Peer Review File

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Reviewer A

The study of the gut microbiota and its application in different pathologies has experienced a boom in the last few years, because of its interesting applications.
In this case, its application was focused on a pathology that, although it has a low prevalence in the population, its effects on affected patients have, in a high percentage, fatal consequences; for this reason, I congratulate the authors.
However, there are several points to be taken into account in the paper, some of which, in my opinion, pose a serious objection to the work.

Major comments:

Comment 1: The authors performed the transplantation of the intestinal microbiota of the donor mice by intraoral administration, which implies that the fecal pellet must pass through the gastrointestinal tract, with the gastric juices (highly acidic). This makes me doubt about the survival of the microorganisms; can the authors be sure that the microorganisms remain alive? They attribute the results to the action of the bacteria, however, could it not be due only to their by-products, which could resist the passage through the gastrointestinal tract?
Reply 1: We are very grateful for Reviewer A's valuable comments. The trend of Firmicutes/Bacteroides in feces in the dexmedetomidine-treated group compared with the saline control group was consistent with that before fecal transplantation. The above results indicate that the FMT experiment is successful and microorganisms remain alive. On the other hand, FMT is one of the most commonly used approaches for investigating the relationship between the gut microbiota and diseases and many studies approved its effects on modulating the composition of microbiota (1-3).
1. Zhang PP, Li LL, Han X, et al. Fecal microbiota transplantation improves metabolism and gut microbiome composition in db/db mice. Acta Pharmacol Sin 2020;41:678-685.
2. De Palma G, Lynch MD, Lu J, et al. Transplantation of fecal microbiota from patients with irritable bowel syndrome alters gut function and behavior in recipient mice. Sci Transl Med 2017;9:
3. Bokoliya SC, Dorsett Y, Panier H, et al. Procedures for Fecal Microbiota Transplantation in Murine Microbiome Studies. Front Cell Infect Microbiol 2021;11:711055.

Minor comments:

Comment 2: Due to the complexity of each of the three experiments detailed by the authors, it would be useful for the reader to describe them graphically and schematically.

Reply 2: We are very grateful for Reviewer A's valuable comments. We have described the experiments graphically in the revised manuscript.
Changes in the text: We added a figure to show the animal experimental protocol. (see Page 8, Figure 1)".

Comment 3: In general, the text of the results includes a lot of numerical information, which complicates the reading. I suggest that the values be expressed in a table, and those that, according to the authors judgment, are important, do include them in the text.
Reply 3: We are very grateful for Reviewer A's valuable comments. According to the reviewer's suggestion, we removed most of the numerical information from the text of the results and added tables to show the data and only include the important value in the text.
Changes in the text: We added tables to show the data and only include the important value in the text. (see supplementary data)

Comment 4: The histologic images are quite small, which impedes the visualization of any relevant information in them.
Reply 4: We are very grateful for Reviewer #A's valuable comments. We appropriately zoomed in on the histological images for a clearer visualization
Changes in the text: We appropriately zoomed in on the histological images for a clearer visualization (see Figure 3B, Figure 4A and Figure 5B)

Comment 5: The mortality rate should be included in the description of the results. Indicate the final number of each animal included in each group and the number of dead animals, not only indicate it as a survival rate.
Reply 5: We are very grateful for Reviewer #A's valuable comments. We showed the morality rates and the number of dead animals in the text
Changes in the text: We have modified our text as advised (see Page 13, line 14-18, and Page 16, line 2-6)

Comment 6: How were the fecal pellets obtained? Were they directly collected from the housing cages? or were they harvested by a surgical procedure?
Reply 6: We are very grateful for Reviewer #A's valuable comments. We euthanized the mice and carefully exposed the small intestine. We cut the distal ileum of the small intestine and fecal samples were collected by extruding the intestine in 2 ml autoclaved tubes.
Changes in the text: we have modified our text as advised (see Page 8, line 12-14)

Reviewer B

Comment 1: An elegantly designed study to elucidate the mechanism by which dexmedetomidine modulates the immune response to I/R injury. Randomization of mice was mentioned explicitly for experiments 2 and 3 but not for experiment 1. Is this an oversight or were the mice not randomized in the first experiment?

Reply 1: We are very grateful for Reviewer #B's valuable comments. It's an oversight that we didn't show the randomization in experiments 1. We have modified our text as advised. We apologize again for the trouble caused by our mistakes.

Changes in the text: we have modified our text as advised (see Page 8, line 5)

Comment 2: In the methods section, it is noted that only mice surviving 3 hours after I/R are included in the study. I am curious as how many animals were lost in each respective group prior to the 3-hour mark. Can you describe the timeline of intraperitoneal injection of Dex and NS and the induction of I/R injury in more detail? Were the mice kept sedated for the duration of the experiment or were they closed and allowed to return to their cages after 3 hours? Was tissue sampling for histology taken after three hours or later during the experiment? In the methods section, it is stated that mice were observed at intervals with gaps up to 24 hours. Does this mean that samples (blood and tissue) were taken from mice that had been deceased for up to 24 hours?

Reply 2: We are very grateful for Reviewer #B's valuable comments, no mice died within 3 h of intestinal I/R. We apologize again for the trouble caused by our writing errors. Dex or NS were injected intraperitoneally 24 hours before establishing I/R and we showed a detailed experimental protocol in the Figure 1. During the study period, mice were kept sedated and maintained body temperature at 37°C with a heating pad, and were injected subcutaneously with 0.5 ml of normal saline for fluid resuscitation and returned to their cages immediately after reperfusion. All samples, including blood and tissue, were collected 3 hours after reperfusion. We apologize again for the trouble caused by our writing errors.

