

Format for ANSWERING REVIEWERS

January 20, 2015

Dear Editor,



Please find enclosed the edited manuscript in Word format (file name: 15712-review.doc).

Title: Overexpression of HMGB1 A-box reduced LPS-induced intestinal inflammation via HMGB1/TLR4 signaling in vitro

Author: Fu-Cai Wang, Jing-Xuan Pei, Jun Zhu, Nan-Jin Zhou, Dong-Sheng Liu, Hui-Fang Xiong, Xiao-Qun Liu, Dong-Jia Lin, Yong Xie.

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 15712

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

Format has been mostly updated.

Except results in abstract, we didnot present P values and provide relevant data to illustrate how the statistical values were obtained, because our study was divided into eight groups and every group included four proinflammatory mediators and the mRNA and protein levels of five molecules, so our data were too many to be presented.

If you have to offer, we will follow-up supplement.

The new revision of the file (ESPS Ms NO: 15712.doc) marked red.

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

2.1 Reviewed by 2902724

(1) Ethyl pyruvate inhibits HMGB1/TLR4 signaling and cytokine release from LPS stimulated SW480 and THP-1 cells. Effect of EP on transfected cells was not investigated? There is no effect of overexpression of HMGB1-Box A on HMGB1/TLR4 signaling and cytokine release in transfected SW480 cells?

The purpose of our study was to investigate the effects of two HMGB1 inhibitors (overexpression of HMGB1 A-box and ethyl pyruvate) on the HMGB1/TLR4 signaling pathways and the secretion of some proinflammatory cytokines in SW480 activation by LPS and co-culturing with THP-1 cells. A comparative study of parallel was to investigate the effects of overexpression of HMGB1 A-box and ethyl pyruvate, so we did not investigate the effect of EP on mock-transfection cells and pA-box-transfection cells.

(2)HMGB1-Box A (released by overexpressing SW480 cells) can inhibit LPS induced cytokine release by THP-1 cells, by inhibition of TLR-4 signaling. This is not the case in the absence of LPS stimulation?

In physiological conditions (for example the absence of LPS stimulation), the cytokine release and activation of TLR4 signaling by THP-1 cells were at a lower level of dynamic balance. Our results indicate that HMGB1 A-box antagonized HMGB1-induced inflammation only under pathological and not physiological conditions.

(3)The paper would be much clearer if the authors would have summarized the main findings as done above?

This is a good idea. In order to make the paper more clearly, we have added some explanation in the introduction part.

(4)The discussion part is too long and contains a lot of repetition. This part should be shortened and be more to the point?

Thank you for your guidance! We have deleted some repetition content of the discussion. But, according to review by 3000672 (The ideas in the discussion are adequately divided in small parts with clear focus on the purpose and the achieved results.), the discussion is also divided into four parts.

(5)The part of the co-culture is somewhat confusing since it is not described in the materials part. Were all cells pre-treated with EP? Were THP-1 cells stimulated with LPS or not? These items are not clear from figures 6 and 7.

Thank you for your guidance! We have increased the details about transwell experiments in the materials and methods.

(6)The conclusion is not phrased correctly since the effect of inhibition by Box A was not investigated in SW480 cells?

Thank you for your guidance! We have revised the conclusion: Our findings suggest that HMGB1 A-box, not only EP, can reduce LPS-induced intestinal inflammation through inhibition of the HMGB1/TLR4 signaling pathways.

(7)The first sentence in real-time PCR in methods part is not correct: RNA is not treated as indicated in table 1. mRNA levels were measured with real-time PCR using primers as mentioned in table 1.

Thank you for your guidance! We have done modification in the corresponding position.

2.2 Reviewed by 2740122

(1) The authors show in their study that ethyl pyruvate, not only A-box, reduces LPS-induced intestinal inflammation through inhibition of the HMGB1/TLR4 signaling pathway, though the exact mechanism remains unclear. Why is this information left out from the conclusions in the abstract?

Thank you for your guidance! We have revised the conclusion: Our findings suggest that HMGB1 A-box, not only EP, can reduce LPS-induced intestinal inflammation through inhibition of the HMGB1/TLR4 signaling pathways.

(2) In the discussion part, in the fourth section in the middle of the first paragraph the authors write following: "After recombinant A-box protein is injected into patients (!!!) with collagen induced arthritis...."

This is a clerical error. "patients" should be replaced by "mice".

2.3 Reviewed by 3000672

Thank you for your comments!

3 References and typesetting were corrected.

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

Sincerely yours,

Xie Yong

Yong Xie, MD, PhD

Department of Gastroenterology,

the First Affiliated Hospital of Nanchang University,

17 Yongwaizheng Street, Nanchang, Jiangxi, China.

Fax: 0791-88692507

E-mail: xieyong_med@163.com