Format for ANSWERING REVIEWERS

January 11, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 7494.doc).

Title: Changes In Intestinal Microflora In Rats With Acute Respiratory Distress Syndrome. A frontier report.

Author: Yan Li, Xiang-Yong Liu, Ming-Ming Ma, Zhi-Jiang Qi, Xiao-Qiang Zhang, Zhi Li, Guo-Hong Cao, Jun Li, Wei-Wei Zhu, Xiao-Zhi Wang

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 7494

The manuscript has been improved according to the suggestions of reviewers and all revisions I made have been highlighted by red colour.
For Co-first author and Corresponding author are not accepted, so I have delete the Corresponding author Xiangyong Liu .

1 Format has been updated and a copyediting service has been used provided by professional English language editing company named Jing-Yun Ma Editorial Office which is suggested by *World Journal of Gastroenterology*, and the number is 2014-01-0040. The editorial certificate is in the attached files.

2 Revision has been made according to the suggestions of the reviewer
Reviewer A: The paper of Yan Li et al. "Changes in intestinal microflora in rats with acute respiratory distress syndrome." is a very well done and written study which has a great importance in this field and contribute to better understanding of possible relationship between the acute respiratory distress syndrome and intestinal microbiota. his study may be classified into grade A, language grade A. The paper could be accepted in the present form.
Reviewer B: Li et al reported a series of specific intestinal microflora in the Acute Respiratory Disetress (ARD) and Acute Lung Injury (ALI). The investigational significance is high due to very strong clinical and translational potential. However, the overall quality of studies is lower, data need to be revised for publication.
Major points:
1)The overall problems are that description in either Figure legends or results are not clear enough to be understood by audiences, the paper need to be edited by a professional editor.
Answer: We've consulted Shanghai Personal Biotechnology Co., Ltds about the detail meaning of results revealed by high-throughput sequencing technology in intestinal microflora between the two groups. Under the guidance of professional teachers, we have conducted professional analysis and description for relevant figures and tables to achieve professional requirements.
2)Cited literatures should be confirmed one by one.

Answer: I have added PubMed citation number and DOI citation to the reference list and list all authors and revise throughout.

3)Please consider if authors can measure rat intestinal berrier by everted gut sac to exactly reflect the bacteria – induced IEC barrier dysfunction.

Answer: Thanks for suggestion on everting gut sac to reflect the bacteria – induced IEC barrier dysfunction.

DAO level has been used as a marker of intestinal mucosal integrity.

eg. Yao Q,Ye X,Wang L,Gu J,Fu T,Wang Y,Lai Y,Wang Y,Wang X,Jin H,Guo Y.Protective effect of curcumin on chemotherapy-induced intestinal dysfunction.Int J Clin Exp Pathol 2013;6(11):2342-2349.[PMID: 24228095].

D-lactate levels in the blood can reflect the status of intestinal mucosal damage.

eg. Wu QJ,Zhou YM,Wu YN,Zhang LL,Wang T.The effects of natural and modified clinoptilolite on intestinal barrier function and immune response to LPS in broiler chickens.Vet Immunol Immunopathol 2013;153(1-2):70-76.[PMID: 23453767]

Gut sac is widely used to evaluate absorption and metabolism of intestinal mucosa.

Eg. Ravi PR,Aditya N,Kathuria H,Malekar S,Vats R.Lipid nanoparticles for oral delivery of raloxifene: Optimization, stability, in vivo evaluation and uptake mechanism.Eur J Pharm Biopharm 2013.[PMID: 24378615]

Parsa A,Saadati R,Abbasian Z,Azad Aramaki S,Dadashzadeh S. Enhanced Permeability of Etoposide across Everted Sacs of Rat Small Intestine by Vitamin E-TPGS.Iran J Pharm Res2013;37-46.[PMID: 24250670]

Mançanares CA,Leiser R,Favaron PO,Carvalho AF,Oliveira VC,Santos JM,Ambrósio CE,Miglino MA. A morphological analysis of the transition between the embryonic primitive intestine and yolk sac in bovine embryos and fetuses.Microsc Res Tech. 2013;76(7):756-766.[PMID: 23650099]

It is a good suggestion to combine gut sac to bacteria – induced IEC barrier dysfunction.

Our article highlights changes in the intestinal flora in ARDS rats by high-throughput sequencing, so we will not consider gut sac method to instruct IEC barrier dysfunction for the moment. However, it is a problem worthy of study in depth and our future research will focus on this aspect in detail.

4)In addition, bacteria data are novel, but the way was presented inappropriately in the paper. The best way is calculate if there is a statically correlation between LPS –induced lung injury and bacterial abundance in the intestine.

Answer: 16S rDNA high-throughput sequencing can reveal changes to microbial diversity in normal and ARDS rats by using PCoA of the weighted UniFrac matrix from 6 samples. The percentage of variation explained by the principal coordinates is indicated on the axes which can indicate correlation between LPS –induced lung injury and bacterial abundance in the intestine.

Minor points:

1)In Figure 1, 2 and 3. Add micro rulers. Figure 2 is not significant and quality is poor, replace them with higher power magnification, for example, 400 *

Answer: I have added micro rulers to Figure 1, 2 and 3,and replace Figure2 with 400 magnification.

2)In Figure 4. Mitochondria pathology seems not significant in the Figure B compared to Figure 4A.

Answer: It is true that mitochondria pathology seems not significant in the Figure 4B compared to Figure 4A, only occurred slight change, so I replace it with slight swollen mitochondria with uncompact structure.

3)In Figure 5 A and B. The pan graph is not clear at all, replace it.

Answer: I have replaced Figure5 with more clear graph according to revisions.

4)Table 4 should be analyzed by column or line graphs combined with statistics

Answer: Statistically significant flora and percentage of more than twice number

of species are shown at the phylum and genus level. Vertical axis represents sequence reads, horizontal axis represents statistically significant flora. We can analyze significant flora between two groups by column graph with statistics.* $p<0.05$ for Normal group vs Model group.

3 References and typesetting were corrected

Answer: References and typesetting were corrected according to the Format for references, including PubMed citation number and DOI citation to the reference and all authors, and I have delete Reference 15th and Reference 18th to meet the requirements.

Thank you again for publishing our manuscript No.7494 in the *World Journal of Gastroenterology*.

Sincerely yours,

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