Author's response to reviews

Title: Daming Capsule restores endothelial dysfunction induced by high-fat diet

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Author's response to reviews: see over
Jan 6, 2012

RE: MS: 1109106496627699
Daming Capsule restores endothelial dysfunction induced by high-fat diet
Rong Zhang, Huifang Niu, Ning Wang, Lihua Sun, Yi Xu, Ruibo Zhao, Xiang Ban,
Yao Yu, Baofeng Yang and Jing Ai

Dear the Editor and Reviewers:

Thank you for your letter dated Dec 8, 2011 and the encouragement to submit a revised version of our manuscript. And we really appreciate the Editor and Reviewers for their positive and constructive comments and suggestions on our manuscript. Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied comments carefully and addressed the Reviewers’ concerns. Please find detailed answers to the specific questions in the Response to Reviewers.

We have already stated under the Methods section that all experimental procedures and protocols used in this investigation were in accordance with the regulations of the ethic committees of Harbin Medical University (Page 5, line 7-8 in the manuscript). We consulted native English speakers to help us copyedit the paper. We tried our best to improve the manuscript and made some changes in the manuscript. These changes will not influence the content and framework of the paper. And here we did not list the changes but marked in red in the revised paper.

Once again we appreciate for Editor/Reviewers’ warm work earnestly, and hope that the correction will meet with approval.

Sincerely,

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Response to Reviewers

Reviewer: Luiz Carlos de Abreu

We thank the Reviewer for their valuable comments and insights. We have attempted to address all of the comments and we hope that the Reviewer will find our response satisfactory. Our revisions are printed in red font in the manuscript and our point-by-point responses are presented below.
Comments:

1. Keywords: Please, consult key words in the National Library of Medicine (http://www.nlm.nih.gov/mesh/MBrowser.html)

We really appreciate that the Reviewer provided the important information on the standardization of key words. We normalized the key words and replaced “high fat” with “high-fat”, “endothelial” with “endothelium” and “eNOS” with “endothelial nitric oxide synthase” (Page 2, line 23). We also replaced all “high fat” with “high-fat” in the manuscript (Page 1, line 1; Page 2, line 5; Page 4, line 15; Page 11, line 6; Page 13, line 24; Page 17, line 18; Page 18, line 16).

2. Abstract: The authors should include the objective of the manuscript in the Background.

According to the Reviewer’s helpful advice we added the objective of the manuscript in the Background. (Page 2, line 4-7)

3. Abstract, Method: The sentence “In this study, we tested the hypothesis that DMC would restore endothelial dysfunction produced by a HF diet. Importantly, we also investigated several mechanisms involved in mediating the effects of DMC on vascular reactivity such as the role of K+ channels and eNOS using appropriate inhibitors.” is more appropriate in the Background.

Thanks for the Reviewer’s correction, and we have moved the sentence to the Background and modified it as the objective of the manuscript in the abstract. (Page 2, line 4-7)

4. Method: Why the authors did not investigate iNOS by Western blot?

It is really an excellent question. We focused on eNOS in our study based on the supports that the NO cascade and eNOS are best known for their role in endothelium-mediated vasorelaxation, and eNOS is seen as a protective enzyme and has been established as a key regulatory signaling molecule in the vasculature (Isenović et al, Cardiovasc Hematol Disord Drug Targets. 2011; Arnal et al, Cell Mol Life Sci. 1999; Chung et.al, Exp Mol Med. 2011; Albrecht et al, J Pathol. 2003). We really appreciate the reviewer’s suggestion. Indeed, many studies indicated that iNOS was relevant to vascular injury (Behr et al, Atherosclerosis. 1999; Kibbe et al, Cardiovasc Res. 1999; Olukman et al, J Diabetes Complications. 2010) and oxidative stress induced the expression of iNOS and subsequent generation of high concentration of NO, which could interact with reactive oxygen species (ROS) causing vascular dysfunction (Olukman et al, J Diabetes Complications. 2010; Rocha et al, Vascul Pharmacol. 2011; Kviety et al, Free Radic Biol Med. 2011). These are
consistent with the Reviewer’s good point on ROS (mentioned in comment 8) and worth further studying. Furthermore, oxidative stress and iNOS are involved in the attenuation of endothelin-1 mediated vasoconstriction observed in isolated mesenteric arteries from high-fat fed rats (Sweazea et al, Horm Metab Res. 2011). That means peripheric arteries could present a different effect just like the reviewer’s suggestion (mentioned in comment 7). In the future work, therefore, we will investigate the role of ROS and iNOS on vasoreactivity and the effect of DMC on them. That would be an interesting and attractive story. We are also referring to these future studies in the discussion on page 13, line 8-16.

5. Results, Figure4D: The figures 4A, 4B and 4C are good. I suggest the authors to change this figure for a better one.

As the Reviewer’s suggested, we have replaced it with a better one as shown in Fig. 4D.

