



Clara Balsano, PhD,
Wan-Long Chuang, MD. PhD,
Editor-in-Chief,
World Journal of Hepatology

November 28, 2014

Dear Dr. Balsano and Chuang,

Please find enclosed the edited manuscript in Word format (file name: 13667-review.doc).

Title: Control of oxidative stress in hepatocellular carcinoma: helpful or harmful?

Author: Akinobu Takaki, Kazuhide Yamamoto

Name of Journal: *World Journal of Hepatology*

ESPS Manuscript NO: 13667

The manuscript has been revised and the format has been updated according to the suggestions of the reviewers. Changes are shown in the manuscript in red underlined text and our detailed responses to the reviewers are listed below.

We hope that the manuscript will now be deemed suitable for publication in *World Journal of Hepatology*.

Sincerely,

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Responses to the reviewers

1. Reviewer 69855

In this review article, the authors reviewed the literature in recent years and try to explain the relationship between oxidative stress and hepatocellular carcinoma (HCC). Although it is a common topic, the authors cited most recent publications and stated the mechanism of oxidative stress increases the risk of HCC and possibility of anti-oxidant treatment for HCC. The references cited were rich and new, the writing is fluent, and the statement is reasonable. There is no ethic problem concerned. According to these features, I am recommending to publish it.

Response: Thank you very much for your comment.

2. Reviewer 01518980

The work is a review of oxidative stress in HCC. Specific comments 1. The article is poorly focused and many irrelevant topics are discussed. For example, the opening section has a detailed discussion of the side effects of current HBV/HCV therapy. There is a detailed description of the HBV replicative cycle. Functions of HBX are covered in depth, etc. 2. In contrast there is very little discussion of the supposed focus of this review, the role of oxidative stress in HCC. The HBV section concludes simply that antioxidant therapy is not indicated. For HCV there is no conclusion at all about the involvement of oxidative stress in HCC with this virus. 3. Several statements are not true. The involvement of oxidative stress in NAFLD is overstated and based on a very limited selection of the literature. Phlebotomy for iron removal is not accepted as being beneficial for NASH or HCV. 4. Therapies such as metformin that are not antioxidants are discussed. 5. Minor comments. Single sentence paragraphs should be eliminated. The figure is very busy.

Response

1. We deleted the descriptions of the side effects of HBV/HCV therapy and HBV replicative capacity as suggested.

2. We added more information about HBV-related HCC as follows (Page 8, Line 2):

“Such persistent aberrant cytokine production and the resulting ROS affect hepatocarcinogenesis.

The HBV genome encodes several gene products including DNA polymerase (Pol), capsid protein (core), envelope proteins L, M, S, and the multifunctional protein HBx, among which, the oncogenic potential of HBx protein has been analyzed in detail.”

Page 10, Line 3: “Mutational analysis of HCC-related HBV has emphasized the importance of HBx, suggesting oxidative stress involvement in the development of HCC. The envelope protein pre-S is also involved in the development of HCC and the pre-S region probably affects the hepatocarcinogenesis pathway via oxidative stress.

Variants of pre-S in the pre-S2 start codon and/or deletions of the 5'-terminal of the pre-S2 region and a pre-S1 mutation with deletions of the 3'-terminal of pre-S1 have been identified in HCC-associated HBV[50, 51]. These pre-S region mutant products induce the accumulation of mutated L protein in the endoplasmic reticulum (ER) of hepatocytes. The ER is a membranous network that functions in the synthesis and assembly of secretory and membrane proteins that correlate with the cellular stress response known as ER stress. Numerous experiments have confirmed the potential pro-oncogenic role of pre-S mutated gene products via accumulation in ER together with enhanced ER stress and ROS[51]. Pre-S mutated proteins accumulating in the ER can trigger c-Raf-1/Erk2 signaling, resulting in AP-1 and NFkB activation and enhanced proliferation of hepatocytes and an increased incidence of liver tumors in transgenics[52]. Pre-S2 mutated proteins also have non-ER related functions interacting with Jun activation domain-binding protein 1 (JAB1) that result in cyclin-dependent kinase inhibitor p27 degradation and cell cycle progression [53].

Oxidative stress must be involved in HBV related hepatocarcinogenesis possibly via HBx and weakly via pre-S related functions.”

Page 11, Line5: “Anti-oxidants should logically be useful for treating HBV-related HCC because HBx and pre-S proteins seem to have promising effects on oxidative stress. However, several studies have found weaker effects on oxidative stress in human serum as well as in vitro, which might lower expectations for this type of therapy.”

3. Page 17, Line 11: We changed this to, “Although iron depletion in NASH remains a matter of debate, it might function as an antioxidant treatment strategy.”

4. Page 17, Line 17: We state that, "Metformin is an anti-diabetic drug that also has anti-oxidative properties."
5. Single-sentence paragraphs have been deleted and the figure has been simplified.

2. Reviewer 00068720

The paper reviews the importance of oxidative stress in hepatocarcinogenesis and of control strategies for the optimal survival of patients with chronic liver disease (CLD) and hepatocellular carcinoma. However, antioxidant therapy has not yielded favorable results, and a balance between oxidative and anti-oxidative responses are important. So the harmful about control of oxidative stress in hepatocellular carcinoma should be described in detail, some additional explanations for further research would improve the overall content of this manuscript. ? ? 1. The "Mechanisms of hepatocarcinogenesis" part should be more organized, and give more evidence for oxidative stress increases hepatocarcinogenesis. ? 2. In the "Preferential antioxidative drugs to treat HCC" part, the author should put forward future direction of clinical trials, or prospects of each drugs. ? 3. The references are not in consistent form.

Response

1. The following statements have been added (Page 7, Line 5): "Oxidative stress could be induced via chronic hepatic inflammation regardless of etiology. Acute liver injury and hepatic inflammation induce ROS via the activation of neutrophils and Kupffer cells. Such cells invade liver parenchyma, indicating that hepatocytes could also be affected by the induced ROS. Although the plasma membrane blocks free superoxide diffusion, membrane superoxide dismutase can convert superoxide anions to H₂O₂ after internalization into hepatocytes^[20]. Mitochondria generate ROS as byproducts of the beta oxidation pathway for fatty acid metabolism through electron leakage from mitochondrial electron transport, resulting in the activation of oncogenic pathways^[21]."
2. The references and typesetting were corrected.

Thank you again for your comments and recommendations.