March 18, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 6631-Review) and the editorial certificate from American Journal Experts: http://www.aje.com in PDF format (file name: EDITORIAL CERTIFICATE).

Title: Recent insights into the mechanisms of alcohol fatty liver disease

Author: Jinyao Liu

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 6631

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

1 Format has been updated

2-1 Revision has been made according to the suggestions of the reviewer-1 (Reviewer code: 02822528)

(1) The author stated that TNF-α overproduction induced AFLD. It is well known that TNF-α overproduction is closely coupled with alcoholic liver injury. However, the role of TNF-α in the induction of AFLD remain uncertain. Whether it is a primary player or whether its appearance is secondary to hepatic injury? It seems more likely that TNF-α promotes the progression of alcoholic liver disease rather than initiates fat accumulation in the liver. Please commentate.

I agree that TNF-α promotes the progression of alcoholic liver disease rather than initiates fat accumulation in the liver. I commentated that at the end of page 11 with the following additional paragraph "Collectively, the previous studies have shown that TNF-α overproduction is closely coupled with alcoholic liver injury. However, the role of TNF-α in AFLD induction remains

uncertain. It seems more likely that ethanol-induced TNF-α overproduction regulates lipid metabolism-associated transcription factor gene expression (SREBP and PPAR-α) as well as induces PAI-1 and Egr-1 gene expression, promoting the AFLD progression"

(2) Although endotoxin-induced systemic inflammatory state can reduce PPAR-α expression, the author did not cite any reference to directly support his statement that TNF-α overproduction is responsible for the decrease of PPARα activity in alcoholic fatty liver. It is better for the author not to state that activated Kupffer cell-derived TNF-α over-production is responsible for the dysregulated PPARα activity.

This is a statement of fact. I re-edited sentence "Alcohol activates the innate immunity and induces an imbalance of the immune response followed by activated Kupffer cells-derived tumor necrosis factor (TNF)-α overproduction, which is responsible for the changes in the hepatic SREBP-1, PAI-1, Egr-1, and PPAR-α activity" to "Alcohol activates the innate immune system and induces an imbalance of the immune response, which is followed by activated Kupffer cell-derived tumor necrosis factor (TNF)-α overproduction, which is in turn responsible for the changes in the hepatic SREBP-1 and PAI-1 activity" in Abstract

(3) On page 5, line 1-4, the author took PAI-1 as transcriptional factor. However, PAI-1 is not a transcriptional factor.

I agree that PAI-1 is not a transcriptional factor, and re-edited sentence "and then focuses on the roles of lipid metabolism-associated transcription factors, such as sterol regulatory element-binding protein (SREBP)-1, peroxisome proliferator-activated receptor (PPAR)-α, plasminogen activator inhibitor (PAI)-1, and early growth response (Egr)-1, in the pathogenesis of AFLD" to "and then focuses on the roles of lipid metabolism-associated transcription factors (sterol regulatory element-binding protein [SREBP]-1) and plasminogen activator inhibitor and peroxisome proliferator-activated receptor [PPAR]-α), plasminogen activator inhibitor (PAI)-1, and early growth response (Egr)-1 in the pathogenesis of AFLD" on page 5, line 2-5.

(4) On page 9, line 20, the sentence "TNF-α has been shown to increase hepatic lipid synthesis by an increase in lipolysis" is confusing.

I renewed the reference and re-edited sentence "TNF-α has been shown to increase hepatic lipid synthesis by an increase in lipolysis" to "TNF-α has been shown to increase hepatic fatty acid synthesis

by increases in hepatic acetyl CoA carboxylase (ACC) and fatty acid synthase (FAS) activities" on page 10, line 2-4.

2-2 Revision has been made according to the suggestions of the reviewer-2 (**Reviewer code:** 00187937)

(1) **Major issue that authors should focus on why some heavy drinkers do not progress to alcoholic steatohepatitis, although some mild alcoholics do? This mechanism may explain the complexity and multifactorial reasons underlying in the pathogenesis of AFL.**

I agree with that and added "Taken together, these studies are support the possibility that AFLD is a complex disease where subtle interpatient genetic variations and environment interact to produce the disease phenotype and determine disease progression [37]. This may partly explain why some heavy drinkers do not progress to alcoholic steatohepatitis, while some mild alcoholics develop steatohepatitis" on page 7, 1st paragraph.

