

ANSWERING REVIEWERS



August XX 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 11861-review.docx).

Title: Nitrite, a novel method to decrease ischemia/reperfusion injury in the rat liver

Authors: Bergthor Björnsson, Linda Bojmar, Hans Olsson, Tommy Sundqvist, Per Sandström

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 11861

1 The manuscript has been improved according to the recommendations from the editorial review:

- 1 The language has been reviewed by www.aje.com; please find the certificate attached
- 2 Columns "research report" has been chosen
- 3 The authors' contributions have been stated according to the editor's comment; please find the changes highlighted.
- 4 The presentation of the numerical results and statistical significance in the abstract has been changed according to the editor's comment; please find the changes highlighted.
- 5 The abbreviations list has been configured according to the editor's comment and "IR" has been changed to "I/R" when appropriate for consistency; please find the changes highlighted.
- 6 The headings in the results section have been adapted to the editor's comments; please find the changes highlighted.
- 7 The reference to "unpublished data" in the discussion chapter has been replaced by a reference to the published data.
- 8 The comments have been written according to format for "research report"; please find the changes highlighted.
- 9 References have been added according to the editor's comment

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

- (1) "...the author should show more data for histological analyses such as the number of animals used for each figure" The number of animals has been added in the text ("Histology", line 5-6); please find the changes highlighted.
- (2) "...the scores should be represented using a graph." Figure 9 has been added to graphically present the distribution of the scores.
- (3) "...and more photomicrographs should be shown with higher magnification" Figure 10 (previously Figure 9) has been expanded to include a higher magnification.
- (4) "The Authors have three groups so they cannot use the t-test to assess statistical significance, rather analysis of variance should be used." Thank you for this valuable comment. In this study, sham animals are included only to show the effects of anesthesia and surgery. The aim of the study is to investigate

the possible effects of nitrite on liver IRI. To achieve this, a group of animals receiving nitrite is compared to a group of animals subjected to IRI alone. At no point did we include the sham animals in these statistical comparisons, and the use of ANOVA would only increase the risk of type II error. To clarify this further, an extra comment on this has been added to the Methods (Statistical analysis), line 3-4, "*No statistical comparisons were made between the study groups and the sham group*"; please find the changes highlighted.

- (5) "*The Authors should determine the levels of antioxidant defenses in the liver of the three groups at the end of reperfusion.*" This is an important issue. The study focuses on the possible protective effects of nitrite administered before liver ischemia/reperfusion. The main end point is tissue damage measured through the levels of transaminases in blood as well as with microscopy. Additionally, evidence of anaerobic metabolism and cell membrane damage in microdialysate is used to further support the findings. Analysis of antioxidant defenses, although highly relevant to the topic in general, was not in the scope of this research and was therefore not included here.
- (6) "*The Authors should give at least a speculative explanation about how nitrite administration protects the liver from IRI.*" Possible explanations have been added to the discussion (line 18-19) "*Thus, administration of nitrite may increase the bioavailability of NO and, in that way, contribute to less IRI.*" Additionally, (discussion, line 37-38) further explanations have been added, including "*Dampening of the electron transport chain will decrease the oxidative stress and may therefore be important in reducing IRI*"; please find the changes highlighted.
- (7) "*How do the Authors explain the increase of NO_x in the sham group at the end of the observational period?*" This has now been clarified in the following sentence in the discussion "*In the sham group, an increase in MD NO_x was noted during the experiment, this is most likely consistent with the inflammatory response to laparotomy*"; please find the changes highlighted.
- (8) "*Authors perform histological evaluation and liver function tests at 4 hours and observe a significant difference but have they checked at longer follow ups?*" Thank you for this important remark. Because the study is only looking at the first 4 hours of reperfusion, it is unclear whether the changes observed in this study during the early phase of reperfusion reflect irreversible damage to the liver. This has been added to the discussion (lines 52-55) as "*When interpreting these findings it has to be kept in mind though that the results are obtained after a short period of reperfusion and therefore it is unclear if the damage continues during later phase.*"; please find the changes highlighted.
- (9) "*The Authors state, in the Methods, that animal body temperature was kept between 3 and 39°C during surgery. Is this correct? It seems quite a wide range of body temperatures. If this is a mistake, please correct.*" Thank you for finding this mistake. This has now been changed to the correct "*...between 37 and 39°C*"; please find the changes highlighted.
- (10) "*In the liver transaminase result section authors write that in the sham operated group AST and ALT were normal after reperfusion but if they were sham operated how could they have had a reperfusion*" Thank you for pointing out this writing error, which has now been corrected. Please find the changes highlighted.
- (11) "*The histology scores although different seem to point out very low values, was the damage altogether relatively non significant ?*" Figure 9 has been added to show the distribution of the total scores. For comments on the significance of these findings during early reperfusion, please see 8 above.

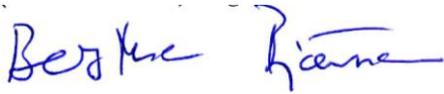
3 Additional changes:

- (1) An author who was missing in the original submission has been added along with his affiliation
- (2) The address of the corresponding author has been changed to match his affiliation address
- (3) Figures have been improved according to "8 TABLES AND ILLUSTRATIONS" in "Format for

research report”

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

Sincerely,

A handwritten signature in blue ink that reads "Bergthor Björnsson". The signature is written in a cursive style with a long horizontal stroke at the end.

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