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World Journal of Gastroenterology

Bari, November 10, 2013

RE: 6114 revised

Dear Editors,
attached please find the revised version of the manuscript entitled "**The pathogenic role of oxidative and nitrosative stress in primary biliary cirrhosis"** for publication in *WJG*.

We thank the reviewers and the Editor for the comments which have been taken into the account for the revision of the manuscript. Accordingly, the new version includes tables and figures better describing the oxidative and nitrosative events having a role in the pathogenesis of the disease. In the introduction, mention to inflammatory processes involved in the onset and perpetuation of cholestatic liver injury has been made. Conclusions and perspectives have been enriched with some working hypothesis on pharmacological intervention.

We hope that the new version of the manuscript will be now suitable for publication in the special issue celebrating the 20th anniversary of *World Journal of Gastroenterology* (11: Cirrhosis: Pathogenesis, prevention, diagnosis, treatment, and evidence-based medicine).

Sincerely,

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WJG-6114 REVISED
Point by Point reply to the reviewers' comments

The authors thank the Editor and the reviewers for their comments. We have taken them into the account and accordingly we have revised the manuscript which now looks greatly improved.

Reviewer 1.

Authors focused on the important mechanism of the liver damage during PBS, the oxidative and nitrosative stress. Although review manuscript present summarization of the current knowledge, several problems and questions raised with the respect to complex pathophysiology of the disease.

Comments

The primary point is that PBC is the inflammatory condition, thus the oxidative stress and NO production are automatically involved. However I miss such association in the manuscript. Detailed relationship to NF-kB, cytokine, immune cell response should be added in this context and thoroughly discussed – is the oxidative/nitrosative stress cause or it is rather accompanying issue of PBS?

This point has been now included in the introduction. In particular, mention to PBC as a chronic inflammatory condition and the association between oxidative/nitrosative stress and inflammation has been made. Moreover, mention to cytokines and immune response has been briefly added, although it is not the main subject of the article. Finally, the significance of stress events as the main phenomenon or rather a second and reactive step in this inflammatory context has been briefly discussed.

Manuscript is very difficult to read and stay oriented. Sometimes seems that information is split to different part of manuscript -e.g. thioredoxin– perhaps separate chapter/paragraph would improve understanding of its position – together with some figure summarizing its function. Better organization of each chapter such as: executive mechanism – regulation – modulation would be very helpful.

A figure in which the activity of thioredoxin in nitrosothiols decomposition is already present in the article. An effort to improve the organization of each chapter has been made as it is now reported in the new version.

I missed figures and schemes to integrate regulation pathways, and inter-relationships. There are only one table and few pictures in the manuscript presenting mainly data of unknown origin and methodological background. The relationships to other mechanism such apoptosis, necrosis, autophagy may improve the picture.

New figures with schemes summarizing pathways and relationships better explaining the pathophysiological mechanisms of damage have been included. Figures containing previously unpublished data have been inserted in the article because we think they could be important to better explain the relationships. Methodological information has been inserted in the legends.

Authors claimed that the pathways offer possible therapeutic approach. Could the authors present the summary (table etc) of current knowledge from preclinical but especially from clinical studies about efficacy of antioxidants or nitric oxide modulators in PBS therapy (or in corresponding animal models). Also, there are some current strategies to treat PBC such as UDCA, how they can modify presented pathophysiological mechanisms?

The potential role of oxidative/nitrosative stress modulating agents have been included in the new version. These are mainly referred to UDCA whose actions are summarized in Table 2.

Changes in bile flow, and involved transporters with the role of responsible nuclear receptors are completely omitted. At least note and citations should be added + relationship to regulatory cascade of oxidative stress and NO production should be addressed. In this place – the question about regulatory role of bile acids in these processes should be specified.

The participation of glutathione and the involvement of nuclear receptors in bile flow formation have been included in the new version.

Separate paragraph about current status of oxidative and nitrosative status detection in patients with PBC and possibilities of its interpretation should be specified.

Interpretation of oxidative and nitrosative stress in patients with PBC is now included in their respective paragraphs.

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Summary tables of clinical studies is required, to support current knowledge about oxidative and nitrosative status and its importance for disease severity and progression – perhaps together with inflammatory markers.
A summary table on the protective effect of UDCA treatment in patients with PBC has been included.
The final sentence of conclusion/manuscript is not very clear – did author mend that modulation of oxidative stress is superior to currently used strategy by UDCA or perspective FXR receptor modulators?
The final sentence has been rephrased in order to include the importance of UDCA on stress and nuclear receptors modulation for the treatment of PBC.
Minor comment
Page 13 – problem with references 5 and 89, ...hepatocyte GMPc ?
These mistakes have been corrected.

Reviewer 2.
The manuscript by Grattagliano et al. provides a survey review of the pathogenetic role of oxidative and nitrosative stress mechanisms in primary biliary cirrhosis. In separate chapters they focus on oxidative and nitrosative stress, interactions of both stress types and on the role of hepatic mitochondrial changes. Overall the authors present a well reasoned and designed review that places oxidative and nirosactive stress, and hepatic mitochondrial changes at the center of a discussion about the pathogenesis of primary biliary cirrhosis.
Critical points:
1. The authors describe a lot of different mechanisms. Therefore, they should provide at least 2-3 figures explaining these mechanisms and concepts. This is especially necessary for the general readers.
Figures containing schemes elucidating some mechanisms of oxidative/nitrosative stress involvement in the pathogenesis of PBC have been included in the revised version.
2. For me is not clear why the authors present Table 1. Are the data from own investigations or from others? This is not explained in the text.
Table 1 contains previously unpublished data from our group. In the new version, methodological information has been inserted in the legend.
3. Also is not clear for me why thy provide figures 1-4 with relatively specific data from selected studies in the review. In addition, the figures are not explained in the text!. As mentioned in 1. the review merits some figures explaining mechanisms and concepts.
We agree with the reviewer. Figures have been inserted to better understand relationships between stress mechanisms. In the new version, methodological information has been inserted in the legends, and reference to figures has been reported in the text.

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