(2) **Minor issues;**

① **In title, instead of 'alcohol' authors should use 'alcoholic'.**

Considering another reviewer's suggestion (Reviewer code: 00159944), I changed the title to "Ethanol and liver: recent insights into the mechanisms of ethanol-induced fatty liver". I hope, it is also suitable for your suggestion.

② **Instead of the 1st reference that is from 2004, authors should use the new reference that reflects the new prevalence of the alcohol intake of all deaths worldwide.**

I used a new reference and re-edited sentence " In 2004, alcohol intake accounted for 3.8% of all deaths worldwide [1]" to "Based on 58 studies from 17 Global Burden of Diseases (GBD) regions, alcohol use disorders accounted for 9.6% (7.7–11.18) of age-standardized disability-adjusted life years (DALYs) worldwide in 2010 [1]" on page 1, line 1-4.

③ **In the last paragraph of introduction, authors should change one of the β1 as β2.**

I revised the manuscript and re-edited the "In addition, our recent research suggested that carvedilol, which blocks the SNS completely via β1, β1, and α1 adrenergic receptors, could block the sympathetic hyperactivity-activated HSC feedback loop to down-regulate TNF-α overproduction, and thereby attenuate the progression of AFLD in rats" to "In addition, our recent research suggested that carvedilol, which blocks the SNS completely via β1, β2, and α1 adrenergic receptors, could block the sympathetic hyperactivity-activated HSC feedback loop to down-regulate TNF-α overproduction, and thereby attenuate the progression of AFLD in rats" in the last paragraph of introduction.

2-3 Revision has been made according to the suggestions of the reviewer-3 (**Reviewer code:** 02541357)

The author suggested that the understanding of these mechanisms could generate therapeutic interventions to reduce the progression of AFLD, however these possibilities were not well explored. He talks about the treatment with IL-6 and carvedilol, although there is no information regarding therapeutics with Pentoxifylline, anti-oxidant agents, anti-TNF-α for example. Also adding brief comments on these therapies under clinical perspective might make the article more interesting and complete for clinicians. Below are some articles which exploit the above mentioned therapies. Ashwin D Dhanda, Richard WL Lee, Peter L Collins, C Anne McCune. Molecular targets in the treatment of alcoholic hepatitis. World J Gastroenterol 2012; 18(39): 5504-5513. Stewart S, Prince M, Bassendine M, et al. A randomized trial of antioxidant therapy alone or with corticosteroids in acute alcoholic hepatitis. J Hepatol 2007;47:277-283. Phillips M, Curtis H, Portmann B, Donaldson N, Bomford A, O'Grady J. Antioxidants versus corticosteroids in the treatment of severe alcoholic hepatitis – a randomised clinical trial. J Hepatol 2006;44:784-790. Spahr L, Rubbia-Brandt L, Frossard JL, et al. Combination of steroids with infliximab or placebo in severe alcoholic hepatitis: a randomized controlled pilot study. J Hepatol 2002;37:448-455. Tilg H, Jalan R, Kaser A, et al. Anti-tumour necrosis factor-alpha monoclonal antibody therapy in severe alcoholic hepatitis. J Hepatol 2003;38:419-425. Naveau S, Chollet-Martin S, Dharancy S, et al. A double-blind randomized controlled trial of infliximab associated with prednisolone in acute alcoholic hepatitis. Hepatology 2004;39:1390-1397. Sharma P, Kumar A, Sharma BC, Sarin SK. Infliximab monotherapy for severe alcoholic hepatitis and predictors of survival: an open-label trial. J Hepatol 009;50:584-591. Menon KV, Stadheim L, Kamath PS, et al. A pilot study of the safety and tolerability of etanercept in patients with alcoholic hepatitis. Am J Gastroenterol 2004;99:255-260. Boetticher NC, Peine CJ, Kwo P, et al. A randomized, double-blinded, placebo-controlled multicenter trial of etanercept in the treatment of alcohol hepatitis. Gastroenterology 2008;135:1953-1960. Whitfield K, Rambaldi A, Wetterslev J, Gluud C. Pentoxifylline for alcoholic hepatitis. Cochrane Database Syst Rev 2009;(4):CD007339. Parker R, Armstrong MJ, Corbett C, Rowe IA, Houlihan DD. Systematic review: pentoxifylline for the treatment of severe alcoholic hepatitis. Aliment Pharmacol Ther 2013 Mar 13. Lebrec D, Thabut D, Oberti F, et al. Pentoxifylline does not decrease short-term mortality but does reduce complications in patients with advanced cirrhosis. Gastroenterology 2010;138:1755-1762.

