Author's response to reviews

Title: Benazepril affects integrin-linked kinase and smooth muscle alpha-actin expression in diabetic rat glomerulus and cultured mesangial cells

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Version: 2 Date: 9 June 2014

Author's response to reviews: see over
DEAR REVIEWERS AND EDITOR,

We are very excited to have been given the opportunity to revise our manuscript, which we entitle, Benazepril affects integrin-linked kinase and smooth muscle alpha-actin expression in diabetic rat glomerulus and cultured mesangial cells. (MS: 9610205971250238).

We carefully considered your comments as well as those offered by the two reviewers. Herein, we explain how we revised the paper based on those comments and recommendations. We want to extend our appreciation for taking the time and effort necessary to provide such insightful guidance.

We hope that this revision improve the paper such that you and the reviewers now deem it worthy of publication. Next, we offer detailed responses to your comments as well as those of the reviewers point by point.

RESPONSES TO THE EDITOR’S COMMENTS:

Referee 1:
http://www.biomedcentral.com/imedia/6182197801292698_comment.pdf

Reviewer's report
Title: Benazepril affects integrin-linked kinase and smooth muscle alpha-actin expression in diabetic rat glomerulus and cultured mesangial cells

Version: 1 Date: 9 May 2014

Reviewer: Hannes Neuwirt

Reviewer's report:

Niu et al. have investigated the impact of an ACE-I on intracellular signaling molecules in GMCs. The data suggest that this involves AKT and ERK1/2 activation and is associated with differential expression of among others ILK or TGFb1.

Major Compulsory Revisions: None

Minor Essential Revisions: The statistics used are commonly considered appropriate for Gaussian distributed data. As the number of animals or in vitro experiments are relatively low, this reviewer suggests to use non-parametric test instead (e.g. Mann-Whitney-U or Kruskal-Wallis).

RESPONSE: Thank you for this great suggestion. We have changed the statistical methods to non-parametric pair t-test (Mann-Whitney-U) instead, which remain consistent across regular t-test statistical methods used in our original revision (line 183).

Please state Catalog numbers of antibodies and primer/probe-sets used for Westers and RT-PCR. Please unify the way how citations are separated from the text (with [citation] or without spaces[citation])
RESPONSE: Thank you for this great suggestion. We have added the catalog number of antibody and probe ID number used for Western blot and RT-PCR. The citations have been unified in this revision.

Discretionary Revisions: None

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
I declare that I have no competing interests

Referee 2:
http://www.biomedcentral.com/imedia/118944149129384_comment.pdf

Reviewer's report
Title: Benazepril affects integrin-linked kinase and smooth muscle alpha-actin expression in diabetic rat glomerulus and cultured mesangial cells

Version:1 Date:11 May 2014

Reviewer: Alexander Kirsch

Reviewer's report:

Niu and colleagues report the results of their studies on the effect of the ACE-I benazepril on the expression of alpha-SMA and integrin-linked kinase. They show comprehensive in vitro and in vivo data on mRNA and protein level of how both benazepril-treated rats and glomerular mesangial cells show a lower of aSMA and ilk levels. Overall, the manuscript is well-written, the data are presented clearly and the analysis seems to have been conducted thoroughly. I do however – apart from some minor issues – see an important issue with the controls the authors chose for their in vivo studies, which I believe warrant caution in the interpretation of their in vivo data.

Major Compulsory Reviews
1. The authors chose untreated, diabetic control rats to compare their ACE-I treated rats to. As expected and nicely shown by the authors, the control rats were diabetic, lower in body weight, had lower blood pressure, and a bit unexpectedly lower blood glucose levels than diabetic control animals. How can the authors exclude that the effect they observed in the ACE-I treated group was really specific to the inhibition of the RAAS rather than secondary to i) lower body
weight, thus less hyperfiltration and less glomerular hypertrophy, ii) less hyperglycemia and most importantly iii) lower blood pressure. All of these factors are well known driving factors behind the development of diabetic nephropathy and must not – I believe – be ignored. The authors should at the very least include a diabetic control group treated with e.g. a thiazide diuretic to lower blood pressure level to a similar degree as seen in the treatment group. Without such controls, the authors’ conclusions about the in vivo effects of benazepril are not truly supported by their data.

RESPONSE: Thank you for this valuable comment. In our original submission, we attempted to describe ILK and α-SMA expression differences between the control group, diabetic group and ACEI group in vivo. As we known, Benazepril is a kind of multifunction drug, including lower blood pressure, less hyperfiltration et al. It is primarily used in treatment of hypertension, congestive heart failure, and heart attacks, and also has beneficial effects in preventing renal and retinal complications of diabetes. It is reported that patients with advanced renal insufficiency taking benazepril showed "substantial" kidney benefits (N Engl J Med, 2006, 354 (2): 131–40). Based on this suggestion, control group treated with thiazide diuretic to lower blood pressure level is critical control for the ACEI group, but only one part of Benazepril function. Accurately, for the therapeutic effect, the real control of ACEI group is diabetic group, but we now add this limitation in our discussion section. Thank you for the suggestion, because it helps a great deal to illustrate that our results remain consistent across the data in vivo and in vitro.

