

Format for ANSWERING REVIEWERS



April 2, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 9423-review.doc).

Title: N-acetylcysteine attenuates reactive-oxygen-species-mediated endoplasmic reticulum stress during liver ischemia-reperfusion injury

Author: Yong Sun, Li-Yong Pu, Ling Lu, Xue-Hao Wang, Feng Zhang, Jian-Hua Rao

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 9423

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

1 Format has been updated

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

(1) **Reviewer 1 comments:** This is a well written article that clearly states the findings. Below there are some comments that should be addressed The abbreviations IR and ROS are not explained the first time they appear. The age or weight of the mice should be stated in material and methods. In the settings of ischemic preconditioning it has been shown that animals respond differently related to age. Way was 90 min. ischemia chosen? How was the dose 300 mg/kg NAC decided upon? " The first sentences in 3.1. may be better to have in the introduction, material and methods and discussion In 3.1 units for GSH, ROS, MDA are missing, this applies to more results later in the article. Further units for "transaminases" is not given and according to material and methods only ALT was measured The abbreviation ALF (acute liver failure?) in the discussion is not explained.

Responses: (a) IR and ROS have been explained; (b) Male C57BL/6 mice weighing 22-25g; (c) Clamp time of 90 minutes is a very common and recognized model in liver ischemia reperfusion injury (IRI) [1, 2]. In addition, our center and other centers also confirmed that this model can trigger ER stress in IR-stressed liver [3, 4]; (d) In fact, we tried different dose NAC (100mg/kg, 300mg/kg, 500mg/kg 1000mg/kg) to treat mice. We found every dose could attenuate liver IRI, but 300mg/kg NAC treatment is most effect on ER stress during liver IRI; (e) Unit for GSH, ROS and MDA has been added in revised version; (f) Unit for "transaminases" is not given; (g) Acute liver failure(ALT) is added in the discussion.

[1] Ji H, Shen X, Gao F, Ke B, Freitas MC, Uchida Y, Busuttil RW, Zhai Y, Kupiec-Weglinski JW. Programmed death-1/B7-H1 negative costimulation protects mouse liver against ischemia and reperfusion injury. *Hepatology*. 2010 Oct; 52(4):1380-9.

[2] Ren F, Duan Z, Cheng Q, Shen X, Gao F, Bai L, Liu J, Busuttil RW, Kupiec-Weglinski JW, Zhai Y. Inhibition of glycogen synthase kinase 3 beta ameliorates liver ischemia reperfusion injury by way of an interleukin-10-mediated immune regulatory mechanism. *Hepatology*. 2011 Aug; 54(2):687-96.

[3] Rao J, Qin J, Qian X, Lu L, Wang P, Wu Z, Zhai Y, Zhang F, Li G, Wang X. Lipopolysaccharide preconditioning protects hepatocytes from ischemia/reperfusion injury (IRI) through inhibiting ATF4-CHOP pathway in mice. *PLoS One*. 2013 Jun 4; 8(6):e65568

[4] Liu J, Ren F, Cheng Q, Bai L, Shen X, Gao F, Busuttil RW, Kupiec-Weglinski JW, Zhai Y. Endoplasmic reticulum

stress modulates liver inflammatory immune response in the pathogenesis of liver ischemia and reperfusion injury. Transplantation. 2012 Aug 15; 94(3):211-7.

- (2) **Reviewer 2 comments:** Yong Sun and co-authors report in their manuscript "N-acetylcysteine treatment attenuates reactive oxygen species mediated endoplasmic reticulum stress and apoptosis during liver ischemia reperfusion injury" experimental data that highlight the potential use of NAC in the treatment of IRI. Overall, the authors did a nice job and presented a well written manuscript. The experimental techniques are appropriate, and the authors present their findings in an adequate way. Overall, I have only a few mini comments that should be addressed in case of publication: 1) Please revise the manuscript carefully for grammatical errors. 2) Some abbreviations are not explained at first appearance. 3) The authors should briefly mention in the discussion limitations of their in vitro and in vivo experiments in mice regarding the applicability of their results on humans.

Responses: (a) We carefully revised grammatical errors; (b) All abbreviations were explained at first appearance in revised version; (c) we discussed the limitations of our experiments in discussion.

3 References and typesetting were corrected

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

Sincerely yours,

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