

May 24, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 18167-review.doc).

Title: Recq15 protects against lipopolysaccharide/D-galactosamine-induced liver injury in mice

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Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 18167

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

Response: Yes.

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

(1) The role of all the authors in the study must be detailed; the contributions of at least two of them are missing.

Response: We agree with your observation. The contributions of all authors have been listed.

(2) Abstract. The aim sentence should include “the effects of Recq15 helicase deficiency” for readers interested in the subject, but not familiar with the RecQ family function.

Response: Per the reviewer’s suggestion, we included the effects of Recq15 helicase deficiency in the aim.

Please see the “Abstract” section: “Recq15 helicase is critical for genomic integrity. Recq15-deficient mice exhibit increased chromosomal instability and elevated susceptibility to cancers.”

(3) Core tip. The administration of LPS/D-Gal should state “intraperitoneal injection or i.p.”

Response: In the revised manuscript, we used the word “intraperitoneally” when discussing the administration of LPS/ D-Gal in the “Core tip” section.

Please see the “Core tip” section: “Wild type and Recq15-deficient mice were intraperitoneally injected with lipopolysaccharide and D-galactosamine (LPS/D-Gal).”

(4) Introduction. Abbreviations for the syndromes, which are used later, should be introduced when first mentioned.

Response: We agree with this critique. We checked all the abbreviations and ensured they were introduced when first mentioned.

(5) Materials and Methods. The Liver injury induction describes the use of different doses of

LPS/D-Gal, but did not state clearly the reason for the use of one or another, neither the length of the treatments. This aspect should be explained for an easier comprehension of the procedures.

Response: We included details of the LPS/D-Gal treatment in the revised manuscript for a better understanding of the procedures.

Please see the “Materials and Methods” section, paragraph 3 (Liver injury induction): “For the mortality assay, mice were intraperitoneally injected with 20 µg/kg LPS and 400 mg/kg D-Gal, and mortality was recorded for 72 h. To induce acute liver injury, mice were intraperitoneally injected with 10 µg/kg LPS and 300 mg/kg D-Gal. Mice were scarified 1 or 6 h after LPS/D-Gal administration.”

(6) The method for quantification of the data of TUNEL assays should be explained. How many fields were examined per animal, etc.

Response: Per the reviewer’s suggestion, the TUNEL quantification method was included.

Pleased see the “Materials and Methods” section, paragraph 8 (Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay): “For each mouse liver section, the number of TUNEL-positive cells in five randomly selected fields was counted.”

(7) qRT-PCR methodology should include whether Trizol extraction is followed by DNase treatment or not. The concentration of the primers used in the assays should be also included, either in the text or in Table 1. Were experimental efficiencies calculated or jus assumed to be 2? This aspect should be clarified.

Response: Total RNA was treated with DNase after extraction. This information was added. The primer concentrations were also included in the text, as the reviewer suggested.

Pleased see the “Materials and Methods” section, paragraph 9 (Quantitative real-time PCR (qRT-PCR)): “Total RNA was isolated from liver tissue using TRIZOL reagent (Invitrogen), and was treated with DNase to remove contaminating DNA before cDNA synthesis.” “The primer concentration used in the PCR assay was 0.5 µM.”

(8) Lysis of liver tissues was done by incubation only?

Response: We apologize for the error. The liver tissues were lysed by sonication. We corrected this mistake in the “Materials and Methods”.

(9) TBS plus Tween is normally abbreviated as TTBS. The percentage of dry milk used should be expressed as (w/v).

Response: Per the reviewer’s suggestion, we changed TBST to TTBS. The percentage of dry milk was expressed as (w/v), as the reviewer suggested.

(10) Primary antibodies should be specified together with their source (mouse, rabbit, etc.). Moreover, antibody dilutions used in western blot should be included at least for primary antibodies.

Response: We agree with this critique. The antibody source and dilutions used in western blot were added.

