ESPS PEER-REVIEW REPORT

Reviewer's code: 00182114

Author concluded that treatment with glutamine prevents gut mucosal injury after partial portal vein ligation model. I ask some questions. 1. Please tell me the portal pressure of SO,SO+G,PPVL and PPVL+G. 2. I think that portal hypertension is a key factor of gut injury by PPVL. I think glutamine reduce portal pressure and ameliorate all the intestinal histopathological changes, with reduction of edema and vasodilatation. Please tell me the comment between portal pressure and glutamine. Does glutamine reduce portal pressure in this study? 3. Antioxidant enzymes such as SOD, GPx and CAT play critical roles in oxidative stress protection by converting ROS into less harmful products. How about intestinal level of SOD,GPx and CAT in author's study?

Answering questions from the reviewer **00182114:**

1 and 2. We do not intend to decrease the portal pressure with glutamine, what we want is precisely to evaluate the possibility of therapeutic action of glutamine in the intestine of the animal with increased portal pressure. The pressure will not decrease because the cause of hypertension is a permanent mechanical blockage (the ligature).

3. Catalase does not present good expression in the large intestine of rats, according to previous studies in our service (Dis Colon and Rectum 2001; Toxicology 2007), for this reason we no measure this enzyme, anymore, in our service. We only sdudied the activity of the glutathione peroxidase enzyme.

Reviewer's code: 02789449

Título: Glutamine prevents oxidative stress in a model of portal hypertension The current work evaluates the protective effects of glutamine in a model of portal hypertension induced by partial portal vein ligation. It is a well-writen paper, however the authors should take into account these minor points to improve the manuscript: 1. Abstract: "Largest area of staining", the total

surface measured should be provided. The authors should be consistent in the abbreviations, once abbreviation appears should be kept through the text, such as control group = SO. 2. Materials and Methods In the section Animals, The conditions of humidity, illumination, temperature, number of subjects per cage…should be provided. In the section Evaluation of eNOS and iNOS, the deparaffinization method is borlada explained, however the authors must cut it off. On the contrary, nothing is said regarding the histology methods after the sectioning at 3 microns. This part should be detailed. Regarding the paragrapha explaining the antibodies staining, it will be recommended to adjust to well known protocolos which are effortless and clearest than the one employed here. Analysis of digital images: The systemic method by which the images were taken, before quantification, should be clarified. Discussion The third paragraph should be removed due to the lack of relevant information provided related to the current work. "there are substances with antioxidant properties…" The authors should include a paragraph in which hepatic encephalopathy will be linked to inflammation as it has been previously demonstrated (Physiol & Behavior 149 (2015):247; Advances in Bioscience and Biotechnology 3 (2012): 881). This will provide a broad vision regarding HE involved systems.

Answering questions from the reviewer **02789449:**

1. Abstract: At least 10 random, non-overlapping images of each histological slide with 200X magnification (44 pixel = 1 µm) were captured. The sum means of all áreas, of each group were calculated and these are shown in figures 3 and 5. Correct abbreviations are SO; SO+G; PPVL and PPVL+G. There are two control groups: one that received the vehicle (Nacl), called SO and another that received glutamine, called SO + G. I will make the correct corrections in the text.

2. Materials and Methods: Twenty-four male Wistar rats, weighing between 250 and 350 grams, were used from the State Foundation for Research and

Production in Health (FEPPS-RS). The animals were divided into 4 groups of 6. During the experiment, the animals were kept in plastic boxes of 47x34x18cm lined with wood, in a cycle of 12 hours light / dark and temperature between 20 and 25ºC. Water and feed are given ad libitum. The protocols of this research were approved by the Ethics Committee for the use of animals (CEUA) of PUCRS registry 5985/14.

Regarding the paragraph explaining the staining of the antibodies, the same method was used already by us described in the article published by our group: **World J Gastroenterol 2014 August 28; 20(32): 11406-11414 ISSN 1007-9327 (print) ISSN 2219-2840 (on line**).

The systemic method used to take the images, before quantification, already by us described in the article published by our group: **World J Gastroenterol 2014 August 28; 20(32): 11406-11414 ISSN 1007-9327 (print) ISSN 2219-2840 (on line**).

3. Discussion: The third paragraph of the discussion was removed. We find interesting to include a paragraph describing the inflammation associated with hepatic encephalopathy. We use the suggested references for the subject.

Best regards,

Gilmara Pandolfo Zabot

Gustavo Franco Carvalhal

Norma Possa Marroni

Francielli Licks

Renata Minuzzo Hartmann

Vinícius Duval da Silva

Henrique Sarubbi Fillmann