Author’s response to reviews

Title: Uric acid induces kidney injury through inducing fibroblast expansion, Endothelin-1 expression, and inflammation

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Dear Hayley Henderson Ph.D,

Editor of BMC Nephrology

Rev " BNEP-D-16-00242: Uric acid induces kidney injury through inducing fibroblast expansion, Endothelin-1 expression, and inflammation"

Thank you very much for the comments and suggestions for our manuscript. We tried to make some revision based on the comments from the reviewers. Point to point comments and suggestions are listed below with our revisions and answers.

Reviewer 1.

1. Material and method: UAL 7 and UAL 14 groups haven't been stated in the material and method section.
Answer: Thank you for the suggestion. We made revision with statements about the groups in the Material and methods section (page 4 of our manuscript).

2. Results: The low quality picture was difficult to see. Magnification is not clearly stated. There is inconsistency in spelling of podocin and nephrin.

Thank you very much for these comments. We made revision for the picture. We also added information about characteristics of tubular injury score (tubular dilatation and intraluminal cast formation) in the picture (Figure 1 A-B). We stated in the legend about the magnification and added the scale bar in Figure 1. We also added statements in Figures 2 and 3 about the magnification of the picture. In the Material and methods section (tubular injury and glomerulosclerosis score, page 5), we clearly stated the magnification that we used for quantification of tubular injury, glomerulosclerosis and fibrosis.

3. Replicates that were used in RTPCR reaction.

Answer: We used duplicates for the quantification of RTPCR. We added statement about this in the Material and methods section in ET-1 mRNA quantification.

4. Statistical analysis that we use.

Answer: We added statement in Material and method about the statistical analysis (page 6-7).

5. The data provided is not sufficient to support the conclusions.

Answer: Thank you very for this comment.

This is the most difficult comment actually. We tried to add some experimental data for supporting our conclusion. We also added Figure 3 about Western blot and immunostaining of αSMA. Western blot was for examining the profibrotic pathway, Transforming Growth Factor β1 which induced myofibroblast transition in this model. We concluded upregulation of TGFβ1 and
αSMA protein expression in UA group based on the Western blot analysis. Results of this procedure has been added in the Results section (page 7). This data lends support to our conclusion about role of uric acid in fibroblast expansion and myofibroblast transition. We also added immunostaining of αSMA to localize the myofibroblast in interstitial areas. We hope our additional data may add further support to our conclusion.

Reviewer 2.

1. Comment 1: Method and result were clearly demonstrated.

Thank you very much for your comment. We revised our method with addition of Western blot analysis and immunostaining of α- Smooth Muscle Actin (αSMA). Western blot was performed to quantify the protein expression of Transforming Growth Factor-β1 (TGFβ1), α- Smooth Muscle Actin (αSMA) and Endothelin A Receptor (ETAR) (page 6).

2. It is interesting that hyperuricemia induced podocyte damage demonstrated by reduced expression levels of podocin and nephrin and allopurinol ameliorated these changes. It would be assuring if the levels of albuminuria were quantified.

Answer: Thank you very much for the comments and suggestion.

We know that filtration disturbance may be important in supporting this data, such as albuminuria, but our research center and laboratory in Indonesia do not have metabolic cage for quantifying urine. We also collected the urine, however not 24 hours urine. So, we cannot add the albuminuria or functional data that used urine. We hope our RTPCR examination of podocyte markers and glomerulosclerosis might be enough for supporting podocyte injury and filtration disturbance.

3. The authors used daily intraperitoneal injection of uric acid, however, I am concerned about the daily variation of the uric acid concentration. Is there any data for the trough value of uric acid in this model? Is there any reason that you did not use uricase inhibitor?

Answer: Thank you very much for your comments and suggestion.
It is the best if we could quantify the concentration of uric acid every 3 days or 7 days. We can get the variation of the concentration. However, we used mice in this experiment, and this is very tough to get the blood from the mice. So, we just quantify the concentration of uric acid in 7 and 14 days (just before euthanizing).

Uricase inhibitor may become good choices in studying hyperuricemia. We had difficulties in preparing and supplying the chow with uricase inhibitor in our country. We realized that our center still needs to be better developed for experimental research. Hopefully in the future we can get uricase inhibitor.

4. The role of uric acid as a cause of renal damage is under an active debate, and there are many previous reports. Please clearly and separately state the novel data and the data you confirmed in this model.

Comments: Thank you for these comments.

We added additional experiment with Westernblot for quantification of TGFβ1 as profibrotic factor, and αSMA (marker of myofibroblast) and ETAR (Endothelin A Receptor) protein expression for supporting our conclusion about the effect of uric acid in fibroblast expansion and myofibroblast transition. We had stated in the discussion "Our immunoblot result showed higher expression of profibrotic factors such as TGFβ1 and ET-1 protein expression which confirm activation of fibrotic pathways after uric acid induction". This is the novel data that confirmed the activation of profibrotic factor after uric acid induction.

We also added in the discussion "Immunostaining of this study also demonstrated fibroblast expansion (Fig 2E) and αSMA positive staining which represented myofibroblast in interstitial area of UA groups (Fig. 3B-D). We proposed in this study that uric acid might induce renal injury through stimulation of fibroblast expansion to myofibroblast transition. Uric acid might induce fibrotic factor, such as TGF-β1 and ET-1, then inducing fibroblast expansion and myofibroblast formation" (page 9, line 27-30). This data supports our conclusion about association between uric acid, fibrotic factors, fibroblast expansion and myofibroblast transition.
Thank you very much for the opportunity to revise our manuscript. We hope that our manuscript may be considered acceptable for publication in BMC Nephrology.

Best regards,

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