

Dear editor,

We deeply appreciate all the approbations and the comments made by the editors and reviewers. We promise we revised the manuscript very carefully according to the comments point by point as following:

This manuscript had been resent to the FirstEditing which is also a professional English editing company for language editing. The manuscript has been thoroughly corrected all of the grammar errors by the native English speaker. The certificate of language editing has been provided.

1. The certificate of funding agency for every grant has been provided.
2. The Audio core tip has been supplemented.
3. The highlights of this article have been finished following the reminders.
4. The references have been checked and there are no repeated references.
5. Figures have been re-checked and revised. The missing labeling has been added and we provided the editable figures as PPT format.

We Thanks again for the attention and the comments from the editors and the reviewers. Would you please contact with me as soon as possible once there are any problems about the manuscript.

We are looking forward to your kind reply.

Sincerely yours,

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Answering reviewers

Name of Journal: *World Journal of Gastroenterology*

ESPS manuscript NO: 38018

Title: Sodium chloride exacerbates DSS-induced colitis by tuning pro-inflammatory and anti-inflammatory LPMCs through p38/MAPK pathway in mice

Author: Hong-Xia Guo, Nan Ye, Ping Yan, Min-Yue Qiu, Ji Zhang, Zi-Gang Shen, Hai-Yang He, Zhi-Qiang Tian, Hong-Li Li, Jin-Tao Li

The manuscript has been improved according to the suggestions of reviewers.

We have carefully addressed the criticisms point -by-point, and our response is indicated below in blue.

Reviewer 1(00002314):

This manuscript addresses an interesting topic and reports a large amount of data. However, these aspects need consideration:

(1) The authors use only one dose of NaCl and it is unclear whether the effect is a non-specific osmotic effect? what is the effect of other salt solutions with the same osmolarity?

Answer: Thanks for your kind question. The dose of NaCl used in our study was chosen according to the references^[1-4]. These references have demonstrated the 2% NaCl in drinking water is suitable for high salt diet effect to some inflammation diseases, including EAE, cardiovascular, hypertension, and IBD. In our previous test, it is true that half of the salt concentration (1%) showed little effect on colitis, while higher concentration salt (4%) will seriously affect mice drinking water which we think is too salty and mice refuse to drink. The aim of this manuscript is to explore the high salt diet

effect to colitis, thus we think the concentration of 2% NaCl in drinking water in DSS induced mice colitis in our study is propriety.

(2) Why was only one concentration tested in vivo and how does it relate to the different concentrations used in vitro?

Answer: Thanks again for your questions. As we answered in the above question, we choose the NaCl concentrations both in vivo and in vitro are according to related references (1-4). In another hand, we think animal experiments and cell experiments were performed relatively independently for proving different aims. The experiment in vivo is to verify high salt's effect to colitis, while the experiment in vitro is to explore molecule mechanisms which should make sure which dose is the most effective.

(3) I found it difficult to read the manuscript because of the English style/grammar, which should be revised: the introduction (especially in the final paragraphs) can easily be shortened; the results section sometimes reports sentences that more easily fit in other parts of the manuscript (e.g. discussion).

Answer: We appreciate the reviewer for kindly comments about the manuscript. We have resent the manuscript to the professional company for language editing and revised the introduction, results section and discussion content according to the suggestions.

(4) The authors may wish to guide the reader more precisely on what novel findings this manuscript adds to the existing literature and on the significance of this in vivo model of IBD (true translational value).

Answer: Thanks again for your kind comments. We have highlighted the novel findings in vivo IBD model in our revised manuscript.

(5) MINOR POINTs - FIGURES: these should be checked because scale bars are missing in several microscopic images (at least in the version available online); - figure 1, panel C: the y-axis has no units; - in several cases symbols/greek letters are missing.

Answer: Thank you for the comments about figures and we have checked the figures. Corresponding modifications have been made according to the opinions. Such as scale

bars, figure 1, panel C: the y-axis units were added, in several cases symbols/greek letters missing were added in the revised manuscript.

Reviewer 2(00503587)

This in vitro study evaluated the effects of NaCl in a murine model of colitis
SPECIFIC
COMMENTS

(1) There are numerous errors of English language usage and grammar: these should all be corrected

Answer: We appreciate your kind comments. We have resent the manuscript to the professional company for language editing and revised the content according to the suggestions.

(2)The INTRODUCTION is too long and should be revised and shortened.

Answer: Thanks for your kind comment. The introduction has been revised and shortened according to your suggestion.

(3)The experimental design included one dose of NaCl only: it would've been helpful to include a dose range. Furthermore, there were inadequate control groups included in this work

Answer: Thanks for your kind question. The dose of NaCl used in our study was chosen according to the references^[1-4]. These references have demonstrated the 2% NaCl in drinking water is suitable for high salt diet effect to some inflammation diseases, including EAE, cardiovascular, hypertension and IBD. In our previous test, it is true that half of the salt concentration (1%) showed little effect on colitis, while higher concentration salt (4%) will seriously affect mice drinking water which we think is too salty and mice refuse to drink. The aim of this manuscript is to explore the high salt diet effect to colitis, thus we think the 2% concentration of salt in drinking water in DSS induced mice colitis in our manuscript is propriety.

(4)All purchased products should be listed with the company and the country of origin.

