Author's response to reviews

Title: Benazepril hydrochloride improves diabetic nephropathy and decreases proteinuria by decreasing ANGPTL-4 expression

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Author’s response to reviews:

Manuscript ID: BNEP-D-15-00140

Title: Benazepril hydrochloride improves Diabetic nephropathy and decreases proteinuria via decreasing ANGPTL4 expression

Dear Dr. Danielle Talbot,

Thank you for your prompt decision letter concerning our revised manuscript (ID: BNEP-D-15-00140). We greatly appreciate for your time in editing our manuscript and the valuable comments and suggestions. Accordingly, we have revised the manuscript in track changes mode. The point-by-point answers to the comments were listed as below. In addition, we corrected some grammatical and clerical errors. We would like to re-submit this revised manuscript to BMC Nephrology and hope it is acceptable for publication in the journal.

Thank you once again for considering the publication of our manuscript and I look forward to hearing from you soon.

Yours sincerely,

Lingyu Xue
Response to the Reviewer #1:

(Comment 1) It is not clear if all the rats developed proteinuria after STZ-treatment and in those that did not if they were excluded. The authors state they measured 24 hr protein excretion and identified > 30 mg/day as ‘success for diabetic nephropathy (DN) but fail to report the specifics.

(Response 1) Thank you very much for your comment. According to your suggestion, we have supplemented the levels of glucose and proteinuria in Results section as following: “The results observed that after treatment with STZ, the glucose levels in the DN (26.910 ± 1.115) and BH (25.611 ± 1.587) groups were both more than 16.7 mmol/L on weeks 8, and the contents of 24 h proteinuria in the DN (42.525 ± 4.157) and BH (31.281 ± 5.123) groups were both more than 30 mg/24 h on weeks 8, which suggested that diabetes and DN rat models were successfully constructed.” (Results section, line 6-11, page 8)

The results of glucose and proteinuria levels have been shown in Figure 1A and B. To further confirm the successful construction of diabetes and DN rat models, we provides the levels of glucose and proteinuria again in the following table, which are corresponding to the Figure 1A and B:

<table>
<thead>
<tr>
<th>Time (weeks)</th>
<th>NC group</th>
<th>DN group</th>
<th>BH group</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>5.060 ± 0.680</td>
<td>26.910 ± 1.115*</td>
<td>25.611 ± 1.587*</td>
</tr>
<tr>
<td>12</td>
<td>4.667 ± 0.584</td>
<td>27.303 ± 2.517*</td>
<td>26.378 ± 0.638*</td>
</tr>
<tr>
<td>16</td>
<td>4.505 ± 0.807</td>
<td>28.160 ± 2.326*</td>
<td>28.331 ± 1.232*</td>
</tr>
</tbody>
</table>

The contents of 24 h proteinuria of rats in different groups (n = 8 for every group at each time point).

<table>
<thead>
<tr>
<th>Time (weeks)</th>
<th>NC group</th>
<th>DN group</th>
<th>BH group</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>18.258 ± 2.587</td>
<td>42.525 ± 4.157*</td>
<td>31.281 ± 5.123*#</td>
</tr>
<tr>
<td>12</td>
<td>17.678 ± 4.634</td>
<td>72.354 ± 5.783*</td>
<td>53.322 ± 3.018*#</td>
</tr>
<tr>
<td>16</td>
<td>18.651 ± 7.214</td>
<td>98.435 ± 3.980*</td>
<td>70.433 ± 4.136*#</td>
</tr>
</tbody>
</table>

(Comment 2) The authors fail to report blood pressure (BP) and thus one cannot exclude the effect of hypertension on ANGPTL-4 findings. The authors should include a group of DN rats treated with a non-Ace inhibitor agent to obtain comparable BP levels as those found in the benazepril-treated group.
Thanks for your comment. We are very sorry for that the blood pressure was not be measured and the influence of hypertension on ANGPTL-4 was not considered in this study, which was a limitation of this study. Despite all this, the present study also could demonstrate that BH might reduce proteinuria and improve DN, which might be associated with ANGPTL-4. We will improve our research in the further study according to your suggestions. In addition, we have supplemented this limitation in Discussion section and revised the conclusion as following:

