Author's response to reviews

Title: The Cardioprotective Effect of Danshen and Gegen Decoction on Rat Hearts and Cardiomyocytes with Post-ischemia Reperfusion Injury

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Version: 3 Date: 23 July 2012

Author's response to reviews: see over
23 July 2012

BMC Complementary and Alternative Medicine

Dear Editor,

2112147058701862 - Cardioprotective Effect of Danshen and Gegen Decoction on Rat Hearts and Cardiomyocytes with Post-ischemia Reperfusion Injury

Thank you very much for your reply dated 25 June in which you advised us that you would be willing to consider a revised version of the above manuscript.

Following the reviewers’ comments, we have seriously revised our original manuscript. We are pleased in re-submitting our revised manuscript via manuscript submission website whereas the responses to reviewers are attached as follows.

Thank you very much.

Yours Sincerely,

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**Responses to Reviewer 1’s comments**

Comment: The quality control of DG decoction is not clear.

Response: In the section of Background, we have mentioned that in our previous publication (Lam et al., 2010), seven components including danshensu, protocatechuic aldehyde, puerarin, daidzein 8-Capiosyl-glucoside, daidzin, salvianolic acid B and daidzein were identified in DG water extracts. Most of these components exhibited various cardio-protective activities. In addition, we have added some information regarding the quality control of DG decoction in Discussion section: ‘Since only water extract was used, the valuable lipophilic tanshinones (cryptotanshinone, tanshinone I and tanshinone IIA) are not contained in the DG extract or only in traces. Therefore, tanshinones did not contribute but water soluble marker compound such as salvianolic acid B could contribute to the cardiovascular activity (Wagner et al., 2011).’

Comment: The novelty of this study are limited.

Response: In the section of Discussion, we have added the discussion on the difference between our and other research group’s study. Firstly, in our study, the I/R injury mainly happens on the reperfusion phase, which accompanied by excessive oxidative stress and ion accumulation after blood or oxygen restore. Secondly, other group’s work was about DG pretreatment protection on rat hearts and myocardium. They explained DG effect on anti-IR injury mainly by activating PKC pathway to inhibit the opening of K$_{ATP}$ on mitochondria. Their results provided the evidence that DG could be used as diet supplementary to improve heart anti-I/R injury potential ability (Chiu et al., 2011a; Chiu et al., 2011b). Our present study focused on DG post-treatment protection on rat hearts and cardiomyocytes with I/R challenge, in both ex vivo and in vitro. Additionally, we paid more attention on the physiological function recovery of rat hearts with I/R injury after DG post-treatment ex vivo. We also provided the evidence that DG directly inhibited the calcium accumulation within cardiomyocytes at the reperfusion injury using live cell recording system under the confocal microscope. Our present results showed that DG could be directly applied in the procedure of blood restoring surgery to attenuate the I/R injury in patient hearts.

Comment: The previous studies of DG protecting the myocardium against ischemia/reperfusion injury, which is more relevant to the present study, should be mentioned in the abstract.

Response: Abstract has been revised accordingly.
Comment: The relationship between the variables being investigated and the cardiac protective effect of DG is not clear. Please clarify. In addition, the update information on the cardiac protective influences of DG, which is important related to the present study has not provided appropriately.

Response: The abovementioned information has been added in the section of Background.

Comment: What was the pH and osmolarity of DG solution? Particularly considering a current ex vivo study. Have the authors dried DG decoction to be powder?

Response: After preparation, DG water extract was freeze dried. It was dissolved in Kerb’s solution (pH 7.4) when experiment was carried out. At the highest concentration we used in this study, the pH value of DG solution is 7.2. We have not measured the osmolarity of DG solution. However, based on the criteria we measured, i.e. contractile force, coronary flow rate, heart specific enzyme activities, no abnormal response was observed when DG solution was injected into the rat heart. Therefore, we believed that the osmolarity of DG solution used in this study is similar to physiological state.

Comment: In Figure 1 A through B, it is not clear when the differences between control and treatments groups are significant. For instance, in Figure 1A, DG (0.4 mg/200 μl) vs. ctrl, are the differences significant at 1 min, 2 min, … or 15 min? or every min? In Figure 2 through Figure 7, what is the meaning of “n=3”?

Response: For Figure 1, we have used Two-way ANOVA to analyze the difference between control group and treatment group. Therefore, both factors including specific parameter and time have been examined in the statistical analysis. We have added the method used in the figure legend. For Figure 2 to Figure 7, the meaning of n=3 is three independent trials. We have revised the figure legend to clarify these points.

Comment: the interpretations of the present data are not appropriate. For instance, the detailed information with regard to the cardiac protective effect of DG from previous studies is needed to show what has been done. What are the new findings made in the present study? What are the study limitations of the present study?

Response: Thanks for the comments. We have revised the Discussion section according to the reviewers’ comments.

Responses to Minor concerns:
1. We have consulted an editor from our university to revise this manuscript and tried our best to correct all grammatical problems.
2. We have deleted/corrected the redundancies in Discussion vs Results section.
Responses to Reviewer 2’s comments

Comment: please give the rationale of choosing the ischemia and reperfusion Langendorff heart model, instead of in vivo models.

Response: The animal model of myocardial infarction (MI) which is mimicked by Langendorff heart model, is facing the high mortality rate of the surgical procedure. In brief, after anaesthesia, orotracheal intubation and thoracotomy, the heart is rapidly exteriorised and the coronary artery is ligated in the proximal segment using a thin thread. The occlusion of the artery can be recognised by blanching of the tissue distal to the ligation. In this complicated surgical procedures, a comparative study showed that the mortality of MI in Sprague-Dawley rats was 36% (see reference). Therefore, we choose Langendorff heart model instead.

Reference: