Author's response to reviews

Title: The protective effects of ginsenoside Rg1 against hypertension target-organ damage in spontaneously hypertensive rats

Authors:

Hui Chen (chenhui_luo@163.com)
Jun Yin (yinjun826@sina.com)
Yanping Deng (shimbiro@sina.com)
Min Yang (yangmin_7777@163.com)
Lingling Xu (candyxxl@163.com)
Fukang Teng (fineskypig1983@163.com)
Defang Li (lidefang@163.com)
Yufan Cheng (chengyufan2009@163.com)
Dong Wang (zhangji-sh@163.com)
Baohong Jiang (jiangbh@mail.shcnc.ac.cn)
Dean Guo (daguomail.shcnc.ac.cn)
Tingting Zhang (tingyu3160@163.com)
Xuan Liu (lillianliucn@yahoo.com.cn)
Wanying Wu (wuwanying902@163.com)
Shuhong Guan (shuhong_guan@hotmail.com)

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Author's response to reviews: see over
Dear editor,

Thank you for the speedy review of our above-mentioned manuscript (1090611313576360). We appreciate the comments from the selected reviewers and respectfully re-submit a revised version of our manuscript, incorporating suggestions from the reviewers, for your consideration. Below, please find our responses to each of the reviewer comments and how the manuscript text was edited accordingly.

We look forward to your response. If any further clarifications or correspondence is needed, please feel free to email me, jiangbh@mail.shcnc.ac.cn.

Respectfully yours,

Baohong Jiang

Shanghai Institute of Materia Medica,
Chinese Academy of Sciences,
Shanghai Zhangjiang Hitech Park,
Haike Road #199, Shanghai 201203, P. R. China
E-mail: jiangbh@mail.shcnc.ac.cn
Point to Point Responses to Editorial and Reviewers’ comments

Reviewer 1

Comment: Hypertension of SHRs depends on the age. So, this study is very important for the age of the animals. How old-weeks of the SHRs did you use? How old-weeks are the rats when you start this experiment? Authors need to indicate the information in Methods section.

Response: Thank you for the question. SHRs and WKYs were all 2 month old at the beginning of experiment. After 8 weeks high salt diet, Rg1 treatment begun. Detection was conducted after another 4 weeks for Rg1 treatment. We add the detail age of SHR in our revised manuscript.

Comment: Authors used 8.0% salt diet through the experiment. Blood pressure of Rg1-treated Rats did not decrease. Please discuss the effects of the salt diet during treatment period.

Response: Numerous studies have demonstrated that high salt intake causes adverse structural and functional effects in the cardiovascular system. Excessive salt intake is often associated with an increase in arterial pressure and, consequently, increases in arterial pressure may partially mediate salt-related adverse effects. In addition to the well-admitted effect of sodium on blood pressure, several clinical and experimental observations are in favour of non-pressure-related effects of salt that could contribute to its influence on cardiovascular outcome. High salt intake caused hypertrophic response, then concentric cardiac-remodeling. High dietary salt led to widespread fibrosis and increased TGF-β1 in the heart and kidney in normotensive and hypertensive rats, suggesting that excessive salt intake may be an important direct pathogenic factor for cardiovascular disease.

Comment: In the text, authors did not discuss heart rate, which indicated Table 1. Authors need to comment it.

Response: Thank you very much for your comment. We used two methods to detect the effects of Rg1 on blood pressure, tail-cuff method and hemodynamics using Mikro-tipped SPR-320 catheter (Millar Instruments Inc). Tail-cuff method was used during the experiment at least three times; Millar catheter record was used before sampling. Neither tail-cuff nor hemodynamics showed the influence of Rg1 on blood pressure. In the previous manuscript, we only reported the blood pressure detected by tail-cuff method to explain there are no effects of Rg1, but neglected to evaluate heart rate during the whole experiment. For heart rate, only the last time detection of tail-cuff method that we reported showed the increase of heart rate for Rg1, the other data including Millar catheter record did not showed the influence of Rg1 on heart rate. Taken all the data together, Rg1 did not regulate blood pressure and heart rate. So in the revised manuscript, we
Comment: In the result section, “… and cell density was reduced in SHR relative to WKY”. Dose this mean a decrease of cell number? How do you estimate this finding? Authors need to explain it.

Response: Thank you for the question. “… and cell density was reduced in SHR relative to WKY” doses not mean the decrease of total cell number. Cell density was identified as cell number (indicated as nuclear number in the manuscript) in the area with fixed size. Shown as the figure followed, there are 13 nucleus in the square for WKY, while there are 6 nucleus in the square with the same size for SHR. But the thickness of vessel wall is different between WKY and SHR. So the density decrease did not mean total cell number decrease.

