Peer Review File

Article information: http://dx.doi.org/10.21037/atm-20-5110

Author Reply: We thank the reviewers for their careful review of our manuscript and have incorporated many of their suggestions delineated below which we believe have greatly improved this review.

Reviewer A: The topic is very interesting and manuscript is well written with excellent figure included, therefore, although very informative, it is easy to read the manuscript in its present form. However, there are certain points that could be addressed and improve the quality of this review manuscript:

Comment 1: Authors should include critical analysis with discussion of strengths and weaknesses of important studies that are cited.
Reply 1: We have attempted to provide more critical analysis to this review

Comment 2: Manuscript is very descriptive, therefore authors should include some quantitation of data cited wherever is possible.
Reply 2: We have provided more quantitative data where applicable. E.g. bile acid concentrations in liver;

Comment 3: A table summarizing important studies with proposed mechanisms and whether data obtained are confirmed in cultures only, in animal models or humans would be helpful.
Reply 3: We now add a table with this information

Reviewer B
- Major (general)
Comment 1: The authors input unpublished data often into this manuscript in support of their selected publications and synthesis of conclusions therein. It is this reviewer's recommendation to remove these mentions of unpublished data if they are not in submission for publication currently.
Reply 1: We have removed all but one reference to unpublished data

Comment 2: There are very few mechanistic views into the process of cholestatic liver injury, although not a fault of the authors, this statement should be removed as part of the abstract.
Reply 2: We have now not only modified the abstract by providing some mechanistic view of how liver cells respond to bile acids but also removed this statement from the abstract

- Major (hepatocyte subsection)
Comment 3: The authors consider the induction of cholestatic liver injury to occur upon the accumulation of bile acids within hepatocytes. This theory is controversial and should be presented as such.
Reply 3: We now mention this controversy in the introduction and later in text

Comment 4: The authors bring light to an interesting new development in the hepatic response to bile acid injury, however, they also disregard the role of receptors and transcription factors that play an anti-inflammatory role such as FXR which was mentioned to "not play a role in bile acid induction of inflammatory cytokines…" Although its role is through suppression of NFκB, this must be mentioned to increase substance of this review. (PMID: 18972444, 27634375)
Reply 4: A section on the role of FXR has been added

- Minor (hepatocyte subsection)
Comment 5: Missing space in page 6 "It is known thatbile acids…"
Reply 5: Corrected

Comment 6: Spell out OSTα/OSTβ first time mentioned.
Reply 6: Corrected

Comment 7: There are new publications that would be beneficial to the topic of hepatocyte inflammatory response following bile acid injury that should be included to strengthen the subsection of this review (PMID: 31887435).

Reply 7: We have now added this reference and information on the inflammasome to the section on immune cell response to bile acids.

Comment 8: There is a focus on TCA in this manuscript, the authors should explain reason of focus on this bile acid early on in the analysis and summary of published studies in this review (mentioned in cholangiocyte subsection).
Reply 8: TCA is a major bile acid in the rodent and human. We now discuss the different bile acid species early in the manuscript

Comment 9: Transition into TLR9 seems abrupt and effort should be made to introduce this study in cohesive manner so reader is not lost during its transition.
Reply 9: We now provide an introduction to make a better transition to the data on TRLs

Comment 10: Page 9 the following sentence should be re-written "Thereafter, bile regurgitated initially into single cells and then spread to adjacent sinusoids, resulting in sinusoidal membrane leakage and hepatocyte death and the formation of bile infarcts."
Reply 10: This sentence has been rewritten

Comment 11: Spell out TNFα first time in page 9.
Reply 11: done

<u>- Major (cholangiocyte subsection)</u>
Comment 12: The authors fail to mention transporters and receptors in cholangiocytes such as FXR, ASBT, and MRP3. These should be included since cholangiocyte pathobiology is greatly affected during cholestatic liver injury (PMID: 21103970, 15133850, 9352879, 23720296, 9389734).
Reply 12: We now include. specific mention of all major bile acid transporters and bile acid receptors in cholangiocytes in this section and add several references.

Comment 13: The role of bile acids interaction with cholangiocytes should be deeper explored. These cells have, as the authors mentioned, a “debatable” response to bile acids which makes it all the more important to demonstrate these phenotypes in this review.
Reply 13: We recognize this concern and have included as much information about the effects of bile acids on cholangiocytes as we are able as this remains an area that needs further research.

- Minor (cholangiocyte subsection)
Comment 14: The authors should retitle subsection or explain what bile acids are considered “cholestatic bile acids” within first couple of sentences.
Reply 14: The term “cholestatic” has been removed from the heading of this section

Comment 15: No mention of CFTR and its role for bicarbonate secretion with AE2.
Reply 15: CFTR is now mentioned in connection with activation of AE2

- Major (immune subsection)
Comment 16: There is no mention of innate immune cell response during cholestatic liver injury. The authors are emplored to include this phenomenon since it deepens the scientific impact of the review/ (PMID: 30150987, 30150987, 30143751, 11861947).
Reply 16: We have expanded text on the role of the innate immune system in cholestatic liver injury recognizing that there is limited information in the liver in contrast to intestine.

- Minor (stellate cell subsection)
Comment 17: The authors do not mention ferroptosis in this subsection which has been implicated in HSC activation during BDL.
Reply 17: We have not been able to locate this reference.