ANSWERING REVIEWERS

July, 16, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 2429-review.doc).

Title: ACE and ACE2 in kidney disease

Author: Sonoo Mizuiri, Yasushi Ohashi

Name of Journal: *World Journal of Nephrology*

ESPS Manuscript NO: 12177

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

Rsponse to reviewer 00506327

1. Page 9, the summary sentence at the end of the page is correct for glomerular but not tubular ace 2 regarding concurrence in biopsies from patients with type 2 diabetes and glomerular findings first reported in the db/ db model of this disease by Ye at al JASN 2006. That is ace is increased and ace 2 decreased similar to human biopsies as stated at the glomerular level. Differences at the tubular level are difficult to find by immunostaining but by western blot and enzymatic activities the studies in mice and rats with diabetes , db/db and STZ show decreased ace and increased ace 2, please see the recent review by Soler et al in NDT where all this is discussed. ref 12. 2. page 4 ,

   Response: We agree with the reviewer's comment and we included ref 32, 33 in the new text (P10, L3-L6) and rewrote the summary sentence as below (P11, L6-L11).

   "In summary, human biopsy studies indicate that established diabetic nephropathy is associated with increased ACE expression and decreased ACE2 expression at both the glomerular and tubular levels. However, the majorities of animal studies indicate that diabetes is associated with increased ACE and decreased ACE2 similar to human biopsies as stated at the glomerular level, whereas in kidney tubules ACE2 is clearly upregulated and ACE is downregulated."

   We also included ref.33 in Table 1.
2. it should also be stated that ace2 was localized in podocytes and ace in glomerular endothelial cells by immunogold staining by Ye et al ref 13 3. page 6,

   Response: We thank for the reviewers' comment. According to your suggestion, we rewrote the localization of ACE2 (P5, L4 –L6).
3. a ratio is only a ratio and cannot be regulated as such. It may be convenient but each enzyme is regulated differently even if the final purpose is the joint regulation of the peptides each one hydrolyzes.

   Response: We agree with the reviewer's comment and we rewrote as below (P7, L6-L8 and P7 L8 from the bottom-L6 from the bottom).

   "Batlle et al. reported that whilst the ACE/ACE2 ratio is a convenient tool, it might be misleading because each enzyme is regulated independently[11], even if the final purpose is the joint regulation of the peptides each one hydrolyzes."

"However, renal ACE/ACE2 ratio data should be interpreted with caution, since

ACE/ACE2 ratio is only a ratio and is regulated independently (P6, L3-L4)."

4. Please modify the sentence. 4. page 14, the authors cite work with XNT used as presumed ace2 activator but do not seem aware of recent work showing that both XNT and Dice actually are NOT ace 2 activators. Please see Haber et al Hypertension 2014 this should be cited in the context of the study cited showing that XNT was not effective ...
   Response: We thank for the reviewers' comment. We cited Haber et al.'s report (ref 61) and rewrote about ACE2 activator in the new text (P16, L6 from the bottom-L3 from the bottom).

Response to reviewer 00608278

Provide more information of the clinical implication including biomarker etc. 4. Please carefully check the reference format. For example, give numbers and several references' style.
Response: We thank for the reviewers' comment. We added the section of clinical implication of circulating ACE2 and urinary ACE2 in the new text (P14, L8 from the bottom-P15, L1 from the bottom). We carefully checked references.

1 Format has been updated

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

3 References and typesetting were corrected

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

Sincerely yours,

Sonoo Mizuiri

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