Changes in the text: we have modified our text as advised (see Page 7, line 22 and Page 8, line 1-4)

Comment 3: In the methods section concerning the grading of histological sections, the scoring system is referred to as a modified Chiu score (with reference to the original paper from 1970). In the original system, the scoring is from 0-5. With Park's modification (the most commonly used), it continues to 8, with the highest grades denoting transmucosal and transmural infarction. Please cite a suitable paper describing the modified system used in this paper to aid the interpretation of your results. Also, describe the assessment procedure by the technicians - such as how many fields were assessed and how many different tissue sections. In the results section, the NS + I/R group is shown the have a mean score of 7.25 implying that almost all animals had at least complete transmucosal

infarction. None of the slides shown in the manuscript have such extensive damage. Please comment on the discrepancy

Reply 3: We are very grateful for Reviewer #B's valuable comments. We re-cited a suitable paper to describe the modified system (Liu KX, Li YS, Huang WQ, et al. Intensive Care Med 2009;35:933-42.) and described the assessment procedure by the technicians. And we re-uploaded the slide images to show the damage.

Changes in the text: we have modified our text as advised. The specific content is as follows: "The pathological scores of the intestinal mucosal injuries were evaluated by randomly choosing six fields of intestine tissue according to the modified Chiu scoring system (25) and the average scores were used to determine mucosal damage. The technicians were blinded to the mouse's treatment". (see Page 9, line 17-19)

Comment 4: When were the histological sections recovered during the experiment? From which part of the intestine were segments recovered for histology? Was the harvesting location standardized for all groups? Was any consideration given to the presence of feces in the lumen when harvesting segments for histology? The presence of luminal feces can degrade the mucosal interface during formalin fixation. This potential bias, if systemic, is not offset by a blinded technician. Grootjans work on IRI shows a very rapid recovery of the mucosal integrity in surviving animals/humans albeit with shortening of the villi. In this study all animals are said to have survived a minimum 3 hours post-IRI. Please comment on the appearance of the mucosa in the slides presented in the manuscript with regard to Grootjans group's now well documented mucosal adaptation after IRI.

Reply 4: Thanks for the Reviewer #B's valuable comments. Our study focused on the damage of ileal tissue 3 h after intestinal I/R in mice, and found that the ileal mucosa was severely necrotic with hemorrhage. As for when the histological sections recovered during the experiment is a shortcoming of our study, it has been supplemented in the limitations section of the Discussion.

The distal ileum of the small bowel is collected, so the collection location is standard. When the ileum was fixed with formalin, we used cold phosphate-buffered saline (PBS) buffer to wash the ileal section, and no feces were present in the lumen.

We have added comments on mucosal appearance regarding the now well-documented mucosal adaptations of the Grootjans group after IRI to the discussion section of the manuscript. We agree with the Grootjans group's study on IRI as mentioned by the reviewer that demonstrated that the mucosal integrity was rapid recovery within 120 min of reperfusion in animals/humans. However, consistent with our findings, Wang et.al found that severe necroses with hemorrhage of colon mucosa were detected at three hours reperfusion and tended to recovery at 6 hours reperfusion. The

restoration of intestinal mucosal integrity is a limitation of this study, which we will further explore in future studies
Changes in the text: we have modified our text as advised (see Page 9, line 14-16) The specific content is as follows: "In present study, we detected I/R caused severe intestinal pathological damage, which was contrary to Grootjans's study, which demonstrated that the mucosal integrity was rapid recovery within 120 min of reperfusion in animals/humans (40). However, the period of ischemia in our study was 60 min and reperfusion for 180 min, and evidence has showed that the sensitivity to ischemia varied from different parts of intestine. Wang et.al found that severe necroses with hemorrhage of colon mucosa were detected at three hours reperfusion and tended to recovery at 6 hours reperfusion. Besides, the constructions of blood supply in mice, rats and human beings were different.(see Page 17, line 14-22).
"The restoration of intestinal mucosal integrity is another limitation of this study, which we will further explore in future studies". (see Page 20, line 15-16).

Comment 5: Studies have shown that small animal models are not always easy to extrapolate how ischemic damage can be expected to behave in large animal models or humans. In the discussion please comment on the relevance of the experiment and what potential therapeutic avenues this may open. Does the pre IRI-injection of the drug (or any other pre-IRI attempt to modulate GI-microbiota) influence its potential as a therapeutic in clinical practice?
Reply 5: Thanks for the Reviewer #B's valuable comments. This study provides a theoretical basis for the clinical treatment of intestinal I/R injury by Dex or intestinal microbiome. We comment on the relevance of the experiment and what potential therapeutic avenues this may open. And pre IRI-injection of the drug influence its potential as a therapeutic in clinical practice. Also this was the limitation of our study and we could focus on the clinical studies in the future.
Changes in the text: We have modified our text as advised (see Page 20, line 1-8)

Comment 6: While the manuscript is reasonably well-written it could benefit from some syntactic improvements prior to publication.
Reply 6: Thanks for the Reviewer #B's valuable comments. We re-polished our manuscript.