UNIVERSIDAD AUTÓNOMA DE NUEVO LEON
FACULTAD DE MEDICINA
Departamento de Bioquímica y Medicina Molecular

Monterrey, NL México September 4th, 2015

Lian-Sheng Ma,
President and Company Editor-in-Chief
World Journal of Gastroenterology

Dear Editor,

Thank you for sending us the reviewers' comments on our manuscript entitled **"RELATIONSHIP BETWEEN OXIDATIVE STRESS MODULATION AND HCV THERAPY"** (ESPS Manuscript NO: 19163) and forwarding your helpful comments. We did a major revision on the manuscript and we wish to submit the revised manuscript for consideration for publication as an invited review article in your prestigious journal. We hope this new version is closer to meet the WJH acceptance criterions.

Attached you will find the new manuscript which contains the changes suggested by the reviewers, they were highlighted in the manuscript with underlined or deleted. In addition you will find a point –by-point response to the reviewers whit the changes described before. Mainly, the title was changed, epidemiological information was modified in introduction section, we include more key words, we include more recent references and the suggested by reviewers, we reviewed the grammar and writing errors as suggested by the three reviewers.

All the authors of this manuscript certify that:

a) This manuscript contains original work, has not previously been published and is not being considered for publication elsewhere.
b) All listed authors participated meaningfully in the study and all have seen and approved the final manuscript.

In addition, as you suggested we properly prepared, named and included the new manuscript and all accompanying documents as follow:

1 19163-Revised manuscript
2 19163-Answering reviewers
3 19163-Copyright assignment
4 19163-Audio core tip
5 19163-Conflict-of-interest statement
6 19163-Google Scholar
7 19163-CrossCheck
8 19163-Language certificate

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Thanks for your invitation and consideration, I am looking forward to hearing from you soon.

Sincerely,

Dr. Ana María Rivas-Estilla PhD
Chief of the Laboratory of Molecular Infectology
Department of Biochemistry and Molecular Medicine

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POINT- BY- POINT RESPONSE TO REVIEWERS (3)

Ms. Ref. No: **19163**

Title: Relationship between oxidative stress modulation and HCV therapy
(New title: Oxidative stress modulation in HCV infected cells)

World Journal of Hepatology

Remarks: Date: September 15th, 2015

Reviewers' Comments to Author:

REVIEWER #1:

Reviewed by 00071702:

Major concern: Oxidative stress imposed either directly by the virus or by the host immune response is considered an important pathogenic mechanism in HCV infection. Moreover, HCV is potentially lymphotropic, invading and propagating in cells of the immune system. It is known that synthesis and maturation of HCV proteins occur at the level of the ER but HCV proteins accumulate at the point of contact between the mitochondrial outer membrane and the ER. By transient fusion of the membranous sub-compartments, the viral proteins migrate from the ER to the mitochondria. Therefore, a direct interaction of HCV proteins with the mitochondrial machinery in hepatic and extra-hepatic sites is strongly posited. Impairment of mitochondria-nuclear cross talk through involvement of PI3 kinases has been recently demonstrated by Bhargava et al., 2011. This and other such recent advances with regard to redox signaling and HCV infection need to be included to provide a holistic understanding of the subject. This is certainly lacking in the current form of the manuscript.

Response: The reviewer is right, in the new version we included the suggested information and references. (Please see page 7 and 8).

Bhargava A, Raghuram G V., Pathak N, Varshney S, Jatawa SK, Jain D, Mishra PK. Occult hepatitis C virus elicits mitochondrial oxidative stress in lymphocytes and triggers PI3-kinase-mediated DNA damage response. *Free Radic Biol Med* [Internet] 2011;**51**:1806–14 [PMID: 21893189 DOI: 10.1016/j.freeradbiomed.2011.08.009]

Available from: http://dx.doi.org/10.1016/j.freeradbiomed.2011.08.009

Minor concerns: Abstract: The authors must avoid generalized statements in the abstract (first 2-3). Major implication on patho-physiology, drugs in pre- and clinical trials and potential leads in clinical translation must be highlighted.

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Response: We included the suggested information in abstract section (please see page 3)

Core tip: Immune perturbation following the infection and subsequent implications must be included.