Insufficient details provided currently

Answer: We would like to thank you for your suggestions. All purchased products have been added with the company and the country of origin in revision manuscript.

(5)The DISCUSSION could be revised (to improve readability) and slightly shortened also

Answer: Thanks for your kind comments. We have revised the manuscript according to your suggestion and the discussion has been slightly shortened.

Reviewer 3(01557050)

Dr. Guo and Dr. Li, et al reported 'Sodium chloride exacerbates DSS-induced colitis by tuning pro-inflammatory and anti-inflammatory LPMCs through p38/MAPK pathway in mice. The article is well-presented. The reviewer has some comments. Comments

(1) In this study, the authors selected 2% NaCl treatment. Please explain how the authors selected this dose of NaCl.

Answer: Thanks for your kind question. The dose of NaCl used in our study was chosen according to the references^[1-4]. These references have demonstrated the 2% NaCl in drinking water is suitable for high salt diet effect to some inflammation diseases, including EAE, cardiovascular, hypertension and IBD. In our previous test, it is true that half of the salt concentration (1%) showed little effect on colitis, while higher concentration salt (4%) will seriously affect mice drinking water which we think is too salty and mice refuse to drink. The aim of this manuscript is to explore the high salt diet effect to colitis, thus we think the concentration of 2% NaCl in drinking water in DSS induced mice colitis in our manuscript is propriety.

(2) In Figure 7, interestingly, IL-1, IL-6 and iNOS increased low doses in NaCl, whereas decreased high doses in NaCl. Then, IL-10 and Arg1 increased dose dependently. Please explain the reason or your speculations of these results in Discussion more in details.

Answer: Thanks again for your kind question. We think this is due to the concentration is too high to cause the cell inhibition reaction through feedback. Under low

concentration NaCl, IL-1, IL-6 and iNOS were up-regulated, while the negative adjustment factor expressions were low. When the salt concentration rised to a certain dose, high levels of pro-inflammatory factors IL - 1, IL - 6 and iNOS induced the cell protective response through feedback, and caused the up-regulation of negative adjustment factor IL-10 and Arg1, and inhibit the expression of pro-inflammatory factor.

(3)In page 8 and 11, please confirm □.

Answer: Thanks for your kind remind. We have confirmed the page 8 and 11.

Reviewer 4(02941507)

Reference 37 suggests that..."A third common colitis model, administration of dextran sodium sulfate, was hopelessly confounded by the high sodium content of the dextran sodium sulfate". The authors should discuss this serious statement.

(1) The authors should explain why they choose to use the DSS instead of the TNBS model of colitis.

Answer: Thank you for the question. Both DSS and TNBS are well-established animal models of mucosal inflammation that have been used for over two decades in the study of IBD pathogenesis and preclinical studies^[5]. Therefore, it is feasible to establish an animal model of colitis with DSS. DSS and TNBS cause different pathogenic mechanisms. TNBS - induced colitis are "CD-like" and DSS-induced colitis are "UC-like"^[6, 7]. They have distinctive features both histologically and clinically^[8]. Our lab has shown more lights on DSS induced colitis studies in previous days.

(2)The authors should explain why they choose the concentration of 2% NaCl in their experimental model.

Answer: Thanks for your kind question. The dose of NaCl used in our study was chosen according to the references^[1-4]. These references have demonstrated the 2% NaCl in drinking water is suitable for high salt diet effect to some inflammation diseases, including EAE, cardiovascular, hypertension, and IBD. In our previous test, it is true that half of the salt concentration (1%) showed little effect on colitis, while higher concentration salt (4%) will seriously affect mice drinking water which we think is too

salty and mice refuse to drink. The aim of this manuscript is to explore the high salt diet effect to colitis, thus we think the concentration of 2% NaCl in drinking water in DSS induced mice colitis in our manuscript is propriety.

(3)The authors stated that “NaCl has been shown to exert pro-inflammatory effect in many diseases including EAE, cardiovascular, lung related diseases, and IBD[19, 36, 37]. However, with the exception of references numbered 19 and 37 both referring to the influence of salt on IBD (experimentally), reference 36 is unrelated with the above mentioned assumption.

Answer: We do appreciate your kind comments and feel so sorry made a mistake of the references. We have rechecked and corrected the error in the revised manuscript.

(4)Reaching the conclusion that “These findings suggest that the control on the intake of NaCl is very important for treating IBD” the authors should bear in mind the results of the study of Khalili H, et al (Front Immunol. 2016 Dec 7;7:554), claiming that “Among a total of 194,711 women over a follow-up of 3,220,247 person-years with documented 273 cases of CD and 335 cases of UC... we found that dietary intake of potassium (Ptrend = 0.005) but not sodium (Ptrend = 0.44) was inversely associated with risk of CD. Although, both dietary potassium and sodium were not significantly associated with risk of UC, there was a suggestion of an inverse association with dietary potassium (Ptrend = 0.08)”. The authors should emphasize that clinical and experimental studies are required in order to fully clarify the role of salt in IBD.

Answer: We would like to thank the reviewer for kind reminding. The clinical studies are different from the animal model. We have added “Although results obtained in the present study indicate that excessive NaCl intake can promote the inflammation in mice with the DSS-induced colitis, the causality of high salt diet and IBD still needs to be confirmed by further investigations. More clinical and experimental studies are required to fully clarify the role of salt in IBD.” in the discussion of the revised manuscript.

References

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