Response to reviewers

We would like to thank our reviewers for going through the manuscript in such detail. Their questions and comments helped us revise our manuscript comprehensively. As detailed below, we addressed all the reviewers' comments.

Reviewer 1:

Comment 1: Specific Comments to Authors: I would like to thank the handling editor for offering me the opportunity to review the manuscript entitled " 5-HT2B receptor induced visceral hyperalgesia via TRPV1 in diarrhea-predominant irritable bowel syndrome"I would also like to commend the authors for their scholarly work, which presents Overall, the manuscript under review appears scientifically and technically valid. The study design follows standard methodology for a randomized controlled trial and has expermintal part not mentioned in the title otherwise all chapters of the study are written well.

Response 1:

Thank you very much for your good advice ! According to your comment, we have revised the title as following: *Serotonin receptor 2B induces visceral hyperalgesia in rat model and patients with diarrhea-predominant irritable bowel syndrome.*

Comment 2:

As the revision process results in changes to the content of the manuscript, language problems may exist in the revised manuscript. Thus, it is necessary to perform further language polishing that will ensure all grammatical, syntactical, formatting and other related errors be resolved, so that the revised manuscript will meet the publication requirement (Grade A).

Authors are requested to send their revised manuscript to a professional English language editing company or a native English-speaking expert to polish the manuscript further. When the authors submit the subsequent polished manuscript to us, they must provide a new language certificate along with the manuscript.

Response 2:

Thank you very much for your good advice ! According to your comment, we have sent our revised manuscript to a professional English language editing company and revised our manuscript following the instructions. A new language certificate along with the manuscript was provided.

Comment 3:

In general, do not use non-standard abbreviations, unless they appear at least two times in the text preceding the first usage/definition. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, and mAb, do not need to be defined and can be used directly.

The basic rules on abbreviations are provided here:

(1) Title: Abbreviations are not permitted. Please spell out any abbreviation in the title.

(2) Running title: Abbreviations are permitted. Also, please shorten the running title to no more than 6 words.

(3) Abstract: Abbreviations must be defined upon first appearance in the Abstract. Example 1: Hepatocellular carcinoma (HCC). Example 2: *Helicobacter pylori* (*H. pylori*).

(4) Key Words: Abbreviations must be defined upon first appearance in the Key Words.

(5) Core Tip: Abbreviations must be defined upon first appearance in the Core Tip. Example 1: Hepatocellular carcinoma (HCC). Example 2: *Helicobacter pylori* (*H. pylori*)

(6) Main Text: Abbreviations must be defined upon first appearance in the Main Text. Example 1: Hepatocellular carcinoma (HCC). Example 2: *Helicobacter pylori* (*H. pylori*)

(7) **Article Highlights:** Abbreviations must be defined upon first appearance in the Article Highlights. Example 1: Hepatocellular carcinoma (HCC).

Example 2: *Helicobacter pylori* (*H. pylori*)

(8) Figures: Abbreviations are not allowed in the Figure title. For the Figure Legend text, abbreviations are allowed but must be defined upon first appearance in the text. Example 1: A: Hepatocellular carcinoma (HCC) biopsy sample; B: HCC-adjacent tissue sample. For any abbreviation that appears in the Figure itself but is not included in the Figure Legend textual description, it will be defined (separated by semicolons) at the end of the figure legend. Example 2: BMI: Body mass index; US: Ultrasound.

(9) Tables: Abbreviations are not allowed in the Table title. For the Table itself, please verify all abbreviations used in tables are defined (separated by semicolons) directly underneath the table. Example 1: BMI: Body mass index; US: Ultrasound.

Response 3:

Thank you very much. According to your comment, we have spelled out all abbreviations in the title, in the abstract, in the key words, in the core tip, in

the main text, in the article highlights and in the figures. And we have highlighted the revised abbreviations such as *serotonin receptor 2B (5-HT$_{2B}$ receptor), diarrhea-predominant irritable bowel syndrome (IBS-D), transient receptor potential vanilloid type 1 (TRPV1)* with yellow color in the revised manuscript. It's our carelessness for the missing of core tip and article highlights. Now we have added the core tip and article highlights in the revised manuscript as following:

Core tip: Higher expression of the serotonin receptor 2B (5-HT$_{2B}$ receptor) was found in patients with diarrhea-predominant irritable bowel syndrome (IBS-D) than in that of controls, which was correlated with abdominal pain scores. Exogenous 5-HT$_{2B}$ receptor agonist administration increased visceral hypersensitivity, which was alleviated by successive administration of a transient receptor potential vanilloid type 1 (TRPV1) antagonist. IBS-D rats receiving the 5-HT$_{2B}$ receptor antagonist exhibited inhibited visceral hyperalgesia. 5-HT$_{2B}$ receptor-induced visceral hyperalgesia may be mediated by TRPV1 channels. The analgesic effect of 5-HT$_{2B}$ receptor antagonist in IBS-D rats could be used as a novel treatment for IBS-D.

ARTICLE HIGHLIGHTS

Research background

Patients with diarrhea-predominant irritable bowel syndrome (IBS-D) experience a significant reduction in their quality of life. While the exact pathogenesis of IBS-D remains incompletely understood, research indicates that serotonin receptor 2B (5-HT$_{2B}$ receptor) plays a critical role in many chronic pain conditions. The role of 5-HT$_{2B}$ receptor in the altered gut sensation of diarrhea-predominant irritable bowel syndrome (IBS-D) was not investigated.

Research motivation

This study is to identify the role of 5-HT$_{2B}$ receptor in the altered gut sensation via

transient receptor potential vanilloid type 1 (TRPV1) channels in IBS-D.