Response: As reviewer suggested, we included the suggested information in core tip section (please see page 4)

Introduction: There is a lack of continuum in epidemiological details discussed in the 1st paragraph and available therapeutic options discussed in the 2nd. Include 2-3 sentences with regard to disease severity, prevalence and endemicity in beginning of the 2nd paragraph followed by why is it necessary to design newer molecules and therapies. HCV and oxidative stress: In this segment the authors must provide a complete understanding without invoking ER at the first place. Thrust on implication of redox-stress in both hepatic-extra hepatic sites must be categorically discussed, this will help the readers. Table: Inclusion of a table delineating various anti-oxidant therapies so far considered along with their mode of action, target, possible implication and failure must be included. References: Try and include more recent references.

Response: We are in agreement with the reviewer, in recent years a large number of information about the role of oxidative stress and viral replication have been published, we included some of this reports and most recent references about this topic (Please see page 7 - 9).
In addition Table 1 was improved including compounds tested as antioxidant therapies, its mechanism of action, dose range and its reference in the same table (Please see page 34).

Reviewer #2

Reviewer No. 02996776

This paper is a review where Lozano-Sepulveda *et al.* try to answer some questions about the relationship between viral and cellular proteins and the resulting regulation of oxidative stress in case of HCV infection. Authors describe molecular mechanisms of HCV-induced oxidative stress, while highlighting the effect of some molecules that modify the levels of cellular oxidative stress in HCV infected cells. It's worth to discuss data about the usefulness and the interaction of antioxidant therapy in case of HCV infection.
The objective of this review was explicitly stated, and through the text, the authors tried to answer the question with a kind of scientific rigour, by citing some of the important references to explain the regulation of Redox-system in normal and infectious situations. Figures and table are quite explaining the flow of idea and the conception of the review. The manuscript is concise, clear and comprehensive.
However, some minor issues should be considered prior to the publication
Minor issues:

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- In the introduction, the authors give a whole statistical review of liver diseases in USA. Is there any interest behind this? This is not explained, because HCV infection and associated liver diseases are major health problem worldwide, and there are even countries with higher prevalences. Furthermore, even the authors are not from USA to say that they are dealing with the problematic in their country!! So, please reconsider this paragraph.

Response: As the reviewer suggested we shortened information about USA epidemiology and rewrited the paragraph including global epidemiology of the disease.

- Please provide keywords

Response: We added keywords in page 2.

- Please review the text, there are many spelling mistakes.

Response: We checked the manuscript for spelling mistakes and also we add the English certificate.

- In abstract section, you need not to abbreviate HCV, twice (lines 3).

Response: As the reviewer suggested we performed the correction in HCV word in abstract section.

- There are many recent references on the field, please refresh your literature (please take a look following examples).

Response: As the reviewer suggested we included more recent refrences about the topic (please see references section at the end)

- Please number pages and lines

Response: As the reviewer suggested we included the number in each page and row in the new version of the manuscript.

- There are more recent data about the mechanisms of HCV proteins like core, E2 and NS5A in oxidative stress. Please update HCV and oxidative stress- section.

Response: As the reviewer suggested we included more recent refrences about the topic (please see references section at the end)
All these references were included in the new manuscript:

Ivanov AV, Smirnova OA, Petrushanko IY, Ivanova ON, Karpenko IL, Alekseeva E, Sominskaya I, Makarov AA, Bartosch B, Kochetkov SN, Isaguliants MG. HCV core protein uses multiple mechanisms to induce oxidative stress in human hepatoma huh7 cells.Viruses. **2015** May 29;7(6):2745-70.

Seo YL, Heo S, Jang KL. Hepatitis C virus core protein overcomes H2O2-induced apoptosis by downregulating p14 expression via DNA methylation.J GenVirol. **2015** Apr;96(Pt 4):822-32.

Ivanov AV, Smirnova OA, Ivanova ON, Masalova OV, Kochetkov SN, Isaguliants MG. Hepatitis C virus proteins activate NRF2/ARE pathway by distinct ROS-dependent and independent mechanisms in HUH7 cells.PLoS One. 2011;6(9):e24957.

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Ming-Ju H, Yih-Shou H, Tzy-Yen C, Hui-Ling C. Hepatitis C virus E2 protein induce reactive oxygen species (ROS)-related fibrogenesis in the HSC-T6 hepatic stellate cell line.J CellBiochem. 2011 Jan;112(1):233-43.

Dionisio N, Garcia-Mediavilla MV, Sanchez-Campos S, Majano PL, Benedicto I, Rosado JA, Salido GM, Gonzalez-Gallego J. Hepatitis C virus NS5A and core proteins induce oxidative stress-mediated calcium signalling alterations in hepatocytes.J Hepatol. 2009 May;50(5):872-82.

