Responds to reviewers

Dear editor,

Thank you very much for forwarding the referee's reports on our manuscript, and we also appreciate your advice and encouragement. Following your suggestions, we have revised our manuscript according to the referee's comments point-by-point.

Reviewer #1 comments:

Question 1. ABSTRACT: It would be interesting to add in the results the percentage of increase and decrease of the analyzed parameters. In the same way, you could improve the aim of the work, so that the conclusion answers it.

Answer: Thanks for your constructive suggestion, and we revised the abstract according to the comment in red font.

Question 2. INTRODUCTION: Page 6, 3rd paragraph: "…autophagy is associated with intestinal mucosal injury in the mucosa of colitis mice…". I suggest rewriting the sentence; - You ended the introduction with a concluding sentence. Finish the introduction with the objective of the work.

Answer: We have revised this sentence, and finish the introduction with the objective of the work in red font.

Question 3. MATERIAL AND METHODS: -The authors studied only males. Why didn't they include the females? - According to AIN-93, with growing animals (up to 3 months of age), pregnancy and lactation, the recommendations for proteins, lipids and carbohydrates are 19%, 17% and 64% (diet energy), respectively. It is necessary to detail the percentages of the macronutrients. I suggest that the authors detail the composition of all diets (macro and micronutrients) in a table. Maybe, the deficiency of micronutrients in the standard chow can influence the progression of the disease.

Answer: We used male animals to avoid the interference of female estrus cycle. Theoretically, it is more reasonable to be half male and half female, and we should make further improvement in the selection of experimental animals in the future. In

our present study, all mice were fed with a normal diet, and the proteins are ⩾ 20%, lipids are ⩾ 6%, Lysine was ⩾ 1.32%, respectively, which were added in the text in the method section. Regular products NIH standard diets for rat and mouse were 18% protein and 5% fat, so we considered that micronutrients have little effect on the experimental results of our present study. However, the deficiency of micronutrients in the standard chow can influence the progression of the disease. It is necessary to research the relationship between micronutrient deficiency and chronic colitis, which we will perform this in the future. Thanks.

Question 4. Why were the control groups not treated with resveratrol? Could resveratrol replace a drug? Or is it an adjunct to treatment? T*herefore, the importance of the treated control groups; -Did the authors control the animals' water intake? If not, there is no way to guarantee that the administered amounts of resveratrol and DSS were given in full, since there may be excess liquid in the bottles. This is a big bias in your work. Why didn't you choose another method of administration: gavage, mixing in the diet?*

Answer: The control groups with none treated was used to detect the effect of DSS induced colitis. 5-ASA was used as the positive drug to detect the effect of resveratrol. DSS was administered by drinking water, which is an economical and convenient way to establish DSS-induce colitis animal model, and resveratrol administration was performed by gavage. We explained this in details in the text in the method section (figure 1C).

Question 5. Was resveratrol administered in two cycles? It is not clear in the text. Why 28 days? Why 100 mg/kg/d? I have not found any references to justify these doses. Similarly, add references that justify the use of 200 mg / kg / d of 5-ASA.

Answer: Resveratrol was administered in two cycles, as a good suggestion, we explained this more clearly in the text in the method section. The dosage of resveratrol in mice was referred to previous literatures (Capogrosso et al., Pharmacol Res. 2016;106:101-113; Alexandre et al., Eur J Pharmacol. 2016;788:29-36). According to the comprehensive evaluation, the dosage of 100mg/kg/D was selected. Of course, the more appropriate dosage needs to be further studied for determination.

The dosage of 5-ASA was first calculated according to the formula of calculating the body surface area between human (clinical application) and mice (ref. Zhang et al., World J Gastroenterol. 2014;20(41):15310-8.; Li et al., PLoS One. 2015;10(12):e0144101.). then we performed experiments to determine the final dosage.

Question 6. Detail the form of animal sacrifice. How were they killed?

Answer: Mice were euthanized with carbon dioxide at humane end points, and the information was added in the text.

Question 7. Was the animals' body mass evaluated? This information is not included in the methodology.

Answer: The animals' body mass was evaluated, and the information was added in the methodology.

Question 8. Add the codes of all kits used in the study, including antibodies.

Answer: We added the codes of all kits used in the study, thanks.

Question 9. Tables must be aligned on the same page.

Answer: We revised the table in the text.

Question 10. The authors immunostaining for occlusion proteins, but did not even mention their relationship with IBD in the introduction.

