatory cytokines and chemokines(Figure 1)[37,38]. MHE patients present the systemic proinflammatory environment reflected by increased circulatory levels of proinflammatory cytokines such as tumor necrosis factor-

alpha (TNF-α), interleukins (ILs), and interferon, and chemokines such as CCL20, CXCL13 and CX3CL1[39].

Furthermore, the association between MHE severity and increased proinflammatory cytokines has been demonstrated to be independent of the severity of liver cirrhosis and ammonia levels, suggesting that systemic inflammation with its proinflammatory cytokines is potentially implicated in the development of MHE[40].

Blood–brain barrier permeability

The blood–brain barrier is composed of capillary endothelial cells surrounded by capillary basement membrane and astrocytic perivascular endfeet. The BBB separates the systemic circulation and brain, prevents the entry of potentially harmful substances into the brain, and maintains the homeostasis of the brain microenvironment. Circulating proinflammatory cytokines cannot directly cross the BBB and exert their effects on the brain. However, these cytokines, including TNF-α, ILs, and interferon, down-regulate the expression of endothelial tight junction proteins, compromise cerebrovascular endothelial cells, activate astrocytes to an inflammatory reactive state, and alter BBB receptor expression and transport pathways, which consequently impair BBB integrity and further increase BBB permeability(Figure 2)[41,42]. Through the aforementioned mechanisms, the proinflammatory signaling, which is initiated by systemic inflammation, crosses the damaged BBB and underlies the neuroinflammatory response that develops in the cerebrum.

The following figures have been modified:

Figure 1

On the background of liver cirrhosis with hepatic dysfunction, dysbiotic gut microbiota and its byproducts including ammonia and endotoxin cross the impaired intestinal barrier, stimulate innate immune responses in the liver, and

lead to systemic inflammation, hyperammonemia, and endotoxemia. TNF-α: tumor necrosis factor-alpha; ILs: interleukins; IFN: interferon.

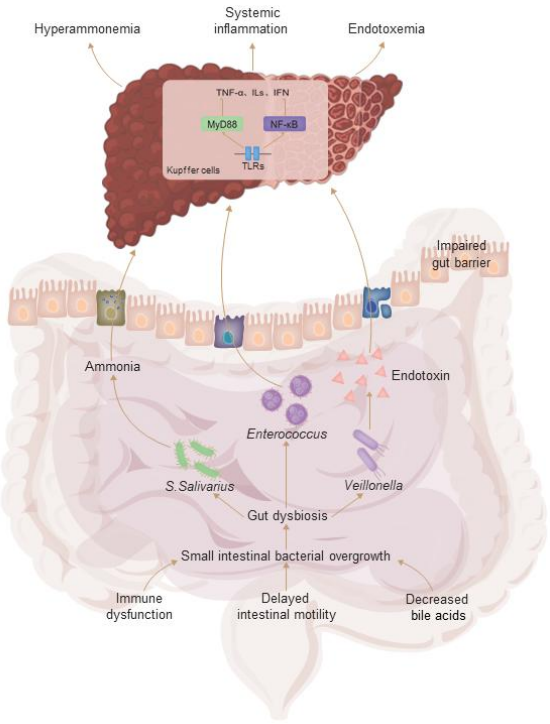
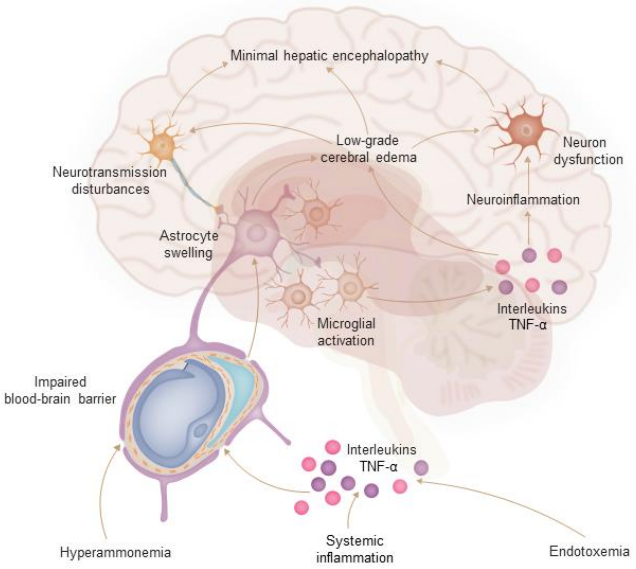


Figure 2

Systemic inflammation, hyperammonemia, and endotoxemia influence the permeability of the blood-brain barrier, resulting in neuroinflammation and low-grade cerebral edema, contributing to the pathogenesis of minimal hepatic encephalopathy.



We have made several modifications to the original manuscript and improved it based on the comments received. We greatly appreciate your help and that of the reviewers regarding improvements to our manuscript. The revised manuscript has been edited by professional editors from Editage®, a professional English editing service. A new language certificate was provided with the revised manuscript. We hope that the revised manuscript is now acceptable for publication in your journal.

With kind regards

Sincerely,

Ming Luo
Attending Physician, Master of Clinical Medicine
Department of Gastroenterology, People's Hospital of Ningxia Hui Autonomous Region, Yinchuan, Ningxia Hui Autonomous Region, PR China.
E-mail address: romean@sina.com

Fei-Hu Bai
Chief Physician, Professor, Doctor of Medicine
Department of Gastroenterology, People's Hospital of Ningxia Hui Autonomous Region, Yinchuan, Ningxia Hui Autonomous Region, PR China.
E-mail address: baifeihu@hotmail.com