Ivanov AV, Smirnova OA, Ivanova ON, Masalova OV, Kochetkov SN, Isaguliants MG. Hepatitis C virus proteins activate NRF2/ARE pathway by distinct ROS-dependent and independent mechanisms in HUH7 cells.PLoS One. 2011;6(9):e24957.

-In “antioxidant therapy” section
There are recent data regarding antioxidant therapy against HCV. Also, it has been recently reported a protective role of amantadine in mitochondrial dysfunction and oxidative stress mediated by HCV. It should be better to report current information about the role of antioxidant therapy.

Response: As the reviewer suggested we included the data in the manuscript and more recent references about the topic (please see references section at the end and page 11 and 19). All these references were included in the new manuscript:

Hsiang CY, Lin LJ, Kao ST, Lo HY, Chou ST, Ho TY. Glycyrrhizin, silymarin, and ursodeoxycholic acid regulate a common hepatoprotective pathway in HepG2 cells. Phytomedicine. 2015 Jul 15;22(7-8):768-77.

Morgan TR, Osann K, Bottiglieri T, Pimstone N, Hoefs JC, Hu KQ, Hassanein T, Boyer T, Kong L, Chen WP, Richmond E, Gonzalez R, Rodriguez LM, Meyskens FL. A phase II, randomized, controlled trial of S-adenosylmethionine in reducing serum alpha-fetoprotein (AFP) in patients with hepatitis C cirrhosis and elevated AFP. Cancer PrevRes (Phila). 2015

Bunchorntavakul C, Wootthananont T, Atsawarungruangkit A. Effects of vitamin E on chronic hepatitis C genotype 3: a randomized, double-blind, placebo-controlled study. J Med AssocThai. 2014

Quarato G, Scrima R, Ripoli M, Agriesti F, Moradpour D, Capitanio N, Piccoli C. Protective role of amantadine in mitochondrial dysfunction and oxidative stress mediated by hepatitis C virus protein expression.BiochemPharmacol. 2014 Jun 15;89(4):545-56.

Yang M, Li N, Li F, Zhu Q, Liu X, Han Q, Wang Y, Chen Y, Zeng X, Lv Y, Zhang P, Yang C, Liu Z. Xanthohumol, a main prenylated chalcone from hops, reduces liver damage and modulates oxidative reaction and apoptosis in hepatitis C virus infected Tupaia belangeri.IntImmunopharmacol. 2013 Aug;16(4):466-74.

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Farias MS, Budni P, Ribeiro CM, Parisotto EB, Santos CE, Dias JF, Dalmarco EM, Fröde TS, Pedrosa RC, Wilhelm Filho D. Antioxidant supplementation attenuates oxidative stress in chronic hepatitis C patients.GastroenterolHepatol. 2012,35(6):386-94.

-**References section**
Reference 5: please give volume number and page numbers.
Response: We included the suggested correction in reference section.

REVIEWER #3

Reviewer 2947057

This review describes interaction of oxidative stress with HCV infection and discusses treatment options. It lists several substances considered as (more or less effective) antioxidants in hepatitis C, and tries to elucidate pathophysiological mechanisms. From a clinical point of view, antioxidants could be interesting in resource-limited settings as an alternative to the costly Directly Acting Antivirals (DAAs) to reduce inflammatory activity.
Unfortunately, most of the data cited are quite old and need to be updated. I recommend to shorten the introduction.
Response: As the reviewer suggested we updated the information and reduced the introduction section.

It seems advisable to focus the epidemiology data on poor-resource regions since patients in most industrialized countries will receive DAAs.
Response: As the reviewer suggested we shortened information about USA epidemiology and rewrited the paragraph including global epidemiology of the disease.

The conclusion should weigh the substances, and the authors should state which one might be most interesting and might be worthwhile further research, either in combination with DAAs or alone.
Response: As the reviewer suggested we highlighted this information about antioxidant compounds and its use together with new DAAS and rewrited the paragraph.

Table 1 should include the literature. It seems difficult to mix in vitro data together with clinical trial results in one table.
Response: Table 1 was improved including compounds tested as antioxidant therapies, its mechanism of action, dose range and its reference in the same table (Please see page 40).

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The style is acceptable, but there are several mistakes in spelling and punctuation. The title is not optimal since “therapy” of HCV is not in the focus of the review. I would suggest “Oxidative stress modulation in HCV infected patients” as the main title.

Response: As the reviewer suggested we changed the title by the new one “Oxidative stress modulation in HCV infected patients”, and we checked the manuscript for grammatical errors.

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