

## RESPONSE TO REVIEWERS

October 23, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format.

**Title: The pathophysiology of cerebral oedema in acute liver failure**

**Author: Scott T, Kronsten V, Hughes RD, Shawcross DL.**

**Name of Journal:** *World Journal of Gastroenterology*

**ESPS Manuscript NO:** 5467

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

1 The format has been updated

2 Revision has been made according to the suggestions of the reviewer as follows:

(1) Reviewer 1

*(i) Their comments on the role of transcranial sonography in this setting are scarce. Please, describe the patterns associated with high intracranial pressure.*

Response – This section has now been expanded.

*(ii) Please, discuss the potential interest of cerebral microdialysis in these patients (mainly as an investigating tool) since it allows measuring different metabolites that can be involved in brain edema.*

Response – This has now been added.

*(iii) The recommendation of blood pressure and Cerebral perfusion pressure in these patients (diastolic blood pressure > 40 mmHg higher than ICP and CPP higher than 70 mmHg) can be very difficult to achieve in these patients. In addition, literature supporting these recommendations is poor.*

Response – We do agree with the reviewer on this point and recommendations are often based on data obtained from experimental ALF animal models and on anecdotal experience from larger liver centres as clinical trial data are scarce. We have revised this section to reflect this.

*(iv) Please, support your recommendations. - Barbiturates can be used as a last resort therapy. However, not all the barbiturates are equal and, in addition, they have hepatic metabolism. Do the authors recommend thiopental or pentobarbital? In TBI, thiopental was more effective in reducing high ICP. Please, discuss.*

Response – There is really only one good published (albeit historical) study of the use of thiopental infusion in 14 ALF patients with raised ICP as assessed by extradural monitoring by Forbes et al..

No major side effects were reported but in our experience barbiturates are rarely if ever used now. This section has been revised to reflect this.

## (2) Reviewer 3

*(i) This is a comprehensive review of the current mechanisms and viewpoints towards cerebral edema in chronic liver failure. The author discussed the already well accepted knowledge like the astrocyte swelling, hyperammonemia too much. My suggestion is to slim the discussion on the already familiar results and focus on the controversy and new progress.*

Response – We think that this reviewer has misinterpreted our review as it only pertains to the discussion of the pathogenesis of cerebral oedema in acute liver failure and not chronic liver failure as referred to above. We have endeavored to slim down the discussion on ammonia and astrocyte swelling as much as possible and to focus on areas of controversy such as the debate around whether cytotoxic or vasogenic mechanisms are more predominant and on new areas of research such as the rapidly expanding field of oxidative stress and neuroinflammation.

*(ii) Studies of recent 3 years are not fully covered in this review. Some of them are very important. The author should complete this. For example (1) Bosoi, C. R.; Parent-Robitaille, C.; Anderson, K.; Tremblay, M.; Rose, C. F. AST-120 (spherical carbon adsorbent) lowers ammonia levels and attenuates brain edema in bile duct-ligated rats. *Hepatology* 53:1995-2002; 2011 (2) Bosoi, C. R.; Yang, X.; Huynh, J.; Parent-Robitaille, C.; Jiang, W.; Tremblay, M.; Rose, C. F. Systemic oxidative stress is implicated in the pathogenesis of brain edema in rats with chronic liver failure. *Free radical biology & medicine* 52:1228-1235; 2012. (3) Bosoi, C. R.; Rose, C. F. Oxidative stress: a systemic factor implicated in the pathogenesis of hepatic encephalopathy. *Metabolic brain disease* 28:175-178; 2013. (4) Montoliu, C.; Cauli, O.; Urios, A.; ElMlili, N.; Serra, M. A.; Giner-Duran, R.; Gonzalez-Lopez, O.; Del Olmo, J. A.; Wassel, A.; Rodrigo, J. M.; Felipo, V. 3-nitro-tyrosine as a peripheral biomarker of minimal hepatic encephalopathy in patients with liver cirrhosis. *The American journal of gastroenterology* 106:1629-1637; 2011. (5) Qu, J.; Lu, X. Hydrogen: a promising novel treatment for hepatic encephalopathy? *Free radical biology & medicine* 63:457-458; 2013.*

Response: Once again the first four references suggested to be added here pertain to hepatic encephalopathy/brain oedema in the context of chronic liver failure and not acute. We were not able to access reference 5 as this has not yet been published and the abstract is not available on Pubmed.

