Format for ANSWERING REVIEWERS

December 25, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 14644-review.doc).

Title: Hydrogen-rich water protects against acetaminophen-induced hepatotoxicity in mice

(Former: Protective effect of hydrogen-rich water against acetaminophen-induced hepatotoxicity in mice via inhibition of oxidative stress and promoting liver regeneration)

Author: Jingyao Zhang, Sidong Song, Qing Pang, Ruiyao Zhang, Yong Wan, Dawei Yuan, Chang Liu

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 14644

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 reviewers

Reviewer 1:

1. Response to comment: (**Abstract: The research background of hydrogen-rich water should briefly address.**)

Response: According to the reviewer's comments, we have added a short introduction of hydrogen-rich water in the revised abstract.

2. Response to comment: (**There are some grammatical errors scattered throughout in the manuscript, for example "3 days after APAP administration, mice were sacri?ced and the liver tissue were obtained to assess the hepatic subcellular structure injury.".**)

Response: According to the reviewer's comments, we have improved the language of the paper by an expert in a language-edit company (American Journal Experts).

Reviewer 2:

1. Response to comment: (**In the abstract, authors should mention the complete experimental design including mortality experiment.**)

Response: According to the reviewer's comments, we have added the mortality experiment design in the abstract.

2. Response to comment: (**In the study design, time of APAP application must be stated (in relation to**

treatment with saline and HRW, resp.))

Response: In the present study, mice were housed in the lab for one week to adapt the new environment. Then APAP administered i.p at 8 a.m. in the first day, followed by NS or HRW treatment at 8 a.m. and 5 p.m. in the following 3 or 5 days.

3. Response to comment: (**Control-HRW group which is very important is missing.**)

Response: According to the reviewer's comments, we have added the Control-HRW group in the revised paper.

4. Response to comment: (**A statistical test used for survival/mortality rates has to be stated.**)

Response: According to the reviewer's comments, we have added the statistical test used for survival/mortality rates in the "**Statistical analysis**" section.

5. Response to comment: (**It is concluded that HRW promotes hepatocyte regeneration. HRW decreased the degree of liver injury/necrosis induced by APAP, thus I would expect a decrease in regeneration (in response to milder injury). Moreover, you measured only 72 h time interval which is not sufficient and appropriate for evaluation of regenerative response of the liver. You cannot conclude that regeneration was increased/delayed/more synchronized after HWR treatment, because authors did not actually monitor course of regeneration.**)

Response: Before we did the experiment, we did the literature research and studying and found that hepatocyte regeneration could be induced 16 hours after APAP administration. In the previous studies, 24 and 48 hours are always the time nodes for evaluation of regenerative response of the liver(Udayan Apte, Am J Pathol, 2009; Runkuan Yang, BMC Gastroenterology, 2012; Bharat Bhushan, Am J Pathol, 2013). In the present study, we chose 72 hours as the time node for detecting the liver regeneration, because the hepatocyte proliferation is remarkable at that time. It is really true that we did not actually monitor course of regeneration, but we could conclude that hydrogen promoted liver regeneration based on the results at 72 hours.

6. Response to comment: (***1*. In the abstract, it seems that MDA and MPO were measured in serum (in APAP group), but it is not true. *2*. In the abstract, authors mention that "HRW also remarkably inhibited … CYP2E1 activation", but they measured protein expression semi-quantitatively (western blot), not the degree of activation.**)

Response: According to the reviewer's and editor's comments, we have re-wrote the abstract part.

7. Response to comment: (**In the abstract, authors should mention the complete experimental design including mortality experiment.**)

Response: According to the reviewer's comments, we have added the mortality experiment design in the abstract.

8. Response to comment: (**In paragraph 2.5, MDA and GSH are included in liver enzymatic activity assays, but MDA is an aldehyde (secondary lipoperoxidation product) and GSH is a tripeptide**)

Response: According to the reviewer's comments, we have changed the "**liver enzymatic activity assays**" as "**Measurement of hepatic oxidative stress**".

9. Response to comment: (**Authors should prefer using term "oxidative stress" instead of "oxidant stress" in the Introduction.**)

Response: According to the reviewer's comments, we have changed **"oxidant stress" to "oxidative stress" in the revised paper.**

10. Response to comment: (**5.Abbreviations should be explained (CYP, NADPH, i-NOS, TNF, BrdU …) when used for the first time in the text. 7. Authors should unify abbreviations (BrdU vs. BrdUrd).**)

Response: According to the reviewer's comments, we have explained the abbreviations when used for the first time in the text in the revised paper.

11. Response to comment: (**GSH and NADPH are named as proteins/enzymes, but it is not true (Introduction).**)

Response: According to the reviewer's comments, we have deleted GSH and NADPH in the introduction

12. Response to comment: (**8. It is not clear if authors measured total or conjugated bilirubin.**)

Response: It is the total bilirubin we detected in the present study.

12. Response to comment: (**In fig. 2A, activity of GSH-px should not be expressed in umol/L.**)

Response: According to the reviewer's comments, we have transform the **umol/L to U/mg.prot** .

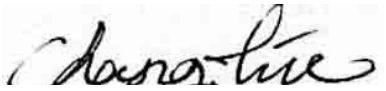
12. Response to comment: (**CYP2E1 is mentioned as the major catalyst involved in the metabolism of drugs (Discussion), but the main isoforms of CYP, responsible for drug biotransformation, are CYP3A4/5/7 (Evans and Relling, Science, 1999). CYP2E1 metabolizes only about 5 % drugs.**)

Response: Chen et al and Gonzalez FJ used null mice and found CYP2E1 plays a role in acetaminophen toxicity in vivo and CYP2E1 is a major CYP contributing to in the metabolism of acetaminophen to NAPQI. Meanwhile, CYP2E1-mediated oxidative stress was an important mechanism in acetaminophen toxicity. (Gonzalez, Drug Metab Dispos, 2007; Chen et al, J Biol Chem, 2008).

(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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