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August 26th, 2016

Scientific Editor:
World Journal Gastroenterology

Re: 28003 Revision Submission

Dear Dr. Jing Yu:

Enclosed please find our revised review article entitled *"**Prolonged Feeding with Guanidinoacetate, a Methyl Group Consumer, Exacerbates Ethanol-Induced Liver Injury**"* which we submit for publication in *World Journal Gastroenterology.*

We thank the reviewers for their constructive comments and valuable suggestions. We have revised the manuscript accordingly to their suggestions and the editors' comments and requests. Our responses to the comment are below the reviewer's individual comments. The changes in the revised manuscript are highlighted in red.

Reviewer 1:
Comment 1: *.Whereas the paper clearly shows the synergism between GAA and ethanol, a need to investigate the mechanisms by which this synergy exerts and increase in TG and cholesterol metabolism resulting in hepatosteatosis is still missing.*
Our Response: *Our study shows that the mechanism of hepatic injury by the combined treatment with GAA and ethanol is primarily due to the profound lowering of the hepatocellular SAM;SAH ratio and hyperhomocysteinemia. We have many seminal publications that show that such lowering of SAM;SAH ratio and elevated homocysteine blood levels can lead to increased hepatic lipid accumulation and increased adipose lipolysis. We had modified the discussion accordingly.*

Comment 2: An important point is to know whether prolonged administration of both GAA and ethanol would lead to hepatocellular carcinoma development and whether the observations are species-specific (rats, mice). Thus, this novel synergy could replace the current experimental models of ASH lacking the NASH component as observed after Lieber de Carli feeding and represent an alternative to this diet alone. Moreover, if HCC development exists in the chronic situation this would a very valuable experimental tool for the study of ASH.
Our Response: Yes, we believe that the combined treatment of GAA and ethanol for a prolonged duration will lead to HCC development in rats and mice as well as in human. Please note that GAA was used as a surrogate for a methyl consumer. When we combine both a methyl consumer like GAA and a methyl stressor like ethanol, there is a pronounced loss of SAM. Such losses have been shown to lead to HCC development (Lu *et a*l *Proc Natl Acad Sci* 2001; 98: 5560-5565; Lu and Mato *Alcohol* 2005; 35: 227-234). Thus, this model could be a very valuable experimental tool for the study of ASH

and HCC development. Furthermore, we agree that this model could replace the current experimental models of ASH lacking the NASH component as observed after Lieber de Carli feeding and represent an alternative to this diet alone.

Thank you for your consideration of our manuscript.

Sincerely,

Kusum K. Kharbanda

Kusum K. Kharbanda, Ph.D.