Answer: We added the relationship of occlusion and ZO-1 with IBD in the introduction section.

Question 11. Page 13: Replace osmic acid with osmium teroxide.

Answer: We have replaced osmic acid with osmium teroxid in the text, thanks.

Question 12. I didn't understand why you used the t-test in statistical analysis. You present more than two study groups.

Answer: Thanks for your comment, and we used one way ANOVA analysis and we are sorry for the mistake. We revised this in the method.

Question 13. replace body weight to body mass; In WB analysis, you did not mention that beta-actin was used as a negative control.

Answer: We used body mass instead of body weight in the text, and we added the information of β-actin in method section.

Question 14. RESULTS - Add the "n" in each figure caption for each analysis; - Figure 2: add the unit of measurements for each protein; - Immunohistochemical analysis could be quantified. This would enrich the work.

Answer: We added the "n" in each figure caption, and the unit of measurements for each protein was added in figure 2. Immunohistochemical staining was analyzed by semi-quantitative method. Thanks for your comment.

Question 15. In figure 7, it would be interesting to show the changes described in the legend of each electromicrograph;

Answer: We showed the changes and markers described in the legend of each electromicrograph in figure 7.

Question 16. DISCUSSION - Add the limitations of the study.

Answer: we added the limitations of the study in the discussion section.

Question 17. The dosage of resveratrol used in the study would be equivalent to which dosage in humans? It would be interesting to discuss this point.

Answer: The dosage of resveratrol in mice was referred to previous literatures (Capogrosso et al., Pharmacol Res. 2016;106:101-113; Alexandre et al., Eur J Pharmacol. 2016;788:29-36). According to the comprehensive evaluation, the dosage of 100mg/kg/D was selected. Of course, the more appropriate dosage needs to be further studied for determination.

Question 18. Review the English language.

Answer: We have polished the English language by professionals.

Reviewer #2 comments:

Question 1. The authors used resveratrol at a dose of 100 mg/kg. How about the dose of resveratrol compared with other studies? Also, the dose of resveratrol appears to be too high for human trial. The authors should discuss the issue.

Answer: The dosage of resveratrol in mice was referred to previous literatures (Capogrosso et al., Pharmacol Res. 2016;106:101-113; Alexandre et al., Eur J Pharmacol. 2016;788:29-36). According to the comprehensive evaluation, the dosage of 100mg/kg/D was selected. Of course, the more appropriate dosage needs to be further studied for determination.

Question 2. There are many reports regarding the protective effect of resveratrol on DSS-induced colitis. What is new in the study? The authors should clearly discuss the issue.

Answer: Many studies reported the protective effect of resveratrol on DSS-induced colitis, but there are few reports about the detail information of the molecular mechanism. In our present study, we showed that autophagy may involve in resveratrol treatment of DSS-induced colitis. Further study on autophagy will help us to understand the role of resveratrol in colitis and provide a theoretical basis for clinical application. We added this information in the discussion.

Question 3. *The authors discussed the discrepancy that resveratrol could reduce or enhance autophagy in DSS-induced colitis and that acute colitis or chronic colitis may be an important point. However, the other possibility, that active phase or healing phase (normal drinking water for 7 days after DSS induction for 7 days in the study) may be important, appears to raise. The authors should discuss the issue.*

Answer: DSS-induced colitis is the most widely used mouse model of colitis due to its rapidity, simplicity, reproducibility and controllability. Acute, chronic and relapsing models of intestinal inflammation can be achieved by modifying the concentration of DSS and the frequency of administration (Chassaing et al., Curr Protoc Immunol 2014; 104: Unit 15.25). Our study utilized DSS-induced colitis refers to these references (Nishiyama et al., Mediators Inflamm. 2012;2012:239617; Liu et al., Int Immunopharmacol. 2013;17:314-320; Köhnke et al., Biomed Res Int. 2013; 2013:748160). Our results of HE staining also revealed that the model was consistent with the pathologic assessment of chronic colitis. Thanks very much.

Question 4. *The experimental design in the study showed oral intake of both DSS induction and resveratrol treatment. How about the oral intake of DSS volume between DSS groups and DSS+RES group? Also, the authors should show how to take DSS and resveratrol, mixture or separate?*

Answer: Thanks for the good suggestion, the method in our text was unclear and we revised the administration of DSS or RES in the text (figure 1C). In the DSS model

group, chronic colitis was induced by two cycles of giving drinking water containing 3% DSS for 7 days and normal drinking water for 7 days. Resveratrol treatment group (DSS+RES) mice was also performed by two cycles, first received DSS by drinking water for 7 days, and then treatment of resveratrol 100 mg/kg/d by gavage for 7days, which is considered as a cycle.

