Hydrogen Gas and Preservation of Intestinal Stem Cells in Mesenteric Ischemia and Reperfusion

Editor

Author Response: We are grateful for your insightful comments and the reviewers' valuable suggestions on our manuscript. We have revised the manuscript following comments.

Reviewer 1

Author Response: We are grateful for your comments on our manuscript. We added correct words for abbreviations and ethical approve number of the current study. Please see our other specific responses below.

1. Explain the reason for the differences in the number of animals in the groups.

Author Response: Thank you for pointing this out to us. We planned to examine 30 rats in this study and conducted experimental protocols at the same time as possible, to reduce biases due to environmental changes. Due to the limitation of the number of rats in which hydrogen gas supply device could be used simultaneously, we could conduct the hydrogen protocol in nine rats. To make equal the number of rats as possible, we conducted ischemia protocol in ten rats and reperfusion one in eleven rats.

2. Please explain why you did not add a hydrogen group to the ischemia group.

Author Response: Thank you for valuable comments. Based on previous studies, we thought hydrogen would work on ischemia-reperfusion injury, rather than ischemic injury. Therefore, we set the reperfusion (without hydrogen) group as our main control group. We added the ischemia group as an additional control group to capture the difference in tissue injury between ischemia and reperfusion.

3. Kruskal Wallis test is suitable for the evaluation of data that cannot be normally distributed in multiple groups. In case of difference, the Mann Whitney U test can be used. Which posthoc test did you use after the ANOVA test (Bonferroni, Tukey)

Author Response: Thank you for pointing this out to us. We had conducted Tukey-Kramer as post-hoc test in the original manuscript and additionally conducted Kruskal-Wallis, which showed the same results. To clarify the statistical analysis, the text in the manuscript has been revised to the following:

In lines 232-234 in page 11 in Statistical analysis, "Intergroup comparisons of mRNA expressions and 8-OHdG concentrations were performed using analysis of variance *with Tukey-Kramer as post-hoc test and/or Kruskal-Wallis tests,* as appropriate."

Reviewer 2

Author Response: We are grateful for your comments on our manuscript. Please see our specific responses below.

1. Please, in the Experimental Protocol topic, it is unclear whether the animals were under mechanical ventilation. Please insert in the text.

Author Response: Thank you for pointing this out to us. We did not use mechanical ventilation because previous study identified that hydrogen inhalation under spontaneous respiration could provide enough and steady supply of hydrogen in several organs (PMID 30718910). To clarify we did not use mechanical ventilation, sentences have been revised to the following:

In lines 158-161 in page 7 in Experimental protocol, "The hydrogen group (n = 9) was connected to the respiratory circuit using a gas supply hood that covered the face and head of the rats*, in which spontaneous respiration was maintained without using mechanical ventilation [14]*"

Science Editor

Author Response: We are grateful for your comments on our manuscript. Please see our specific responses below.

1. Language Quality: Grade B (Minor language polishing), Scientific Quality: Grade C (Good)

Author Response: Thank you for your comments. We followed reviewers' comments and editor's suggestions. We added sentences to provide highlights of the latest cutting-edge results with two additional references. Language had been edited by professional English editing services (Enago, Crimson Interactive Pvt. Ltd., Mumbai, India).

Company Editor-in-Chief

Author Response: We are grateful for your comments on our manuscript. Please see our specific responses below.

1. I recommend the manuscript to be published in the World Journal of Gastrointestinal Surgery. Before final acceptance, when revising the manuscript, the author must supplement and improve the highlights of the latest cutting-edge research results, thereby further improving the content of the manuscript. To this end, authors are advised to apply a new tool, the Reference Citation Analysis (RCA). RCA is an artificial intelligence technology-based open multidisciplinary citation analysis database. In it, upon obtaining search results from the keywords entered by the author, "Impact Index Per Article" under "Ranked by" should be selected to find the latest highlight articles, which can then be used to further improve an article under preparation/peer-review/revision.

Author Response: Thank you for your valuable suggestions. We added sentences to provide highlights of the latest cutting-edge results as follows. In addition, we found two valuable references using RCA (PMID 19329995, 26973414) and added them in Introduction and Material and Methods.

In lines 289-296 in pages 12-13 in Discussion, "*In this study, the tissue-protective effects of continuous hydrogen gas inhalation were histologically identified in the model of ischemic-reperfusion injury at mesentery. In addition, hydrogen protected intestinal stem cells from oxidative stress following ischemia–reperfusion injury, which has not been reported as therapeutic effect of hydrogen in previous studies. Notably, the intestinal stem cells were not injured by ischemia alone (ischemia without reperfusion), and therefore, hydrogen would provide tissue-protective effect only when reperfusion happens, rather than only ischemic injury exists*."