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ESPS PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology
ESPS manuscript NO: 15712
Title: Effects of high mobility group box 1 (HMGB1) A- box on HMGB1/TLJing Yu signaling pathways in SW480 and THP-1 cells activated by lipopolysaccharides
Reviewer's code: 02902724
Reviewer's country: Netherlands
Science editor: Jing Yu
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CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	PubMed Search:	[ ] Accept
[ ] Grade B: Very good	[Y] Grade B: Minor language polishing	[ ] The same title	[ ] High priority for publication
[ ] Grade C: Good	[ ] Grade C: A great deal of language polishing	[ ] Duplicate publication	[ ] Rejection
[Y] Grade D: Fair	[ ] Grade D: Rejected	[ ] Plagiarism	[ ] Minor revision
[ ] Grade E: Poor		[Y] No	[Y] Major revision
		BPG Search:	
		[ ] The same title	
		[ ] Duplicate publication	
		[ ] Plagiarism	
		[Y] No	

COMMENTS TO AUTHORS

Review of research paper Effects of high mobility group box 1 (HMGB1) A box on HMGB1/TLR4 signaling pathways inSW480 and THP-1 cells activated by lipopolysaccharides. Fucai Wang, Jingxuan Pei, Jun Zhu, Yong Xie, Nanjin Zhou, Dongsheng Liu, Huifang Xiong, Xiaoqun Liu, Dongjia Lin. In the paper by Wang et al HMGB1/TLR4 signaling was investigated LPS stimulated THP-1 cells cocultured with HMGB1-Box A overexpressing SW480 cells. Also the effect of ethyl pyruvate was investigated. The main finding of this paper are: ? Ethylpyruvate 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. ? 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. Major comments ? The paper would be much clearer if the authors would have summarized the main findings as done above. ? The discussion part is too long and contains a lot of repetition. This part should be

shortened and be more to the point. ? 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. Minor comments: ? The conclusion is not phrased correctly since the effect of inhibition by Box A was not investigated in SW480 cells. ? The first sentence in realtime PCR in methods part is not correct: RNA is not treated as indicated in table 1. mRNA levels were measured with realtime PCR using primers as mentioned in table 1.

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The purpose of the paper to investigate the effects of two HMGB1 inhibitors (HMGB1 A-box and ethyl pyruvate) on the HMGB1/TLR-4 signaling pathway and the secretion of some proinflammatory cytokines in SW480 and THP-1 cells after activation by LPS, is well established and consecutively unfolded. The hypothesis of the potential role of the HMGB1 A-box as a novel approach to the treatment of IBD is presented in an interesting manner, however it is rather ambitious because of the complex pathogenesis of IBD in which activation of innate immune receptors, such as TLRs, is just one of many mechanisms. However the authors have managed to describe theoretical and experimental evidence of HMGB1 A-box as a potential inhibitor of HMGB1/TLR-4 signaling pathways convincingly. Therefore it could be a possible therapeutic strategy for some patients with IBD. The introduction announces the topic clearly and understandably and the literature is well synthesized. The Toll-like receptors are not as novel as the authors have claimed. They were described for the first time in the middle of the `90s. Furthermore, there are data in the literature for both pro- and anti-inflammatory activities of IL-6, which make it more regulatory than

proinflammatory cytokine. In spite of that, in the context of NFkB activation the IL-1β, IL-6, IL-8 cytokines are rather proinflammatory. Another strength of the paper is the appropriate and precise materials and methods section. The methodology is clearly and widely explained. The authors have chosen two cell lines – intestinal epithelial cell line and human monocyte-like THP-1 cell line, the former – overexpressing the HMGB-1 A-box inhibitor by gene transfection technology for the purposes of the study. The authors have performed sufficient variety of methods to evaluate gene and protein levels of investigated parameters. The result section is well-organized and logically sequenced with sufficient number of figures. Commencing with the construction of HMGB1 A-box-overexpressing plasmid cell lines, followed by effects of that over-expression of HMGB1 A-box and EP on HMGB1 and on TLR4/LPS signalling pathways and secretion of cytokines in SW480 cells and in THP-1 cells the results are very convincing. The ideas in the discussion are adequately divided in small parts with clear focus on the purpose and the achieved results. In conclusion, the proposed article is well-written, easy to understand, scientifically consistent and suitable for the World Journal of Gastroenterology. Moreover, the article concerns up-to-date problem in IBD, revealing details about pathogenesis of IBD and suggesting a therapeutic strategy for future development.

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COMMENTS TO AUTHORS

Manuscript 15712 entitled "Effects of high mobility group box 1 (HMGB1) A- box on HMGB1/TLR4 signaling pathways in SW480 and THP-1 cells activated by lipopolysaccharides" by Wang et al. Comments Wang et al. have studied the effects of ethyl pyruvate and HMGB1 A-box on the HMGB1/TLR4 signaling pathways using intestinal epithelial cell line SW480 (overexpressing HMGB1 A-box) in a single culture activated with LPS or in co-cultures with THP-1 cells. They have determined HMGB1, TLR4 and its downstream signaling molecules MyD88 and NF-kappaB p65 protein and mRNA levels by RT-PCR, densitometry and Western blot, and also studied the cytokine levels in the culture supernatants. The authors conclude that HMGB1 A-box and ethyl pyruvate both inhibit HMGB1/TLR4 and downstream signaling molecules MyD88 as well as NF-kappaB p65 and the secretion of several important pro-inflammatory cytokines induced by LPS stimulation. In general, this study is methodologically well performed, the paper well written and has a clear message. The authors have used intestinal epithelial cell line to study the potential effect of above-mentioned compounds and to clarify some pathogenetic molecular mechanisms in IBD.

Therefore, the objective of this study is relevant. There are only a couple of issues that need to be addressed: 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? 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 should be corrected!