Author's response to reviews

Title: Yi Qi Qing Re Gao Formula Ameliorates Puromycin Aminonucleoside-induced Nephrosis by Suppressing Inflammation and Apoptosis

Authors:

Yumin Wen (colline0725@163.com)
Yongli Zhan (zhanyongli88@sina.com)
Huijie Liu (liuhuijie66@163.com)
Tingting Zhao (ttfrfr@163.com)
Liping Yang (relyssia@gmail.com)
Haojun Zhang (zhhj529@163.com)
Xi Dong (yaolishi2001@163.com)
Ping Li (lp8675@163.com)

Version: 4
Date: 11 April 2015

Author's response to reviews: see over
Authors’ Response to Reviewers’ Comments

Article title: Yi Qi Qing Re Gao Formula Ameliorates Puromycin Aminonucleoside-induced Nephrosis by Suppressing Inflammation and Apopotosis

Manuscript #: 7357166115088612

Authors:
Yumin Wen
Yongli Zhan
Huijie Liu
Tingting Zhao
Liping Yang
Haojun Zhang
Xi Dong
Ping Li

Date: 11 March 2015
Dear Dr. Rowles:

Thank you for considering our manuscript entitled “Yi Qi Qing Re Gao Formula Ameliorates Puromycin Aminonucleoside-induced Nephrosis by Suppressing Inflammation and Apopotosis” (7357166115088612) for publication in BMC Complementary and Alternative Medicine. We appreciate the reviewers taking time to evaluate our manuscript. Their comments and suggestions are valuable in helping us improve the paper.

On the following pages are point-by-point responses to comments from the reviewers.

We would like to express our great appreciation to you and the reviewers for comments on this paper. We look forward to hearing from you.

Thank you again!

Sincerely,

Ping Li, MD, PhD
Professor
Reviewer #1: Maria Victoria Aguirre


Response: Thank you for pointing out this oversight. This citation has been added as reference 15 in the Discussion (line 288 page 16).

Comment 2) Results section: Action of YQQRG on the activation of inflammatory markers in the kidney, first paragraph: Include references about inflammatory markers in PAN model.

Response: We would like to thank the reviewer for this good suggestion. References on inflammatory markers were included in the Discussion in the original version of the manuscript. In the revised manuscript we have moved these references to the Results (reference 12-14, line 243 page 14).

Comment 3) Discussion, sixth paragraph. There are confusing sentences about caspase-3 role in apoptosis. The active form of caspase-3 plays a central role in the execution-phase of cell apoptosis. The regulation of programmed cell death depends mainly on the balance between Bcl-2 pro-apoptotic and anti-apoptotic members and on the effects of apoptotic inhibitory proteins (proteins of IAP and XIAP families).

Response:
We completely agreed with the reviewer’s comment. The sentence “The interaction of caspase-3 with Bcl-2 plays an important part in regulating apoptosis and maintaining cellular homeostasis” has been deleted in the revised manuscript. We hope that we have now sufficiently explained the role of caspase-3 in apoptosis. The Bcl-2 family includes both pro-apoptotic and anti-apoptotic members. In this study we examined the anti-apoptotic protein Bcl-2, so the discussion in this paragraph mainly focus on the function of Bcl-2 itself.

Comment 4) Include original magnification or length bars in the following panels: 4(A), 5(A), 6(A), 7(A) and 8

Response: Thank you for indicating this oversight. Micron bars have been added to all panels.
Comment 5) Methods, Statistical analysis. First paragraph: Correct the spelling mistake: "deviation"
Page 5-line 92. Replace "Anti-apoptosis actions" with "anti-apoptotic actions"
Page 15- line 283 - Replace "Anti-apoptosis effects" with "anti-apoptotic effects"

Response: Thank you for comment. We confirmed that our spelling of “deviation” is correct. In any case, we have changed the expression to “mean ± (SD)”, which is the standard in publishing. We have also confirmed that all instances of the adjective “anti-apoptotic” are correct.

Comment 6) In spite the authors have mentioned the bioactive compounds present in each medicinal plant; it would be important to include whether or not seasonal variations affect the percentage of each compound in the final herbal mixture.

