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ESPS Peer-review Report
Name of Journal: World Journal of Gastroenterology
ESPS Manuscript NO: 9451
Title: TLNa Ma signaling and the inhibition of liver hepcidin expression by alcohol
Reviewer code: 00012403
Science editor: Na Ma
Date sent for review: 2014-02-13 08:29
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CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
[ ] Grade A (Excellent)	[Y] Grade A: Priority Publishing	Google Search:	[ ] Accept
[Y] Grade B (Very good)	[ ] Grade B: minor language polishing	[ ] Existed	[ ] High priority for publication
[ ] Grade C (Good)	[ ] Grade C: a great deal of language polishing	[ ] No records	
[ ] Grade D (Fair)		BPG Search:	[ ]Rejection
[ ] Grade E (Poor)	[ ] Grade D: rejected	[ ] Existed	[ ] Minor revision
		[ ] No records	[Y] Major revision

COMMENTS TO AUTHORS

The authors investigated the implication of TLR4 signaling in the activation of hepcidin in mice chronically fed with alcohol. They showed that suppression of hepcidin mRNA levels by alcohol in wild type mice was lost in TLR4 mutant ones. The authors claim that this effect is related to modulation of NFkB and Stat3 signaling in TLR4 mutant mice. Although the data on hepcidin suppression is clear cut, data presented concerning NFkB, Stat3 and SHP is much weaker. Additional data need to be presented to make the story more convincing. Major points: 1. Quantitative data (e.g. densitometry analysis) should be shown for all Western blots. 2. How can the authors conclude that NFkB is not activated in ethanol fed mutant mice. Figure 2 clearly shows strong NFkB phosphorylation in the cytosol but also up-regulation in the nucleus in TLR4 mutants. The reviewer is not convinced that nuclear levels between wild type and mutant mice are really significantly different especially when the relative increase to their respective controls is taken into consideration. What happens to IkB? Is it down-regulated? Does it still bind NFkB in the cytosol?? Is there a possibility that NFkB is activated independently from TLR4? What happens to the TNFR1 which could be implicated in NFkB activation? 3. The authors claim that NFkB DNA-binding is inhibited. Although the p50 subunit can bind DNA (which is absent in mutant mice), additional experiments investigating p65 DNA-binding are required as this sub-unit contains the principal DNA binding domain of active NFkB (p50-p65 heterodimers) and western blots have focused on this sub-unit to suggest inhibition. In addition, the examination of some typical NFkB responsive genes should be done to further sustain some functional inhibition of NFkB . 4. Reduction of Stat3 phosphorylation after ethanol exposure has already been reported. As Stat 3 is not a direct target of TLR4 it is somehow surprising that TLR4 mutants show higher levels of p-stat3. Are there any

functional consequences of these differences? Is Stat3 DNA-binding altered? Is activation of key Stat3 target genes different in both groups?? 5. Modifications of signalling pathways in the liver by ethanol are likely cell specific. To the reviewer's knowledge, hepcidin is principally synthetized by hepatocytes. It would, therefore, be of interest to do some immunohistochemistry analyses in order to investigate which cells are affected by these modifications (kupffer cells?, hepatocytes?, sinusoidal endothelial cells? Etc.). 6. Although there is protein-protein interaction between SHP and NFkB, sustaining that there are significant differences between wild-type and mutant animals, in particular in nuclear extracts and immunoprecipitates is not possible from the presented data in figures 5 and 6. Minor comments 1. The methods section does not mention how cytosol and nuclear extracts have been prepared. At least some information should be given. 2. The discussion over-interprets the results. The data are not as strong as the authors want to make them and conclusions should be formulated more cautiously. For example, a formal interaction between TLR4, Stat3 and hepcidin transcription has not been established by this study; or, alcohol induced dissociation of the SHP-NFkB complex has not been clearly demonstrated in this paper etc.

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ESPS Peer-review Report
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CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
[ ] Grade A (Excellent)	[ ] Grade A: Priority Publishing	Google Search:	[ ] Accept
[ ] Grade B (Very good)	[Y] Grade B: minor language polishing	[ ] Existed	[ ] High priority for publication
[Y] Grade C (Good)	[ ] Grade C: a great deal of language polishing	[ ] No records	[ ]Rejection
[ ] Grade D (Fair)	[ ] Grade D: rejected	BPG Search:	[Y] Minor revision
[ ] Grade E (Poor)		[ ] Existed	[ ] Major revision
		[ ] No records	

COMMENTS TO AUTHORS

Major 1. Do you have respectively semiquantitative graph for Figure 2 to Figure 6. It is necessary to give readers semiquantitative graph, besides statistical symbol is also important. 2. How many animals are there in the different groups? The number of animals should be point out in the section of Materials and Methods and the footnote of each graph. 3. This study investigate the TLR4 signaling and the inhibition of hepatic hepcidin expression in mice under chronic ethanol feeding. And the inhibition of hepcidin may cause iron overload together with inflammation response. Therefore an inflammation response outcome in mice is necessary, do you have any histopathological changes photo? or data for hepatic inflammation cytokines concentration? Minor 1. In figure 4, TLR4 signaling inhibition the phosphorylation of stat3, however, what is the factor that increase stat3 phosphorylation in the ethanol group with TLR4 mutant type? 2. The first paragraph of discussion showed the role of TLR4 signaling pathway in ALD together with the aim of the study, however this has described in the section of introduction, please simplify or delete it.