Please see the “Materials and Methods” section, paragraph 2 (Reagents and antibodies):

“Caspase-3 (rabbit polyclonal, 1:1000), ERK (rabbit polyclonal, 1:2000), phospho-ERK (rabbit polyclonal, 1:2000), JNK (rabbit polyclonal, 1:1000), phospho-JNK (mouse monoclonal, 1:2000), phospho-p65 (mouse monoclonal, 1:1000), phospho-H2A.X (γ -H2A.X, rabbit polyclonal, 1:1000), β -actin (rabbit polyclonal, 1:2000), and GAPDH (rabbit polyclonal, 1:3000) antibodies were obtained from Cell Signaling Technology (Waltham, MA, USA).”

(11) How was quantification of western blots performed should be included. Software for densitometric scanning, etc.

Response: Western blot quantification was performed using Image J. This information was added to the “*Materials and Methods*” section, as suggested.

(12) Results. In general, comparisons are only established between LPS/D-Gal treated wt and Recq15-deficient samples, no mention to control levels (saline treatment) is normally done.

Response: Although the reviewer makes a good point, the data from the saline treatment can reflect whether LPS/D-Gal treatment works. Thus, we kept the control data.

(13) Figure 1B shows liver images of wt and Recq15-deficient animals. The later seem to be larger, but no reference to this aspect is made into the text. These images are presented as an example of severe liver hemorrhage, but this is difficult to see. The authors should evaluate the extent of the hemorrhage in another form, such as liver hemoglobin concentration.

Response: As suggested by the reviewer, we added a reference to “Eukaryotic elongation factor 2 controls TNF- α translation in LPS-induced hepatitis” (*J Clin Invest* 2013; **123**: 164-178) in the discussion of Figure 1B. To further confirm liver hemorrhage, we also performed hematoxylin and eosin staining, which is presented in Figure 1C.

(14) Figure 2B only shows cleaved caspase-3, in my opinion the image should include both cleaved and uncleaved bands together with their sizes.

Response: As suggested, the uncleaved caspase-3 bands were added to Figure 2B.

(15) Cytokines were only examined by qRT-PCR and in my opinion the actual levels of the cytokines should be measured.

Response: We agree with this critique. We measured TNF- α and IL-6 levels, and found that serum TNF- α and IL-6 levels were also significantly increased in the knockout mice after treatment, as compared to WT mice. These data were added to Figure 3, and the text in the “results” was modified accordingly.

(16) Western blots of ERK and JNK and their phosphorylation are shown (Figure 3D), and the text states the existence of significant or non-significant differences, depending on the protein examined. However, no quantification of the data is included, and whether the correction is established against GAPDH or between phosphorylated and unphosphorylated proteins is not explained.

Response: Per the reviewer’s suggestion, we quantified the ERK, JNK, and p65 phosphorylation with Image J. These data were added to Figure 3.

(17) In my opinion, examination of oxidative stress requires additional measurements including: 1) activities of catalase, NOX and glutathione reductase; and 2) ROS and glutathione levels.

Response: To further examine oxidative stress, we performed additional measurements, including an evaluation of catalase and glutathione reductase activity, as the reviewer suggested. We found that, in Recql5-deficient mice, catalase and glutathione reductase activity significantly reduced after treatment. These data were added to Figure 4, and the text in the “results” was modified accordingly.

(18) Given the role of Recql5 helicase in DNA repair, some insights into DNA damage.

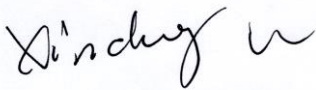
Response: Per the reviewer’s suggestion, we detected the levels of γ -H2A.X, a DNA damage repair biomarker. We found that γ -H2A.X levels significantly increased in Recql5-deficient mice after treatment, as compared to WT mice, indicating increased DNA damage in knockout mice. These data were added to Figure 2C and the text in the “results” was modified accordingly.

3 References and typesetting were corrected

Response: Yes.

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

Sincerely yours,



05/24/2015

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