Minor Essential Reviews

1. Spelling, Grammar

a. Page 4, line 5 from the bottom: “intraglomerular” --> “glomerulus”

RESPONSE: Thank you for this point. We have changed this word in this revision (line 75).

b. Page 5: “renal protective” --> “renoprotective”

RESPONSE: Thank you for this point. We have changed this word in this revision (line 89).

c. Page 6 and throughout the rest of the manuscript: ‘diabetes nephropathy’ --> ‘diabetic nephropathy’

RESPONSE: Thank you for this point. We have changed this word in this revision (line 104 and throughout the rest).

d. Page 7, last paragraph: “… was carried out in four different the types of medium…” --> “…was carried out in the four different types of medium…”

RESPONSE: Thank you for this point. We have changed this word in this revision (line 152).
e. Page 10, last line before first bold heading: “the beneficial effects of benazepril were mainly mediateD…”

RESPONSE: Thank you for this point. We have changed this word in this revision (line 217).

2. Please indicate the number of independent experiments performed for western blotting. There are only exemplary blots shown with one replicate, the quantification however has SEM, therefore there must have been other experiments.

RESPONSE: Thank you for this point. For western blot, the films were scanned and quantitative analysis from three independent experiments. We have added the number of independent experiment in this revision, (line 177-179).

3. Section on Renal Histology: The authors’ description of their histological findings is a little vague. Please describe what is meant by:
a. “lost the typical structures of the cortical tubulues”
b. “some features of healing”
c. When stating that there were fewer inflammatory cells, is this based on subjective impression or were the number of cells counted. If they were not and this is feasible, it should be done.

RESPONSE: Thank you for this point. For renal histology, we have rewritten those sentences, removed the uncertain description (line 1967-202). Also we added the quantification of glomerular staining for PAS, CD68 and CD3ε (shown in Fig 1G, K and O).

4. Discussion
a. The authors should mention the limitations of their study.

RESPONSE: Thank you for this point. We added the limitation in the discussion section.

Discretionary Reviews
1. The discussion focuses a lot on other literature but gives only a relatively brief impression of what the authors think their data mean.

RESPONSE: Thank you for the suggestion. We added the limitation in the discussion section, it helps us to illustrate that our results remain consistent across the data in vivo and in vitro.

Level of interest:An article whose findings are important to those with closely related research interests

Quality of written English:Acceptable
Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
I declare that I have no competing interests.

Editorial Request:
1. Competing Interest - please include a 'Competing interests' section between the Conclusions and Authors' contributions. If there are none to declare, please write 'The authors declare that they have no competing interests'. Please consider the following questions and include a declaration of competing interests in your manuscript:
Financial competing interests
? In the past five years have you received reimbursements, fees, funding, or salary from an organization that may in any way gain or lose financially from the publication of this manuscript, either now or in the future? Is such an organization financing this manuscript (including the article-processing charge)? If so, please specify.
? Do you hold any stocks or shares in an organization that may in any way gain or lose financially from the publication of this manuscript, either now or in the future? If so, please specify.
? Do you hold or are you currently applying for any patents relating to the content of the manuscript? Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript? If so, please specify.
? Do you have any other financial competing interests? If so, please specify.
Non-financial competing interests
? Are there any non-financial competing interests (political, personal, religious, ideological, academic, intellectual, commercial or any other) to declare in relation to this manuscript? If so, please specify.

RESPONSE: Thank you for this point. We added “Declaration of competing interests” section before Authors' contributions, and there are none to declare.

2. Line numbering - Please revise your manuscript to include line and page numbers. Authors are asked to ensure that line numbering is included in the main text file of their manuscript at the time of submission to facilitate peer-review. Once a manuscript has been accepted, line numbering should be removed from the manuscript before publication. For authors submitting their manuscript in Microsoft Word please do not insert page breaks in your manuscript to ensure page numbering is consistent between your text file and the PDF generated from your submission and used in the review process.

RESPONSE: Thank you for this point. We added line number follow the gridline in this revision.

We would be grateful if you could address the comments in a revised manuscript and provide a cover letter giving a point-by-point response to the concerns.
Please also ensure that your revised manuscript conforms to the journal style (https://connect.yale.edu/owa/UrlBlockedError.aspx). It is important that your files are correctly formatted.

We look forward to receiving your revised manuscript by 13 June 2014. If you imagine that it will take longer to prepare please give us some estimate of when we can expect it.

You should upload your cover letter and revised manuscript through http://www.biomedcentral.com/manuscript/login/man.asp?txt_nav=man&txt_man_id=9610205971250238. You will find more detailed instructions at the base of this email.

Please don't hesitate to contact me if you have any problems or questions regarding your manuscript.

With best wishes,

Miss Maria Merrie Jul Ladag
on behalf of Prof Alexander Rosenkranz

e-mail: editorial@biomedcentral.com
Web: http://www.biomedcentral.com/

To submit your revised manuscript

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