Comment: Figure 1 and 3 are not distinct. Please use more large images.

Response: We submit the larger images this time.

Reviewer 2

Comment: Figure 1(F). There is a need to explain why the lower dose (5mg/kg) has significant inhibitory effect, compared to SHR, while the higher dose 20 mg/kg has no effect. Maybe because the difference in dose was two wide (4 fold). Ideally next higher dose 10 mg/kg should have been tried particularly when the very high was shown to be ineffective.

Response: Thank you for your question. We used three dosages (5 mg/kg, 10 mg/kg and 20 mg/kg) to evaluate the protective effects of Rg1. There are no significant difference between 10 mg/kg and 20 mg/kg Rg1 on vascular protection. So we only report the results of 5 mg/kg and 20 mg/kg Rg1 in the manuscript. At present stage, we also cannot explain these phenomena. We hope we would explore more mechanism in depth in future.

Comment: Figure 1. There is no point to compare Rg1 treated group (SHR-Rg1) with WKY group. This group (SHR-Rg1) needs to be compared primarily with SHR group, showing that there is no significant (NS) effect compared to SHR group. There is a need to explain the rise in HR induced by the test material which may account for the lack of visible effect on BP.

Response: Yes, there is no direct description about the comparison of Rg1 treated group with WKY group in the Results. We add the description in Figure 1 legend (###p<0.001 compared with WKY). The remodeling of aorta is very significant, even treated with Rg1. Tail-cuff and hemodynamics were used to evaluate the effects of Rg1 on blood pressure and heart rate. No regulation of Rg1 on blood pressure was found. For heart rate, only the last time detection of tail-cuff method that we reported showed the increase of heart rate for Rg1, the other data
including Millar catheter record did not showed the influence of Rg1 on heart rate. Taken all the data together, Rg1 did not regulate blood pressure and heart rate. So in the revised manuscript, we delete the data of heart rate.

**Comment:** Why high salt diet (8%) has given to the rats, while the authors have used spontaneously hypertensive rats (SHR).

**Response:** As the most abundant and bioactive compound of Panax notoginseng, protective effect of Rg1 against organ damage induced by hypertension are warranted. Numerous studies have demonstrated that high salt intake causes adverse structural and functional effects in the heart and kidney. To set up the heart and kidney damage model in relative short period, high salt diet was used.

**Comment:** Can the authors provide more details about the importance of protecting organ damage by Rg1, where it does not lower blood pressure.

**Response:** Myocardial fibrosis is commonly observed in hypertrophied heart during hypertension. The myocardial matrix becomes less distensible, as the formation of the adducts in collagen to resist normal turnover. Therefore, monitoring cardiac fibrosis and markers of collagen synthesis, degradation and the use of drugs that reverse collagen accumulation might represent a novel opportunity to alter the natural history of hypertensive heart disease. We add these sentences in the Discussion.

**Comment:** Can the authors provide more information about the traditional use of the plant and also more information about the Rg1 and provide references, if available.

**Response:** Panax notoginseng is well known for its efficacy in promoting blood circulation, ameliorating pathological hemostasis, alleviating pain. The main active components of Panax notoginseng include more than 30 different types of saponins, among which Rg1 and Rb1 are found in the highest content. A number of clinical and physiological effects of Rg1 have recently been described, such as inhibition of tubular epithelial to myofibroblast transition (Xie et al., 2009), improvement of myocardial dysfunction in rats with burn injuries, amelioration of hepatic microcirculatory disturbances, anti-hyperglycemic activity (Yang, 2010), and improvement of endothelial cell function. We add above information in the Background.

**Comment:** Page 1, line 1: The first sentence of the abstract should be corrected. Like “Although a number of antihypertensive drugs are available for the management of hypertension, yet the organ damaged induced by hypertension is not resolved.”

**Response:** We corrected the sentence according reviewer’s suggestion.

**Comment:** Page 1, line 7: Please add “induced” between “hypertension” and “complications

**Response:** We corrected the sentence according reviewer’s suggestion.

**Comment:** Page 6, Data analysis, “Please mention the name of the software used for statistical analysis”

**Response:** SigmaPlot software was used for statistical analysis for our research.
Comment: Page 12, line 7: Discussion. Please replace “in other hand” with: on the other hand
Response: We corrected the sentence according reviewer’s suggestion.

Comment: Page 15, Figure legends. Figure 4 has been mentioned twice separately for the same graph. Both should be mentioned under one figure without separate headings.
Response: Sorry for our carelessness, we did the correction in the revised manuscript.

