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Dr. Shui Qiu,

Science Editor, Editorial Office

World Journal of Nephrology

Dear Sir:

We thank both, reviewer and editor for their thoughts about our paper and for their useful comments to improve the clarity and quality of the manuscript entitled: "Immunohistochemical Expression of Intrarenal Renin Angiotensin System Components in Response to Tempol in Rats Fed a High Salt Diet"

According to the Editor and Reviewer requests, all these points are responded. Below we have listed reviewer's and editor´s comments, concerns and requirements, followed by our response and the corresponding changes introduced in the revised version of the manuscript (highlighted in yellow).

I look forward to hearing from you.

Yours sincerely,

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Reviewer 1#

COMMENTS TO AUTHORS

Cao et al. reported that a high sodium intake induced an imbalance between the hypertensive and anti-hypertensive components of the renal RAS in rats, leading to hypertension. Chronic antioxidant, tempol supplementation could improve the disbalance between the natriuretic and anti-natriuretic components of the renal RAS and decrease hypertensive blood pressure levels.

This study includes important results; however, there are some critical issues to be addressed.

Response:

We thank the reviewer for the positive comments and we included all the corrections in the revised version of the manuscript.

Reviewer concern:

Major 1. In order to confirm the results of immunohistochemistry in kidney sections, staining artifacts should be avoided. The authors should site the relevant references where the antibodies used in this study worked well in nephron segments, especially in tubules. If the authors applied the antibodies to the kidney firstly, positive and negative histological control should be made.

Response:

We thank the reviewer for pointing out this important issue. In fact we have purchased these antibodies from well known and prestigious laboratories as Peninsula, CA, USA, Santa Cruz Biotechnology, Inc USA and Abcam, Cambridge, Ma, USA, as it was stated in the manuscript. We have followed strictly instructions and recommendations from laboratories, for the use of these antibodies in rat research tissues. As requested by the reviewer, we have incorporated in Methods section a paragraph stating that the the antibodies used in this study worked well in nephron segments, especially in tubules, based on six citations (numbered 20 to 25) of the literature, that are included in the References Section of the revised version of the manuscript.

The following paragraph was included in Methods (page 8, lines 23-25):

"The antibodies used in this study worked well in nephron segments, especially in tubules [20-25]."

Reviewer concern:

2. In order to compare the results of immunohistochemistry among groups, the authors should use quantitative or semi-quantitative analysis of the staining areas of the target molecules.

Response:

We understand the reviewer concern. To compare the results of immunohistochemistry among groups, we have used quantitative analysis of the staining areas of the target molecules by means of image analysis software, which returns values of integrated optical density in a system previously calibrated with positive and negative controls. (Image-Pro Plus ver. 4.5 for Windows, Media Cybernetics, LP. Silver Spring, MD, USA), and the results were quantified and shown in bars in each corresponding figure.

To clarify this issue, and mention the quantitative method of analysis used, we have added the following paragraph in methods section (page 8, lines 25-28):

"Immunoreactivities for Ang II, ACE 2, AT1R, AT2R, Ang (1-7) and MasR in renal tissue are expressed as integrated optical density (IOD) ± SEM using a model for automated computer image analysis to quantify IHC stains in hematoxylin counterstained histological se sections [26].

Reviewer concern:

3. Since immunoblotting was not performed, the phrase "protein level using immunohistochemistry" (P3, L13-14) seems not to be appropriate. The results just showed "staining intensity".

Response

We thank the reviewer for pointing out this error. We have corrected the redaction in the revised manuscript, deleting "protein level" and changing it by "staining intensity". (page 3, lines 14, 18 and 20).

Reviewer concern:

4. The authors cannot lead the conclusion that "decreasing AngII degradation by down-regulation of ACE2" (P15, L4 from the last line) because of no mechanistic findings.

Response:

We thank the reviewer for pointing out this concern. We have corrected the conclusion in the revised manuscript, changing by "favouring increased Ang II and down-regulation of ACE2", a conclusion based only in the objective results.

The following paragraph was included in page 14, line 17-19:

"In conclusion, our results show that a high sodium diet may alter the physiological balance between opposing components of the renal RAS, favouring increased Ang II and down-regulation of ACE2."

Reviewer concern:

Minor

1. "Changes in in the homeostasis (P3, L8 from the last line)" should be "changes in the homeostasis".

2. "Although is well known (P6, L5)" should be "Although it is well known".

3. “rabbit Anti-MasR (P8, L17)” should be “rabbit anti-MasR”.

5. “SHR (P15,L17) should be “Spontaneously Hypertensive Rats”.

4. “Table should be placed in another sheet with headings”.

Responses:

1. The word “in” repeated twice was corrected and one “in” was deleted (page 3, line 22).

2- “is” was replaced by “it is” (page 6, line 6)

3- “Anti” was replaced by “anti” (page 8, line 18)

4. Table 4 was placed separately in another sheet with headings (page 24).

5. Full name of Spontaneously Hypertensive Rats was included (page 14, line 15).

We thank again the reviewer and hope to have satisfied all his concerns and requirements.

Reviewer2#
COMMENTS TO AUTHORS
Nutcracker phenomenon, also known as left renal vein entrapment, is characterized by impeded outflow from the left renal vein into the inferior vena cava due to extrinsic compression, often accompanied by demonstrable lateral dilatation and medial (mesoaortic) narrowing. Most typical nutcracker morphologic features result from compression of the left renal vein between the aorta and the superior mesenteric artery, known as anterior nutcracker. Less often, a retroaortic or a circumaortic renal vein is compressed between the aorta and the vertebral body, which is called posterior nutcracker

We think that this comment must correspond to another manuscript and not to our manuscript. In fact, in our work we described the “ Immunohistochemical Expression of Intrarenal Renin Angiotensin System Components in Response to Tempol in Rats Fed a High Salt Diet” and has not any relationship with nutcracker phenomenon, which is a pathology characterized by impairment of renal circulation by extrinsic compression and is not produced by high salt diets.