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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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 19 September in which you advised us to respond to the reviewers’ comments.

Following the 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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Referee 1

Comment: My concern is that the symbol * labeling on the Fig 1 and figure legend confused readers regarding with when the differences between control and treatment groups are statistically significant. To my understanding, the * indicates that the differences of data at every min between DG (0.4mg/200 μl) and control group shown in Fig 1 A to D are statistically significant. However, by my observation, for example, the data shown in Fig 1B at 1, 2, 3 and 4 min appear to be very close and seems to be no significant different statistically. The authors should be very careful to use the symbol and describe when the data are significantly different. The authors’ response by adding “Two-way ANOVA…” is not adequate, please delete this sentence.

Response: Thank you for your comment. We used Two-way ANOVA because there are two independent factors to be considered, different time points and different treatments. The symbol * indicated that there is significant difference between specific treatment group and control group. The significant difference considering all the time points (i.e. from 0 min to 15 min) on a whole but not necessary on every minute.

The sentence “Two-way ANOVA…..” has been deleted.

Comment: In Fig 4, *p<0.05 and ***p<0.01 maybe # p<0.05 and ###p< 0.001. Please check the mistyping. In Fig 7, please delete “analyzed by two-way ANOVA and”, which is redundant vs. Statistical analysis.
Response: We have corrected the mistyping in the legend of Figure 4. The sentence in the legend of Figure 7 “analyzed by two-way ANOVA and….” has been deleted.
Referee 2

Major concern:

Comment: The animal model used in this study is not in vivo which is mainly accepted as the ischemia/reperfusion model. The authors cite the paper1 to support the rational not to use in vivo model, is because modality of ischemia/perfusion surgery in SD rat is as high as 36%. However, this paper1 does not really support the present study. In the paper1 , 36% modality rate is based on the observation 1 month after the ligation of left anterior descending coronary artery. The present study is an acute study, in vivo model is supposed to be better than ex vivo.

Response: Thank you for your comments. We agreed that paper 1 we cited may not support the choice of Langendorff heart model as our model is acute while the cited one is chronic model. However, Langendorff heart model allows detailed and continuous analysis of intrinsic mechanic such as the contractile force and coronary flow in our experiment and also continuous measurement of heart damage as indicated by the release of lactate dehydrogenase (LDH) and creatine kinase (CK). It is the reason why Langendorff heart model has been the most widely adopted models of mammalian cardiophysiology for basic and pre-clinical drug research. With the results we obtained in this study, we can have more detailed information to understand how the Danshen and Gegen Decoction protect the heart from ischemia and reperfusion process. Moreover, we have more confident in obtaining positive outcome in heart protection in in-vivo model of coronary artery ligation which may perform in the future.

Comment: Western blotting data
Figure 3, 5A and 5B: actin bands appear the same. However, Figure 5B is from cytosol, while Figure 3 and 5A are from total cell. The actin bands should not be the same.

**Response:** The actin bands in Figure 3 and 5A are from whole cell extract and they are the same. However, the actin band in Figure 5B is not the same as Figure 3 and 5A. They look similar because all the lanes are well normalized.

**Comment:** Figure 3: the blotting bands of troponin 1 are hardly distinguished from the background. How were the differences between the samples determined.

**Response:** When we analyzed the band intensity using densitomter, a more distinguished band in each lane could be observed. The band was selected and the intensity was measured. The relative quantity (expression level) of each band was shown in the lower panel of Figure 3.

**Comment:** Figure 5B: the background in the same gel looks not the same (eg. Ctrl compared to Trolox).

**Response:** We think you mean the right panel of Figure 5B. Since protein expression level in mitochondria is very low (when compared to whole cell extract and cytosolic fraction), the band intensity is relatively weaker and the background is relatively higher. Under this circumstance,
the uneven distribution of background color is amplified. We guaranteed that each blot shown in the manuscript is a complete one. No cut and paste has been done on the blot.

**Minor concern:**

**Comment:** I am having difficulties in finding the troponin 1 antibody on the Cell Signaling website (http://www.cellsignal.com/). Could the authors please indicate the antibody used.

**Response:** The catalog number of Troponin I in Cell Signaling is #4002.

**Comment:** The catalog number of the antibodies used in the Methods should be added.

**Response:** Thank you for your comment. The catalog number of the antibodies used has been added in the Methods.

**Comment:** Spelling mistakes


**Response:** Thank you. The typos have been corrected.