“Unfortunately, this study has some limitation. Previous study showed that STZ could not only induce DN but also hypertension [1]. Because this study was focused on the effects of BH on DN, the blood pressure was not be measured and the influence of hypertension on ANGPTL-4 was not considered. Therefore, the effect of hypertension on ANGPTL-4 should be investigated in the further study. Despite all this, the present study also could demonstrate that BH might reduce proteinuria and improve DN, which might be associated with ANGPTL-4. In addition, this study only can reveal that ANGPTL-4 might be associated with the treatment of BH on DN; however, whether BH improved DN by targeting ANGPTL-4 is still unclear. Thus, the further study focused on the overexpression or deficiency of ANGPTL-4 should be performed to investigate the causal relationship between ANGPTL4 expression and the improving effect of BH on DN.

Conclusion

In conclusion, this study is a strong demonstration that BH might reduce proteinuria and improve DN, which might be closely associated with ANGPTL-4. However, the specific mechanism is not fully understood, which need to be exploited in further study.” (Discussion section, line 1-18, page 12)

Response to the Reviewer #2:

(Comment 1) The experimental work up is well made, however, the authors fail to prove a clear causal relationship between ANGPTL4 expression and nephropathy worsening or improving which they should be available to provide due to their multiple time point examinations....

(Response 1) Thank you very much for your comment. It is true that this study only can reveal the association of ANGPTL4 expression with the improving effect of BH on DN; however, the causal relationship is still unclear. We have supplemented this limitation in the Discussion section as following: “In addition, this study only can reveal that ANGPTL-4 might be associated with the treatment of BH on DN; however, whether BH improved DN by targeting ANGPTL-4 is still unclear. Thus, the further study focused on the overexpression or deficiency of ANGPTL-4 should be performed to investigate the causal relationship between ANGPTL4 expression and the improving effect of BH on DN.” (Discussion section, line 7-12, page 12)

(Comment 2) ANGPTL-4 is reported to be secreted by podocytes. Are the authors able to demonstrate ANGPTL-4 expression inside the podocytes by for instance electron microscopy?
Thanks for your comment. In this study, the ANGPTL-4 expression in the renal tissue was detected by qRT-PCR and HE. Because this study was aimed to investigate the effects of BH on proteinuria and ANGPTL-4 expression, the location of ANGPTL-4 expression was not explored. We will evaluate whether ANGPTL-4 is expressed inside the podocytes using electron microscopy in the following study according to your suggestion.

Did the authors by any chance measure ANGPTL-4 also in urine and serum?

We are very sorry for that ANGPTL-4 level was not measured in urine and serum but only in the renal tissue. However, the present study also could demonstrated the association of ANGPTL4 expression with the improving effect of BH on DN. In the following study, we will further evaluate whether the ANGPTL-4 levels in urine and serum are consistent with the renal tissue.

In the manuscript p values regarding Table 3 were p<0.001 , in the Table 3, p values are reported as 0.000. Were they really 0.000?

Thanks for your comment. In the Table 3, p values are reported as 0.000, which can be explained that the p value is close to 0 and specific values cannot be exactly calculated. We confirmed that the p values with 0.000 in Table 3 are true.

Results in Figure 1 and 4 would better be reported in a Box and Whisker Plot fashion.

It is true that a Box and Whisker Plot fashion is also a pattern to show the results in Figure 1 and 4. However, in this study, all data were expressed as means ± standard deviation (SD). We compared the mean values of the levels of biochemical indicators and the mRNA level of ANGPTL-4 among different groups and all these data had small SD. So we think that the results in Figure 1 and 4 shown in histogram also can demonstrate our conclusion.

Once again, thank you very much for your comments and suggestions.

References