Question 5. The authors mentioned the details of DAI score and histological score. Are these same or different compared with previous reports?

Answer: DAI was consistent with the previous report that resveratrol could decrease the DAI score compared with DSS group, which showed that resveratrol may have a better effect on colitis. The histological score showed resveratrol may alleviate intestinal mucosal barrier dysfunction in DSS-induced colitis mice more effective than 5-ASA. The results revealed that the treatment effect of resveratrol on colitis is significant, which may be used as clinical alternatives. This information was added in the text, thanks.

Question 6. *Figure 6 and 7 showed that resveratrol could enhance autophagy in DSS-induced colitis. However, these results did not elucidate whether resveratrol may reduce inflammation by the enhancement of autophagy. The study using autophagy inhibitors may be needed. The authors should discuss the issue.*

Answer: As a good suggestion, it is perfect to use autophagy inhibitors to explain this issue, but we did not perform it in the present study due to time and fund limitation. In the next study, we will do further study to explore the role of autophagy in colitis treatment of resveratrol.

Question 7. *There are some mistakes or concerns in the manuscript. Abstract: dextran sulphate (? sulfate) sodium Title: DSS-induced ulcerative colitis, ulcerative is needed? Figure 3: no units of protein levels Figure 4 and 5 have scale bars. Bar size may be different among 5x, 20x, and 40x? Reference No. 31 is not shown in the manuscript. [32] may be changed to [31] in the manuscript. Reference No. 33 is not shown in the references. [33] may be changed to [32] in the manuscript.*

Answer: We revised the title and dextran sulfate sodium in the abstract and text. We added units in figure 3 and scale bars in figure 4 and 5, and the references were also

rearranged, thanks.

Reviewer #3 comments:

Question 1. The treatment of colitic animals with resveratrol needs to be described in more detail in the Method section. It is not clear how they treated animals. Were the colitic animals dosed by i.v. or i.p.?

Answer: Thanks for this comment, and we explained the administration of DSS or RES in the text as figure 1C.

Question 2. *Figure 2: the figure legend of lacks the description as how the body weight (%) was calculated.*

Answer: We added the formula of body weight calculation in the method section in red font.

Question 3. *Figure 3: the title of "TNF" needs to be described in full*.

Answer: We added the full name of TNF in figure 3.

Question 4. Figure 4: please describe if the Y axis label "Protein level" is a relative level or not, and how they are calculated.

Answer: The protein level was detected by enzyme-linked immunosorbent assay, and quantitative concentration of protein was determined by a standard curve. We added the unites of the protein level, thanks.

Question 5. Please add the scale for the HIC images in Figures 5-6.

Answer: We added the scale for images in Figures 5-6.

Question 6. Did the authors measure the protective effect of resveratrol on parameters related to the acute intestinal inflammation (e.g. the Myeloperoxidase (MPO) activity assay) and the intestinal permeability (e.g the FITC-labeled dextran intestinal permeability assay)?

Answer: Our present study did not measure the effect of resveratrol on parameters related to acute intestinal inflammation. As a good suggestion, we will detect the effect of resveratrol on acute colitis in the future study, which may better explain the profile of resveratrol in colitis. Thanks very much.

Reviewer #4 comments:

Question 1. In Figure 2, the colon length of mice from each group was measured and

compared. At the end of the chronic colitis induction, the mice should be aged at 10 weeks, and the average colon length in control is only 6 cm, which is kind of lower than other studies. Please provide the original images of the harvested colons.

Answer: The average colon length was slightly lower than others, but this is the real result of the experiment, and we provide the original images of the harvested colons in figure 2.

Question 2. The authors should also describe how the colons were prepared and which portion (proximal, middle, or distal) of colon was used for H&E staining and IHC staining. In Figure 4A, the colon from control group seems from proximal part, while the rest seems from middle colon. DSS induced colitis is most severe at the distal colon, please explain why distal colon was not used for the comparison.

Answer: Clinically, ulcerative colitis occurs in any part of the colon, which is more likely to occur in the distal colon. Inflammation occurs in the proximal, middle and distal colon in the mouse model of DSS. We evaluated the degree of colonic mucosal damage based on the pathological score of random fields of vision in the relatively severe area of colitis, and the pictures in the text represent the colitis close to the average colon score.

