Format for ANSWERING REVIEWERS

August 26, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: ESPS Manuscript NO: 12723-review.doc).

Title: Pathogenesis and therapeutic approaches for non-alcoholic fatty liver disease

Author: Hye-jin Yoon, Bong-Soo Cha (Corresponding author)

Name of Journal: *World Journal of hepatology*

ESPS Manuscript NO: 12723

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

2 Revision has been made according to the suggestions of the reviewer

(1) Reviewer No. 2444976

The authors have written a review on drug mechanism and treatment outcome in NAFLD. The paper is well written but needs minor English review.

Response 1: We had a professional editor proofread our manuscript.

I have several comments regarding specific drugs:

Pioglitazone- there is a concern re this class of drugs concerning risoglitazone and an increase in cardiac mortality.

Response 2: Thank you for your important suggestion. We agree and changed the content about rosiglitazone. Instead of rosiglitazone, we only introduce pioglitazone as a treatment drug for NAFLD. [1,2] Then, we added a comment about the safety of pioglitazone in regards to cardiovascular outcomes with attachment of articles that can prove this.[3,4] You can confirm this content in the manuscript.

Metformin-the authors should consider a comment that there is no evidence of an effect on hard clinical end-points such as mortality

Response 3: Thank for your valuable advice. We added a comment that explains the limitation of metformin in reduction of progression to liver cirrhosis, HCC, and death, because metformin is very limited in regards to histologic improvement of hepatic steatosis and steatohepatitis.[5]

Vitamin E-the authors should insert a comment that it is recommended for treatment in non-diabetics since in diabetics there are concerns re an increase in mortality in diabetics.

Response 4: Thank for your valuable advice. We inserted a comment that vitamin E is not suitable in treatment of NASH patients with diabetes, because high dose vitamin E supplement is related with increased mortality in chronic disease, such as type 2 diabetes.[6,7]

Lipid-lowering- I think there is a need to mention that initial resistance to using statins due to a concern following reports of hepatic side-effects with early statins . There is now sufficient data from real world use of statins to confirm that hepatotoxicity is extremely rare.

Response 5: Thank you for your important advice. Statins are very important drugs, but there are concerns for its use in chronic liver disease. As you recommended, we inserted a comment that several studies report statins rarely induce serious liver injury.[8-11]

Clistazol- I suggest the authors include a reference showing a decrease in hepatic steatosis, inflammation an fibrosis in a CDAA mouse model. Fujita et al Gut 2008;57:1583-91.

Response 6: Thank you for your valuable recommendation. We inserted a comment about the article that you recommended to us. [12] You can confirm this comment in the manuscript.

PUFA-I suggest they cite in addition a good review by Bouzianis et al Nutr Rev 2013;71:753-71. MUFA-there is evidence that a high MUFA diet attenuates hepatic steatosis in obese rats. Hak et al Prostaglandins Leukot Essent fatty Acids 2013:89;301-40.

Response 7: Thank you for your valuable recommendation. We cited the article as you recommended. You can confirm this citation in the manuscript. [13] However, we did not find Hak et al.'s article. So we cited another article [14]that shows that MUFA attenuates hepatic steatosis in obese rats.

(2) Reviewer No. 2715281

This study is an extensive review on the therapeutic approach for non-alcoholic fatty liver disease. As such, the information presented is interesting; however, the paper has several limitations:

1. Although the meaning is mostly clear, quite a few sentences are grammatically incorrect. The manuscript needs editing by someone with expertise in technical English editing, and particular attention should be paid to English grammar, spelling, and sentence structure so that the paper is clear to the reader.

Response 1: Thank you for your important suggestion. We received English review via expert. And we correct the manuscript according to expert's opinion.

2. Some of the references that are cited do not seem to be those of recent studies. This review should consider all published data on the therapeutic approach for non-alcoholic fatty liver disease available.

Further, the authors should consider citing more (recent) studies in their paper. A few examples are listed below:

Response 2: Thank you for your recommendation. We inserted the articles that you recommended.

