Author’s response to reviews

Title: Protective effect of Shenmai injection on doxorubicin-induced cardiotoxicity via regulation of inflammatory mediators

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Version: 2 Date: 27 Nov 2018

Author’s response to reviews:

Dear Editors,

Thank you for your letter regarding revision of the manuscript entitled “Protective effect of Shenmai injection on doxorubicin-induced cardiotoxicity via regulation of inflammatory mediators” (BCAM-D-18-01258R1). We had edited the manuscript carefully following the reviewers’ advice.

We hope the revised manuscript is now satisfied for publication. We are looking forward to hearing from you, kindly acknowledge us by e-mail. Thank you for handling our manuscript.

Thanks.
Yours sincerely,
Yanfei Xin.

Respond to the comments made by the reviewers

Firstly, we thank the reviewer for the good advices. Our responses to the comments of reviewer are listed below:
Luiz Ferreira (Reviewer 1):
1. Abstract p. 2 Line 38, "Pretreatment with SMI elicited a dose-dependent cardioprotective effect in DOX-dosed mice as evidenced from normalization of serum AST and LDH…"
Data does not support this conclusion. In most cases, the treatments are different from control and similar to the DOX group.
Thanks for the reviewer’s carefulness. We have corrected the sentence, as shown below. Pretreatment with SMI elicited a dose-dependent cardioprotective effect in DOX-dosed mice as evidenced from normalization of serum inflammatory mediators, as well as improving cardiac function and myofibrils disarrangement.

2. Abstract p. 2 Line 50, Key words: Correct the word "aniti-inflammation". Suggestion: anti-inflammatory. We corrected the word "aniti-inflammation" to "anti-inflammatory" following the reviewer’s suggestion.

3. Background p.3 Line 5, "Unfortunately, DOX has too serious adverse effect of cardiotoxicity so that has limited its clinical use". Please correct the sentence. Suggestion: "DOX has serious cardiotoxicity effects that limited its clinical use". We corrected the sentence following the reviewer’s suggestion. Thanks.

4. Background p.3 Line 48, "..DOX-induced cardiotoxicity via a regulation of innate immune.." Please correct the sentence. Suggestion: "… regulating the innate immune response." We corrected the sentence following the reviewer's suggestion. Thanks.

5. Methods p.6 Line 1, "...(IL-6), monocyte chemotactic protein 1 (MCP-1) and Interferon-γ (INF-γ), in serum were measured." How the authors knows that the cytokines are relate to heart inflammation. Why do not evaluate the cytokines in cardiac tissue? Thank you for your suggestion. It has been proved that myocardial inflammation is positively correlated with the content of inflammatory factors in serum. We selected inflammatory cytokines in serum in the present study. In the further study, we will detect the levels of inflammatory factors in both of heart tissue and serum following the reviewer’s suggestion.

6. Methods p.6 Line 44,"Statistics. Data are expressed as means ± S.D..." This information is different from the figure legend. In the figure legends they are describe as mean ± SEM. Please, confirm the correct the information and review all the statistical analyses. Thanks. We review all the statistical analyses and corrected the errors. All data are expressed as means ± S.D in the manuscript.

7. Results p.7 Line 2, "General observations. All experimental mice are still alive." Please correct the sentence. Thanks for your suggestion. We have corrected the sentence, as shown below. At the end of the treatment period, all mice were alive.
8. Results p.7 Line 7, "DOX also caused a significant decrease (P < 0.05) in heart weight and heart-to-body weight."
According to Table 1 there is no statistical difference between control and DOX group in relation to heart-to-body weight. Please review this result.
It is right no statistical difference between control and DOX group in relation to heart-to-body weight. We corrected it in the manuscript.

9. Results p.7 Line 9, "The mice in SMI pretreated group, especially in the DOX + SMI (H) group, be weakened DOX-induced decrease in heart weight (P < 0.05).
There was no statistical difference in relation to DOX group. The group DOX+Aft-SMI (H) showed statistical difference. Please review this information, maybe the group is wrong.
Thanks for your issues. We rewrote the section, as shown in the following paragraph.
At the end of the treatment period, all mice were alive. However, the mice in the DOX treating groups appeared weak, with hair erection, a hunched posture and weight loss. As shown in Table 1, DOX also caused a significant decrease (P < 0.05) in heart and body weight. Pretreated with 5 g/kg of SMI significantly improved DOX-induced decrease in body weight (P < 0.05). Although, heart weight was not founded any trend of recovery. Ascites was present in DOX, DOX+SMI (L), DOX+SMI (M), and DOX+Aft–SMI (H) groups but not in the control, SMI (H), and DOX+SMI (H) groups. Compared with the mice injected with DOX only, the mice in the DOX+SMI (H) group had a clearly lower mean volume of ascites (P < 0.05).

10. Results p.7 Line 30, "embodied in CO and EF parameters in DOX + SMI (H) and DOX + SMI (M) Groups (Figure 3 H-K)."
Please clarify this information and verify statistics. In relation to EF, most of SMI groups does not showed statistical difference in relation to DOX group.
Thanks for your issues. EF is a typo. We corrected it with FS in the sentences.

11. Results p.7 Line 52, "Hearts sections of DOX-treated mice revealed exhibit vacuolar and.."
Exclude the word "revealed".
We corrected it.

12. Results p.7 Line 52, "As grades scores showed in the Fig.5 H, there was a good dose-effect histopathological improvement in the groups of mice pretreated with 0.5, 1.5 and 5 g/kg of SMI."
Please correct the number 15 for 1.5. Add the information about the focal myofibrillar loss.
Thanks for your suggestion. We corrected it.

13. Results p.8 Line 25, "Pretreatment of SMI significantly decrease the expression of iNOS in DOX-injected mice."
Please change the word "decrease" for decrease. The Western blot analyses there was statistical difference only between DOX and DOX + SMI (H). Please clarify this information.
We have corrected it, as shown in the following sentence. Pretreatment with 5 mg/kg of SMI significantly decrease the expression of iNOS in DOX-injected mice.
14. Discussion p.9 Line 45, "DOX invited a significant increase in NF-κB binding activity."
Please change the term "invited" to led. We corrected it following the reviewer's suggestion.

15. Discussion p.9 Line 47, "pro-inflammatory mediators expression, including TNF-a, IL-1b and NO."
Please correct the terms TNF-a and IL-1b. Use the same abbreviations throughout the manuscript (TNF-α and IL-1β).
Thanks for your suggestion. We corrected it.

16. Table 1, Delete the symbol # in the group DOX on the Heart weight evaluation.
Thanks for your suggestion. We corrected it.

Luiz Ferreira (Reviewer 2):

1. First of all I have objection against the title (Should include the active ingredient not Injection)
Thanks for the reviewer’s suggestion. As be introduced in manuscript, Shenmai injection consists of Panax Ginseng C.A.Mey. and Ophiopogon japonicus (Thunb.) Ker Gawl. It is widely used in China. Ginsenosides been identified as the main ingredient in Shenmai injection. However, it is unclear that which content is the active ingredient to play an anti-inflammatory role.

2. Results in the abstract is ambiguous.
We reedit the section in abstract.

3. Background should be introduction
Thanks. We corrected it following the reviewer's suggestion

4. LDH and CPK are not specific enzyme (where are CPK-MB or LDH-ISO -----ect)
Thanks for your good suggestion. LDH and CK be selected as the biochemical markers in cardiotoxicity in many studies. So did we. We will use CPK-MB or LDH-ISO in the next study as the biomarker. Thank you again.