Question 3. In Figure 5, please provide the negative control for IHC staining. The presented images are blurred, and the staining seems like background, especially in DSS + RES group.

Answer: We added the negative control for IHC staining in figure 5, and we also provided the original figures as PPT to keep high solution.

Question 4. In Figure 7 (TEM images), endoplasmic reticulum and mitochondria should be marked on the images (i.e., as "ER" or "M"). The authors claimed that autophagosome can be observed, however, the result is not convincing, since only one image was presented, without quantification.

Answer: we added the markers in figure 7 to show the dysregulation of endoplasmic reticulum and mitochondria. The observation of autolysosomes by TEM is the most direct method to detect autophagy, although it is not accurate and quantitative. However, there is still controversy in quantitative detection. Reports showed that the

most commonly used method for quantitative detection of autophagy is the ratio of LC3-II / I and the expression of beclin-1 protein. In our study, TEM was mainly used to observe the formation of autophagy and the destruction of organelles. As a good suggestion, we will improve the quantitative detection technology of autophagy in the future, thanks.

Question 5. The authors claimed that treatment of RES increased the production of ZO-1 and occluding in colitic mice. It would be better to also show the TEM images with the tight junctions between neighboring IECs.

Answer: When we observed autophagy by TEM, we do not evaluate the tight junctions. The TEM can just reflect the local characteristics, and the immunohistochemistry of the tight junctions can reflect the overall expression. Therefore, we just used immunohistochemistry to evaluate the tight junctions. As a good suggestion, we will perform further observation on the TEM images with the tight junctions between neighboring IECs. Thanks very much.

Question 6. If resveratrol and 5-ASA were given at the same time when animals received DSS to induce colitis, this is not a treatment, as treatment usually starts when there is already a disease.

Answer: The animals received resveratrol or 5-ASA after DSS for 7days, which was considered as one cycle. We described it unclear and we added detail information in figure 1C, thanks for the comment.

Question 7. Minor comments: The catalog numbers for the antibodies used for Western blotting and immunohistochemistry staining should also be provided. In Table1, under "Body weight loss", "-<" should be "<=". For "Stool consistency" and "Bleeding", what does the "-" mean? For "Inflammatory cytokine assay", how were blood samples (1.0 ml) collected? please provide details.

Answer: The catalog numbers for the antibodies were added in the text. Table1, "-<" was revised. "-" indicates that there are only three grades of evaluation in stool consistency and bleeding, and we added explanation in the text. In the present study, most mice were sacrificed, and blood samples (1.0 ml) were collected from the heart, and some mice with lighter weight mass were collected for about 0.8 ml blood.

Thanks very much.

Question 8. For Figure 2A and 2B, the same color (for the curve) for a specific group should be used in both 2A and 2B, for consistency. For Figure 2A, the significance (*), i.e., P<0.05, should be labeled on the curve at the corresponding day(s). For Figures 4, 5, and 7, what are the bar scales?

Answer: We used the same color in figure 2, and the significance was labelled on the curve. The scales were added in figure 4, 5 and 7.

Question 9. For Figure 5, a quantification of ZO-1 and occluding staining is needed. For Figure 6C, the label "C" is missing.

Answer: Semi-quantification of ZO-1 and occludin staining was added as table 3, and "C" was added in figure 6.

Question 10. Also, the quantification of LC3-II/I ratio should be shown, instead of LC3B protein level. Reference 31 was not cited in the text (discussion).

Answer: The quantification of LC3-II/I ratio should be shown in figure 6，and we revised the title. References were rearranged in the text, thanks.

Question 11. Reference 33 is missing from the REFERENCES.

Answer: we are sorry for the wrong version of the manuscript, and we revised this in the text.

Editor's comments:

(1) I found the authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s).

Answer: we provided the approved grant application form, thanks.

(2) I found the authors did not provide the original figures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor.

Answer: We provide the original figures as PPT file, thanks.

*(3) 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.

Answer: We revised this in the text according to the above comment, thanks.

Thank you very much for your attention and consideration.

Sincerely yours,

Ying-Yu Ma,

On behalf of all contributing authors

Key laboratory of gastroenterology of Zhejiang province, Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College, Hangzhou 310014, Zhejiang Province, China

E-mail: myy011525@163.com