Fan H et al., Arq Bras Endocrinol Metabol. 2013 [15]

Thank you for your valuable advice. We insert a paragraph about GLP-1 agonist, and cite the article that you recommended. [15,16]

Xiao J et al., Hepatobiliary Pancreat Dis Int. 2013[17]

We insert a comment about these articles. The name of article 'Therapeutic approaches to non-alcoholic fatty liver disease: past achievements and future challenges' cited in life style modification paragraph.

Di Minno MN et al., World J Gastroenterol. 2012[18]

The name of article 'Omega-3 fatty acids for the treatment of non-alcoholic fatty liver disease.' is cited in PUFA and MUFA paragraph.

Hokari A et al., World J Gastroenterol. 2012[19]

We insert a paragraph that is explained the MK615 and the article that you recommend is cited in this paragraph.[19-21]

3. The title of this manuscript is not suitable, as it does not accurately reflect the major topic and contents of the study. In this review, the authors investigated not only the current therapeutic options and new candidate drugs for the treatment of NAFLD, but also the mechanism of hepatic fat accumulation, focusing on the role of insulin resistance.

Response 3: Thank you for your important suggestion. We decided to change our article's main title, as this article mainly focused on pathogenesis and treatments of NAFLD. As the Chief Editor limited the words of title (less than 12words), we changed our title to 'Pathogenesis and therapeutic approaches for non-alcoholic fatty liver disease'

4. Use only standard abbreviations. The authors should avoid the usage of abbreviations as much as possible. Avoid abbreviations in the abstract.

Response 4 : Thank you for the valuable advice. We wrote the full terms throughout the Abstract. And we also changed to standard abbreviations throughout the manuscript.

5. Please include a section that emphasizes the contributions of the authors to the study.

Response 5: Thank you for your valuable advice. We included a section that introduces our

contributions to this study in the conclusion paragraph.

6. The article did not explain the pictures and tables in detail. Place explanatory matter in footnotes. Explain in footnotes all non-standard abbreviations that are used in each table. Be sure that each table and each picture is cited in the text. If you use data from another published or unpublished source, obtain permission and acknowledge the source fully.

Response 6: Thank you for the valuable advice. We checked that each table and each picture is cited in the text. We also cited all articles used in the Table.

Figure 1: We explained Figure 1 in detail adding footnotes. We also explained all non-standard abbreviations that are used in Figure 1. We designed Figure1 using Microsoft Power point program. We revised the figure legend as shown below.

Table 1: We summarized data of the manuscript. This table did not follow any other sources.

Figure1. Mechanism of hepatic insulin resistance and the key pathway of drug action

Delivery of FFAs to the liver and skeletal muscle is increased in insulin resistance conditions, and these are metabolized via mitochondrial beta oxidation. Consequently, hyperglycemia and increased hepatic FFA uptake reduce glucose uptake and oxidation in skeletal muscle. Diet and exercise are the main treatment strategies for this pathogenesis; insulin sensitizers and MUFA may contribute to reducing peripheral insulin resistance. Pioglitazone and fenofibrate act on β-oxidation of mitochondria and reduce hepatic steatosis. Accelerated β-oxidation also causes increased production of ROS. Vitamin E can reduce oxidative stress. Adipose tissue inflammation of the liver leads to inflammatory activation of hepatic Kupffer cells via classic response and produce inflammatory cytokines. This is also associated with decreased adiponectin levels and promotes hepatic steatohepatitis. Pentoxifylline inhibits TNF-α and alleviates steatohepatitis. Hyperglycemia caused by insulin resistance up-regulates lipogenic gene expression, such as SREBP-1c and ChREBP, and induces lipogenesis in hepatocytes. Cilostasol may inhibit SREBP-1c.