Research objectives

This study aims to elucidate the role of the 5-HT_{2B} receptor in both IBS-D patients and rat models induced by acetic acid and wrap restraint. The findings are anticipated to offer novel insights into potential avenues for IBS-D treatment.

Methods.

Rectosigmoid biopsies were collected from IBS-D patients and healthy controls. The expression level of 5-HT_{2B} receptor in colon tissue was measured and correlated with abdominal pain scores in IBS-D patients. The IBS-D rat model was induced by intracolonic instillation of acetic acid and wrap restraint. Alterations in visceral sensitivity, 5-HT_{2B} receptor and TRPV1 expression were examined following 5-HT_{2B} receptor antagonist administration. Changes in visceral sensitivity after the administration of the TRPV1 antagonist were recorded.

Results:

A higher expression of 5-HT_{2B} receptor was observed in the colonic mucosa of patients with IBS-D compared to controls, correlating with abdominal pain scores. The IBS-D rats was successfully established through intracolonic instillation of acetic acid and wrap restraint. Administration of the exogenous 5-HT_{2B} receptor agonist increased visceral hypersensitivity, which was subsequently alleviated by successive administration of TRPV1 antagonist. IBS-D rats receiving the 5-HT_{2B} receptor antagonist displayed inhibition of visceral hyperalgesia. Additionally, the percentage of 5-HT_{2B} receptor-immunoreactive (IR) cells surrounded by TRPV1-positive cells (5-HT_{2B} receptor I^{+}) and total 5-HT_{2B} receptor IR cells (5-HT_{2B} receptor I_T) in IBS-D rats significantly decreased with the administration of 5-HT_{2B} receptor antagonist.

Conclusions:

The increased expression of 5-HT_{2B} receptor contributing to visceral hyperalgesia through the induction of TRPV1 expression in IBS-D provides important insights into the potential mechanisms underlying IBS-D-associated visceral hyperalgesia.

Research perspectives

The analgesic effect of RS-127445 in IBS-D rats suggests its potential as a novel

treatment for IBS-D.

Comment 4:

Authors must revise the manuscript according to the Editorial Office's comments and suggestions, which are listed below:

(1) Science editor:

1 Scientific classification: Grade B. 2 Language classification: Grade B. 3 Specific comments: (1) Please provide the Biostatistics statement. (2) Please provide the Institutional review board statement. (3) Please provide the Informed consent statement. (4) Please provide the Language certificate. The English-language grammatical presentation needs to be improved to a certain extent. There are many errors in grammar and format, throughout the entire manuscript. Before final acceptance, the authors must provide the English Language Certificate issued by a professional English language editing company. Please visit the following website for the professional English language editing companies we recommend: https://www.wjgnet.com/bpg/gerinfo/240. (5) Please provide the Figures cited in the original manuscript in the form of PPT. All text can be edited, including A,B, arrows, etc. With respect to the reference to the Figure, please verify if it is an original image created for the manuscript, if not, please provide the source of the picture and the proof that the Figure has been authorized by the previous publisher or copyright owner to allow it to be redistributed. All legends are incorrectly formatted and require a general title and explanation for each figure. Such as Figure 1 title. A: ; B: ; C: . (6) Please obtain permission for the use of picture(s). If an author of a submission is re-using a figure or figures published elsewhere, or that is copyrighted, the author must provide documentation that the previous publisher or copyright holder has given permission for the figure to be re-published, and correctly

indicate the reference source and copyrights. For example, “Figure 1 Histopathological examination by hematoxylin-eosin staining (200 ×). A: Control group; B: Model group; C: Pioglitazone hydrochloride group; D: Chinese herbal medicine group. Citation: Yang JM, Sun Y, Wang M, Zhang XL, Zhang SJ, Gao YS, Chen L, Wu MY, Zhou L, Zhou YM, Wang Y, Zheng FJ, Li YH. Regulatory effect of a Chinese herbal medicine formula on non-alcoholic fatty liver disease. World J Gastroenterol 2019; 25(34): 5105-5119. Copyright ©The Author(s) 2019. Published by Baishideng Publishing Group Inc[6]”. And please cite the reference source in the references list. If the author fails to properly cite the published or copyrighted picture(s) or table(s) as described above, he/she will be subject to withdrawal of the article from BPG publications and may even be held liable. (7) Please don’t include any *, #, †, §, ‡, ¥, @….in your manuscript; Please use superscript numbers for illustration; and for statistical significance, please use superscript letters. Statistical significance is expressed as aP <0.05, bP <0.01 (P > 0.05 usually does not need to be denoted). If there are other series of P values, cP <0.05 and dP <0.01 are used, and a third series of P values is expressed as eP <0.05 and fP <0.01. (8) Please add the Core tip section. The number of words should be controlled between 50-100 words. (9) The “Article Highlights” section is missing. Please add the “Article Highlights” section at the end of the main text (and directly before the References). 4 Recommendation: Conditional acceptance.
Language Quality: Grade B (Minor language polishing)
Scientific Quality: Grade B (Very good)

Response 3:

Thank you very much for your help. According to your advice, the Biostatistics statement, the institutional review board statement, and the informed

consent statement have been provided. The Informed consent statement has been added in the manuscrit as following:

The use of human tissue samples and clinical data was approved by the ethics committee of Dalian Friendship Hospital. All donors were informed of the aim of the study and gave consent to donate their samples. **And we have highlighted the revised contents with yellow color in materials and methods.**

And the language certificate, the figures cited in the original manuscript in the form of PPT have been provided. The Figures cited in the manuscript has not been published elsewhere. Following your instructions, the symbol of *, #, †, §, ‡, ¥, have been deleted and changed to $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ in all the figures.

We are sorry for our carelessness. Now we have added the core tip and article highlights in the revised manuscript and highlighted it with yellow color.

Thank you very much!