Format for ANSWERING REVIEWERS

April 27, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 16956-article.doc).

Title: Ischemic preconditioning ameliorates intestinal injury induced by ischemia-reperfusion in rats

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Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 16956

The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated
Yes.

2 Revision has been made according to the suggestions of the reviewer

(1) In the "methods" of "abstract", the authors didn't mention if the rats were divided "randomly".

Explanation and revision: We greatly appreciate the reviewer's question. In the present study, the rats were randomly divided into three groups (S group, IR group and IP group). According to the reviewer's suggestion, we have amended some content. Please see the revision highlighted in green on page 3, line 6.

(2) In the "materials and methods" section, the authors didn't mention the test method of MDA level, and, the method of testing each factor should be detailed and specific.

Explanation and revision: We should like to express our appreciation to the reviewer for suggesting how to improve our manuscript. According to the reviewer's suggestion, we have carefully checked the manuscript and amended some content the test method of MDA level. Please see the revision highlighted in green on page 6, line 5-12.

(3) In the "materials and methods", the authors should demonstrate: the samples come from which part of intestine and the length of the samples. This information is only appeared in the

"Histopathological examination of intestinal injury" section. So, what about samples in other testing? Did the samples come from the same part of the intestine?

Explanation and revision： We believe that the reviewer's question is very well. In the present study, all the samples were come from the same part of the intestine. A segment of 1.0 cm intestine tissues (from 5 cm of the terminal ileum) was harvested. According to the reviewer's suggestion, we have amended some content. Please see the revision highlighted in green on page 6, line 6，line 15-16 and line 27.

(4) The rat intestinal I/R model should be supported by at least one reference.

Revision: According to the reviewer's suggestion, we have amended some content. Please see the revision highlighted in green on page 6, line 3.

(5) In the "Histopathological examination of intestinal injury" section, I think two expert investigators are not enough.

Revision: According to the reviewer's suggestion, the third expert investigator was invited to evaluate intestinal mucosal damage with the criteria of Chiu's scores. The result is the same as shown in Figure 4, intestinal tissue injury in the IR group was markedly increased than that in the S group ($P < 0.01$). IP obviously decreased intestinal tissue injury comparable to the IR group ($P < 0.05$). And, we have amended some content. Please see the revision highlighted in green on page 7, line 9.

(6) In the "results" section, data should be followed with unit, and, all P values should be listed.

Revision: We should like to express our appreciation to the reviewer for suggesting how to improve our manuscript. According to the reviewer's suggestion, we have carefully checked the manuscript and amended some content. Please see the revision highlighted in green on page 8, line 17-19, line 25-27; page 9, line 3-5.

(7) In figure 5 & 6, the figure of western blot should be marked group. In addition, I think the ICAM-1 result from the western blot figure cannot be recognized as significant difference.

Explanation and revision: We greatly appreciate your careful work on our manuscript. According to the reviewer's suggestion, we repeated the detection of ICAM-1 protein expression by western blot. The result showed that intestinal tissue ICAM-1 protein expression in the IR group was significantly increased than that in the S group (353.33 ±45.19 vs 100.00±25.00, $P < 0.01$). IP evidently decreased

intestinal tissue ICAM-1 protein expression comparable to the IR group (204.67±53.27 vs 353.33 ±45.19, $P < 0.05$). Furthermore, the figure of western blot has been marked group. Please see the revised figure 5 , figure legend 5 and the revision highlighted in green on page 9, line 17-21 .

(8) In the "discussion" section, I think some other studies of IP and its mechanisms of preventing I/R injury should be further discussed, and, some merits and drawbacks of IP application compared with other approaches preventing I/R injury in intestine might be discussed.

Explanation and revision: We should like to express our appreciation to the reviewer for suggesting how to improve our manuscript. Ischemia-reperfusion injury (IR) of the intestine is part of the pathophysiology of many intestinal disorders, such as strangulated hernia, volvulus, necrotizing enterocolitis, mesenteric embolic event, and intestinal transplantation. It is an important factor associated with morbidity and mortality in both surgical and trauma patients [1, 2]. According to the reviewer's suggestion, we have carefully checked the manuscript and amended some other studies of IP and its mechanisms of preventing I/R injury in the discussion. Please see the revision highlighted in green on page 10, line 15-20.

With respect to merits of IP application in intestine, the clinical application of IP is a simple, safe and tolerable procedure, with wide-ranging immunomodulatory effects. Recently, a randomized controlled trial has shown the remote IP powerfully counteracts the injury to the intestinal mucosa caused by a period of ischemia and subsequent reperfusion during elective open infrarenal abdominal aortic aneurysm repair[3]. Regarding drawbacks of IP application in intestine, although IP is one of the most reproducible and powerful phenomena in intestine protection, it has not readily translated to routine clinical use. Therefore, more clinical trials are needed to further investigate intestinal IP use during gastrointestinal surgeries. According to the reviewer's suggestion, we have carefully checked the manuscript and amended the above content in the discussion. Please see the revision highlighted in green on page 13, line 1-9.

References

[1] **Eltzschig HK**, Eckle T. Ischemia and reperfusion-from mechanism to translation. *Nat Med 2*011, **17**: 1391-1401.

[2] **Stringa P**, Romanin D, Lausada N, Machuca M, Raimondi JC, Cabanne A, Rumbo M, Gondolesi G. Ischemic preconditioning and tacrolimus pretreatment as strategies to attenuate intestinal ischemia-reperfusion injury in mice. *Transplant Proc* 2013, **45**: 2480-2485.

[3] **Li C**, Li YS, Xu M, Wen SH, Yao X, Wu Y, Huang CY, Huang WQ, Liu KX. Limb remote ischemic preconditioning for intestinal and pulmonary protection during elective open infrarenal abdominal aortic aneurysm repair: a randomized controlled trial. *Anesthesiology* 2013; **118**:842-852.

3 References and typesetting were corrected
Yes.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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