FFA, free fatty acid; TG, triglyceride; CPT-I, carnitine palmitoyltransferase-I; ACC, acetyl-CoA carboxylase; ATGL, adipose triglyceride lipase; ChREBP, carbohydrate responsive element binding protein; SREBP-1c, sterol regulatory element binding protein-1c; TCA, tricarboxylic acid; ROS, Reactive oxygen species; IRS, insulin receptor substrate; DAG, diacylglycerol; G-6-P, Glucose 6-phosphate; TNF-α, tumor necrosis factor- α; MUFA, monosaturated fatty acids; M1, Kupffer cells activated via classic pathway

Table 1. Treatment with various regimens

n, number; US, ultrasonography; RCT, randomized controlled trial; &, and

(3) Reviewer No. 2444760

The manuscript of 'Therapeutic approach for non-alcoholic fatty liver disease: focus on drug mechanism and treatment outcome' reviews the pathogenesis and treatment of non-alcoholic fatty liver disease (NAFLD), with the emphasis on drug mechanisms and therapeutic outcome. Being summarized by this review, insulin resistance (IR), oxidative stress, and adipokine-based inflammation take the central place in the spectrum of NAFLD, especially non-alcoholic steatohepatitis (NASH). Thiazolidinedione, Metformin, Vitamin E, Pentoxifylline, Fibrates, Ezetimibe, and other options (i.e., Cilostazol, polyunsaturated fatty acids (PUFAs), monosaturated fatty acids (MUFAs)) serve as effective agents for the therapy of NAFLD. These results add a new level to our knowledge about NAFLD, and may be valuable for both experimental research and clinical interference of this ever-growing disease.

Major comments 1. As described by the review, 'The pathogenesis of NAFLD development is closely associated with insulin resistance and dyslipidemia, especially hypertriglyceridemia', and 'Increased serum levels of free fatty acid (FFA) and glucose can cause oxidative stress in the liver……and lead to ectopic fat accumulation, especially in the liver.' These mechanisms related to two-hit hypothesis have already been well established by previous reviews. However, some novel pathological disorders that underlie NAFLD, such as gut microbial alternation , gut-liver crosstalk, endoplasmic reticulum stress, and TLR/MyD88 signaling-mediated innate immune response, are not mentioned.

Response 1: Thank you for the valuable advice. As the reviewer suggested, we added the content about ER stress, TLR/MyD88 signaling-mediated immune response, and gut microbial alternation. We added two paragraphs to demonstrate the pathogenesis of NAFLD via ER stress, Gut-microbial alternation, and TLRs stimulation.
ER stress [23-27]
Gut-microbial alternation and TLRs stimulation [28-35]
2. Drug-based treatment of NAFLD reflects the other topic of this review. Both mechanisms and outcome of TZDs, Metformin, Vitamin E, Pentoxifylline, Fibrates, and Ezetimibe has been detailedly discussed. But this review seems to ignore some up-to-date progression in this field. For example, GLP-1 has recently been uncovered, both experimentally and clinically, to serve as important regulator of serum glucose, dyslipidemia, and NAFLD.
Response 2: Thank you for your valuable advice. We inserted a paragraph about GLP-1 agonist, and cited the article that you recommended. [15,16]
Minor comments 1. Secondary Title, which explains the pathogenesis (insulin resistance - FFA flux and hyperinsulinemia, role of oxidative stress – Mitochondrial dysfunction, inflammation and adipokines) and different therapeutic drugs, is suggested in the text
Response 3: Thank you for your important suggestion. We decided to change our article's main title, because as you mentioned, this article mainly focus on pathogenesis and treatment of NAFLD. Thus, we also felt that changing the main title is more reasonable. Because Chief-editor limited the word of title (less than 12words), we changed our articles main title to 'Pathogenesis and Therapeutic approach for non-alcoholic fatty liver disease'.

3 References and typesetting were corrected

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

Sincerely yours,

Peter Laszlo LAKATOS, MD, PhD
1st Dept. of Medicine
Semmelweis University
Budapest, Koranyi 2A
H-1083-Hungary
Fax: +36-1-313-0250
E-mail: kislakpet@bell.sote.hu

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