Response: We appreciate your suggestion. It is also an interesting concern. Most of the bioactive compounds mentioned in the Discussion are well-researched components of these herbs and are detailed in the Pharmacopoeia of the People’s Republic of China. Even though the actual percentage of each compound may vary depending on season of harvest, these bioactive compounds are the labels of the final herbal mixture. In addition, the herbs used in YQQRG in this study were purchased from Kangmei Pharmaceutical, which is a well-known pharmaceutical manufacturer for its strict quality control in processing Chinese herbal medicines.

Comment 7) For evaluating the functional disturbance of the foot processes a complementary assay with immunofluorescence microscopy with monoclonal antibodies against beta 1 integrin and/or podocin should be done. Moreover, the anti-inflammatory effect of the herbal mixture should be assessed by i-NOS immunohistochemistry and/or renal superoxide anion production.

Response: As the reviewer suggested, we have done podocin immunofluorescence and iNOS immunohistochemistry. The iNOS immunohistochemistry was added and the results are shown in figure 10 and described on page 14, lines 254-263 in the text. However, the anti-podocin antibody we had on hand is a polyclonal antibody from Sigma (P0372). We have attempted to perform both immunofluorescence and immunohistochemistry with this antibody, but the results showed a high level of nonspecific staining. Therefore, we did Western blot instead, results of which have been added as figure 4 and described on page 13, lines 232-239 the text.
Reviewer #2: Zhaohong Chen

Comment 1) It is interesting paper, indicating therapeutic effects of YQQRG and clarifying the mechanism of its anti-inflammation and anti-apoptosis effect on PAN nephrosis. The data are globally convincing. However, it is unclear what the molecular target (receptor) is in podocytes? All the analyzed pathways may be secondary responses (adaptation) of podocytes to injury.

Response: Thank you for your comment. In this study we observed that after YQQRG treatment, inflammation and apoptosis were both suppressed. Based on the literature, the most active components of these herbs have anti-inflammatory and anti-apoptotic effects. Therefore, we deduced the treatment effects of YQQRG might be partly due to its anti-inflammatory and anti-apoptotic actions. In PAN-induced nephrosis, inflammation and apoptosis are not restricted to the podocyte. The tubules and tubulointerstitium are also involved in macrophage recruitment, production of inflammatory mediators, and apoptosis. Thus, it is unlikely that all the effects observed in this study were secondary responses of podocytes to injury. In order to study the specific molecular targets, further in vitro studies or studies on target gene knockout animals are necessary.

Comment 2) 1. PAN can induce either minimal change disease (MCD) or focal segmental glomerular sclerosis (FSGS) depending on the dose of treatment. It is important to know the exact disease process of the PAN rat model described by the authors in order to understand the role of in the reduction of proteinuria. Since YQQRG could potentially improve PAN-induced nephrosis by decreasing podocyte apoptosis. It is important to show the PAS staining of kidney tissue to see whether there is significant focal sclerosis to suggest FSGS rather than MCD.

Response: Thank you for the comment. As mentioned in the Results (page 13, lines 226-228), glomerular lesions were not observed in the PAN-treated groups by PAS staining because our study was a MCD rat model rather than FSGS.

Comment 3) 2. Glomerular filtration rate need to be measured in the animals to be certain that the reason for the reduction in proteinuria is not due to decreased GFR.

Response: Thank you for pointing out this important issue. It’s true that less urinary protein is observed when glomerular filtration rate decreases. Based on our preliminary study (Zhan Y, et al. 2014, reference #8 in this manuscript) on this model, serum creatinine in the PAN model group did not differ from the sham group. Therefore, we felt that glomerular filtration rate is not reduced in our model and the reduction of proteinuria level in this study was not due to decrease in renal function.
Comment 4) Figure 8 is almost unreadable.

Response: Thank you for pointing out this error. We have revised the label “Model 15D” to “PAN 15D” in the second panel of this figure.

Comment 5) The magnification of the top row in figure 9 seems inconsistent

Response: Magnifications of all panels are the same. Glomeruli in the image indicating sham group day 10 seemed smaller than the other two in this row because this was a smaller cross section of the glomeruli. To avoid misunderstanding, we have replaced the original image. Moreover, a micron bar has been added in each panel of this figure.