## (3) Reviewer 4

*(i) There are several relevant aspects of the field which are not properly covered. The manuscript must be modified taking into account the following points: 1. Role of lactate. A relevant contributor to cerebral edema in ALF seems to be lactate. Several articles support this role. For example: Zwingmann et al (2003) show that increased brain lactate synthesis and impaired glucose oxidative pathways rather than intracellular glutamine accumulation are the major cause of brain edema in ALF. A role for lactate is also supported by Rose et al (2007) and Bernal (2010). The role of lactate must be discussed and (at least) the above studies must be mentioned. Zwingmann C, Chatauret N, Leibfritz D, Butterworth RF. Selective increase of brain lactate synthesis in experimental acute liver failure: results of a [H-C] nuclear magnetic resonance study. *Hepatology*. 2003 Feb;37(2):420-8. Rose C, Ytrebø LM, Davies NA, Sen S, Nedredal GI, Belanger M, Revhaug A, Jalan R. Association of reduced extracellular brain ammonia, lactate, and intracranial pressure in pigs with acute liver*

failure. *Hepatology*. 2007 Dec;46(6):1883-92. Bernal W. Lactate is important in determining prognosis in acute liver failure. *J Hepatol*. 2010 Jul;53(1):209-10.

Response – Although we had already made reference to the Zwingmann and Bernal papers mentioned above, we had not included the Rose publication or expanded the discussion on the role that increased lactate may play in cerebral oedema in ALF. This has now been amended.

(ii) *Brain region differences of the effects and mechanisms and in their temporal progression. It has been clearly shown by Cauli et al (2011) in rats with ALF that the type of edema (vasogenic or cytotoxic) and the mechanisms involved in their induction are different in different brain areas, specially between cerebellum and cortex. Cerebellum shows vasogenic edema while cortex shows cytotoxic edema. Moreover, the time course of the appearance and progression of edema is also different in different brain areas. These aspects are very relevant and must be discussed and (at least) the above study must be mentioned. Cauli O, López-Larrubia P, Rodrigo R, Agusti A, Boix J, Nieto-Charques L, Cerdán S, Felipe V. Brain region-selective mechanisms contribute to the progression of cerebral alterations in acute liver failure in rats. Gastroenterology. 2011 Feb;140(2):638-45.*

Response - This important observation had already been discussed in the conclusions and perspectives part of the manuscript including specific reference to this study. We have expanded upon this discussion further including raising the issues of the mechanism of brain oedema varying temporally and between different brain regions.

(iii) *Another aspect which should be mentioned is that "The persistence of hyperammonemia, rather than its level determines brain glutamine levels, which correlate with IP" (Tofteng et al, 2006) Tofteng F, Hauerberg J, Hansen BA, Pedersen CB, Jørgensen L, Larsen FS. Persistent arterial hyperammonemia increases the concentration of glutamine and alanine in the brain and correlates with intracranial pressure in patients with fulminant hepatic failure. J Cereb Blood Flow Metab. 2006 Jan;26(1):21-7.*

Response – This important observation and reference has now been added.

(iv) *In several parts the references provided for some statements are not appropriate. Works confirming data from previous studies are cited to support the ideas. The original articles showing the idea must be cited (instead or in addition). For example:*

4.1. Page 6: *"Neuroinflammation is now widely considered to result from a direct interaction between microglia and ammonia". The article showing that hyperammonemia activates microglia is: Rodrigo R, Cauli O, Gomez-Pinedo U, Agusti A, Hernandez-Rabaza V, Garcia-Verdugo JM, Felipe V. Hyperammonemia induces neuroinflammation that contributes to cognitive impairment in rats with hepatic encephalopathy. Gastroenterology. 2010 Aug;139(2):675-84.*

4.2. Page 10 (end) and 11 (beginning): *"Free radicals such as NO and superoxide can be categorised into reactive nitrogen and oxygen species (RNOS), respectively. In cultured astrocytes and in rat brain in vivo, ammonia triggers their formation through N-methyl-D-aspartate (NMDA)-receptor and calcium (Ca<sup>2+</sup>)-dependent mechanisms". The original article showing that NMDA receptors*

*mediate oxidative stress induced by hyperammonemia in rats in vivo is: Kosenko E, Kaminski Y, Lopata O, Muravyov N, Felipe V. Blocking NMDA rec.*

Response – Thank you for pointing this error out to us. Rodrigo R et al. has now been added. With regard to the Kosenko E et al. paper, this had already been included in the manuscript but reference to it at this point had been omitted in error. We apologise and this has now been rectified.

3 References and typesetting have been corrected.

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

Sincerely yours,

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