I added following brief comment and the related references about the targets for therapy in alcoholic hepatitis and cirrohosis on page 19, paragraph 2 in the "SUMMARY". "Other therapy targets, such as CXC chemokines [143]; pentoxifylline, a phosphodiesterase inhibitor [144]; oxidative stress [144]; and TNF-α [144], for alcoholic hepatitis (AH) were reviewed by Orman [143] and Dhanda [144]. Pentoxifylline also reduces the complications in patients with advanced cirrhosis [145]. There have been some advances in our understanding of the pathogenesis and clinical characteristics of alcoholic liver disease. However, standardized nomenclature and histologic classifications are lacking; the animal models do not accurately mimic advanced alcoholic liver disease; and the pathophysiologic significance of the serum levels of biomarkers is unclear (due to impaired liver clearance and ongoing bacterial infections). Additional detailed studies on these potential targets in humans and animal models are urgently needed"

2-4 Revision has been made according to the suggestions of the reviewer- 4 (**Reviewer code:** 00159944)

Firstly, the title is too large, this reviewer is mainly focused on AFLD and TNF-α, I suggest the author grasp this main idea and fully discuss the relationship between AFLD and TNF-α, whether it is a cause or whether its appearance of AFLD?

I changed the title to "Ethanol and liver: recent insights into the mechanisms of ethanol-induced fatty liver"

Secondly, I suggest the author discuss the difference of AFLD and NAFLD.

I added the discussion and the related references about the differences of AFLD and NAFLD on page 19, paragraph 3 in the "SUMMARY" as following: "The pathophysiological significance of hepatic lipid accumulation in the absence of significant alcohol consumption, defined as NAFLD, is also increasingly recognized and regarded as the hepatic manifestation of the metabolic syndrome (substantially reviewed by Leamy [146] and Mitake [147]). Both AFLD and NAFLD encompass mild fatty liver to steatohepatitis with significant necroinflammation and progressive fibrosis. However, the interaction between alcohol and obesity is poorly understood, and it is unknown whether the combined effects of alcohol and obesity on the progression of liver injury progression are additive or synergistic. It is important to describe the single individual and combined effects of alcohol and the metabolic syndrome on both hepatic steatosis and other organs to understand the differences between AFLD and NAFLD."

Moreover, the author did not point out the different roles of TNF-α in AFLD and other diseases. It is well known that TNF-α participates in the normal inflammatory response and immune response as well as pathological condition. TNF-α overproduction can occur in many diseases, for instance, Rheumatoid arthritis (RA)、ulcerative colitis (UC) and so on. TNF-α inhibitor has been used for the treatment of some diseases, such as RA and UC. What is the difference in the treatment of AFLD and other diseases? There are a series of problems to be solved in practical applications of TNF-α inhibitor. In my opinion, the author should analyze these differences briefly. It is better to make evaluation for the treatment of AFLD.

I discussed and added the related references for the anti-TNF-α treatment in alcoholic liver disease, juvenile idiopathic arthritis, and ulcerative colitis on page 20 at the end of the "SUMMARY" as

following: "TNF-α has also been found to have a crucial role in alcoholic hepatitis, and small preliminary studies have evaluated the effect of anti-TNF therapy in this condition [148]. However, the use of anti-TNF-α drugs in alcoholic hepatitis is still controversial and needs to be investigated further. TNF-α overproduction also occurs in juvenile idiopathic arthritis [149] and ulcerative colitis [150], and a TNF-α inhibitor has been used to treat both conditions. However, neither prolonged nor tapering treatment seems to influence the risk of relapse [149]."

3 References and typesetting were corrected

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

Sincerely yours,

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