Comment: Page 17, References: Please arrange the references according the journal (BMC CAM) specifications.
Response: We checked all the references and arranged the references according BMC CAM specification.

Comment: Table 1 (HR-bpm) P: The value of SHR-Rg1 (20 mg) is significantly higher than SHR (control). This means it should also be significantly higher than WKY. Please check again.
Response: Thank you for your comment. In the revised manuscript we only reported the effects of Rg1 on blood pressure.

Comment: Figure 1; Font size is too small. Please enlarge the size of all figures to make it legible.
Response: We enlarged the size of all figures in the revised manuscript.

Reviewer 3
Comment: In “Background”-the authors should improve the explanation about the reason to investigate the effect of Rg1 in the vascular remodeling and target-organ damage induced by hypertension.
Response: We add more information about Rg1 in the Background of the revised manuscript.

Comment: The chemical structure of Rg1 should be present as Figure and not as Supplementary Material.
Response: We present chemical structure of Rg1 in Figure 1 in the revised manuscript as reviewer’s suggestion.

Comment: In “Methods-Animals and Rg1, page 4”-the authors describe, based on the HPLC (high-performance liquid chromatographic) analysis, that the purity of Rg1 is higher than 99%. In my opinion, estimate the purity using only HPLC analysis is not appropriate. I believe that the purity statement of Rg1 must be certified by the Company Shanghai Yousi Bio-Tech. The HPLC analysis and NMR data of Rg1 could be used to confirm the purity described by Shanghai Yousi Bio-Tech Co., Ltd.
Response: Yes, the purity report of Rg1 was provided by Shanghai Yousi Bio-Tech, and we confirm the purity of Rg1 by HPLC and NMR again.

Comment: In “supplementary Figure 1”-the conditions used in HPLC analysis (flow equipment used, mobile phase, UV detection, etc) should be placed in the Figure captions.
Response: We add the description in the Figure legend.
Comment: In “Supplementary Figure 2” –the equipment used to obtain the 13C NMR data should be cited in the Figure captions (same as 4).
Response: We add the description in the Figure legend.

Comment: Despite the fact that the chemical structure of the ginsenoside Rg1 is very well established in the literature, a table content its 13C NMR data it is not necessary. The authors should write in the text that these data are in agreement with those previously reported in the literature.
Response: We add the description that our 13C NMR data are in agreement with the previous report.

Comment: Carefully check the text for spelling errors (Example-Page 3-Background: Although the blockers of calcium channel and inhibitors of rennin-angiotensin system are widely applied for clinical therapy, But….)
Response: We are so grateful to the Reviewer’s comment and yet understand that there are still errors throughout the text. We have performed multiple rounds of copyediting, not only to incorporate all the Reviewer’s comments but also to minimize the grammatical errors.

Comment: A careful revision of the text by a native speaker is recommended.
Response: We asked a native speaker to revise the whole manuscript and hope this new version is suitable for publication.

Referee 4
Comment: Why they used those two concentrations of Rg1?
Response: Before we start the experiment, we check the reports of Rg1 for its doses. The most common dose for Rg1 (about 1 month treatment) is between 1 to 20 mg/kg (Neurosci Lett. 2010, 478:66-71; Eur J Pharmacol. 2009, 608:42-7). We choose the concentrations of Rg1 according previous report. We used 5 mg/kg, 10 mg/kg, 20 mg/kg three doses for the study. Because 10mg/kg and 20mg/kg are all not so difference, we only present the results of 5mg/kg and 20 mg/kg in the submission.

Comment: Why did they treat only the SHR groups with Rg1? It will be interesting to see what is happening with the cardiovascular system of the WKY group of rats.
Response: Yes, it is a very suggestive comment. Because the research duration of present study is more than 3 months (two month high salt diet, one month Rg1 treatment (with high salt diet), parameter detection). It is impossible for us to get full data before the deadline of revision. We hope to investigate the cardiovascular response of Rg1 on WKY in the near future.

Comment: In the part concerning the methods (Measurement of blood pressure and heart rate in conscious rats) only Systolic blood pressure (SBP) and heart rate (HR) are mentioned, but in Table 1 we can see the data for Diastolic pressure (DBP), I assume that this an oversight.
Response: Yes, thank you for your comment. We correct this oversight in the revised manuscript.

Comment: Also, according to Table 1 the Heart in index is expressed in mg/g, why in mg. The rat
heart weight can be expressed in g.

**Response:** Yes, the rat heart weight can be expressed in g. For heart index, it is easy to express as mg/g than g/g. For example, the heart index of WKY is 2.9±0.2 mg/g (0.0029±0.0002 g/g).