

January 07, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: ESPS Manuscript NO 7486-review.doc).

Title: A murine model to study brain, behavior and immunity during hepatic encephalopathy

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Name of Journal: *World Journal of Hepatology*

ESPS Manuscript NO: 7486

The authors are grateful for all the comments provided by the editor and reviewers on the manuscript. We have improved it according to your suggestions. All changes are highlighted in yellow in the manuscript, as required.

First, all modifications suggested by the editor were performed. These include:

- 1 Addition of running title, post code, author contributions and core tip
- 2 References were fully formatted
- 3 Abstract was fully revised
- 4 Inclusion of COMMENTS section

The reviewers' comments are replied as below:

Reviewer 00071753

1 The authors presented the result in the last 6 lines of the introduction. This inaccuracy should be removed.

We agree and have removed this part of the introduction

2 The numbers of the reference in the text, mainly in the beginning of the method, seems to be wrong

We have thoroughly revised all references and formatting

3 The results presentations are very condensed in just two figures and should be split.

We thank the reviewer for the suggestion, but unfortunately the figure splitting would mean a different article format, and the other reviewers were comfortable with this figure organization. Thus, we respectfully disagree.

4 There are syntax and grammar errors in the manuscript.

The manuscript has been fully revised for grammar, syntax, spelling and punctuation errors.

Reviewer 00181520

1 Please modify the introduction: Omit the part starting from "Mice submitted to such TAA injection regime developed massive liver injury and inflammation....." till the end of the introduction. These are your results. You should not write them in this section.

As suggested also by the first reviewer, we accepted the changes and modified the text accordingly

2 Please modify the discussion: The first paragraph is a repetition of what you wrote in the introduction and methods. Start with the second paragraph straight away.

We have excluded the first discussion paragraph as suggested

3 References: Revise reference number 14. Add the volume and page numbers.

We have revised and formatted all references as suggested

Reviewer 01560721

1 TAA has been used in rat models of ALF and HE. The authors developed a murine model of ALF and HE using TAA. However, I do not understand the efficacy of this new murine model. You should describe the efficacy of this model compared with the previous model using TAA.

Previous animal models in the literature failed to reproduce all the different clinical signs of hepatic encephalopathy using only a single TAA dose [Farjam M, Dehdab P, Abbassnia F, Mehrabani D, Tanideh N, Pakbaz S, Imanieh MH. Thioacetamide-induced acute hepatic encephalopathy in rat: behavioral, biochemical and histological changes. *Iran Red Crescent Med J* 2012]. This might be due to acute TAA administration causing too much liver injury and high mortality rate, precluding the observation of neuronal symptoms of liver failure. In our model we used lower TAA dose for longer periods (48hs) which reproduces a closer clinical pathology.

2 The authors show MR image in the brain. MR revealed a discrete, but diffuse brain edema in TAA-treated mice. However, there are no pathological findings in the brain. You should investigate the pathological findings, especially brain astrocytes.

We find the reviewer comment very interesting, but unfortunately the focus of our manuscript has been around animal response and phenotype, and not the specific cellular effects caused by TAA intoxication. Further studies regarding neuron biology and astrocyte response are certainly needed but we believe it to belong to another work.

3 The authors show hyperammonia in the brain. However, there are poor data in the brain. Do you explain the mechanisms of HE in TAA- TAA-treated mice?

We disagree that the data are poor in the brain. We have provided data regarding brain ammonia, EEG readings and MR imaging in live mice. As mentioned before, this work is not focused on the cellular mechanisms behind HE, but rather on how the mice respond to intoxication and what symptoms can we observe in this model.

4 Minor points 1. In the Introduction, lines 26-31, these sentence "Mice submitted to such...due to liver failure." should be changed to the results section.

The authors agree. Changes have been made as suggested

Sincerely yours,

A handwritten signature in black ink, appearing to read 'Gustavo B Menezes', enclosed within a large, stylized circular flourish.

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