6. Discussion, 1st paragraph: Please, check this sentence: “The principal finding of this study was that DMC could protect the aorta from HF-induced endothelial dysfunction via upregulating the eNOS expression.” Although the authors showed a protective effect of DMC on histological and Western blot analysis, there was no functional effect when vascular reactivity was tested during contraction and relaxation. Thus, I suggest the authors to be careful in this assumption, it would be better to affirm that “DMC could partially protect the aorta from HF-induced endothelial”.

We apologize that our statements made the Reviewer misunderstand the functional effect of vascular reactivity. Our vascular reactivity results showed that treatment with DMC did not improve the loss of vasoconstriction (Fig 1B, shown with red arrow in the graph below). In order to show the vasorelaxation to ACh more clearly we calculated it as (tension PE – tension ACh)/tension PE (the percent change in reactivity to $10^{-4}$ M ACh after $10^{-5}$ M PE). As shown with red arrow in Fig 1C, significant mitigation was seen in the relaxation of the aortic rings in HF+DMC group. Along with the protective effect of DMC on histology and Western blot analysis, we concluded that DMC could protect the aorta from HF-induced endothelial dysfunction. But the Reviewer’s suggestion is more precise. Since DMC did not improve the loss of vasoconstriction, we affirm that DMC could partially protect the aorta from HF-induced endothelial dysfunction (Page 2, line 20; Page 10, line 4; Page 11, line 6; Page 13, line 19).
7. Discussion: Maybe DMC treatment for a longer period would present an effect with more intensity in vascular reactivity. This hypothesis could be raised. Furthermore, peripheric arteries could present a different effect, such as the tail artery.

We thank the Reviewer for these excellent advices. We included a short discussion on this topic on page 13, lines 8-11. As discussed in comment 4, we will make an animal model with the treatment of DMC for a longer period and investigate the role of eNOS, iNOS and ROS on vascular reactivity and function with aortas and some peripheric arteries.

8. Discussion: The authors could propose future studies to investigate DMC effects on arterial pressure, heart rate, cardiac autonomic regulation and reactive oxygen species.

Agreeing with the Reviewer’s suggestion, we have already done the studies of arterial pressure, heart rate and cardiac autonomic regulation in our previous work (Ai et al, BMC Complement Altern Med. 2010; Ai et al, Biol Pharm Bull. 2009). As for reactive oxygen species, it’s a good point and worth further studying. As discussed in comment 4, we proposed it for future work and expanded the discussion (Page 13, line 12-16).


We are very sorry for the negligence and have removed a redundant “of”. (Page 10, line 20)

Reviewer: Karl WK Tsim

We greatly appreciate the time and effort invested by the Reviewer to improve our
work. We have attempted to address all of the comments and we hope that the Reviewer will find our response satisfactory. Our point-by-point responses are presented below.

Comments:

1. The authors should be aware of the quality and clarity of all the figures. The standard of graphical presentation skill is not high enough. The authors should make the results in a more concise way for better illustration.

We apologize for the unclear graphical presentation. We remade all graphs with GraphPad Prism 5 and exported them in a better way of TIF format.

2. For all the experiments, the authors should include DMC alone as the control. It is important to know the background effect of DMC alone. Otherwise, the restoring effect of DMC against high fat diet will not be indicative. This is also applied to those experiments with ATV treatment that ATV must be treated alone as the background control.

Thanks for the Reviewer’s suggestion and we quite agree with it. In previous studies of chronic toxicology, we have ascertained that DMC treated alone has no damage effect on multiple tissues such as heart, liver, kidney, lung, vessels, et al. So we didn’t consider DMC alone as the control. ATV has now become one of the most powerful pharmacological strategies in the treatment of cardiovascular diseases. And there are a lot of relevant researches on ATV. We didn’t consider the group of ATV alone according to the experimental groups in these references (Abdin et al, Eur J Pharmacol. 2011; Rathouska et al, Pharmacol Res. 2011; Xie et al, Clin Exp Hypertens. 2010; Jorge et al, Arq Bras Cardiol. 2005). But it is really a good suggestion. And we will notice the point and observe the effect of ATV alone in future study.

3. In Fig1E, the statistics in HF+DMC and HF+ATV are misleading. The stars should not be placed in order to avoid the confusion that HF and HF+DMC has statistical difference. This is the same as for HF+ATV.

We are very sorry for making the Reviewer confuse with symbols representing statistical significance. In figures we used “*” to show statistical difference vs. control group. And we used “#” to present statistical difference vs. HF group. In Fig 1E, since the vascular contraction to KCl was significantly attenuated in HF group and HF+DMC as well as HF+ATV as compared with control, we placed stars in these three groups. We understand the misleading symbols referred by the Reviewer. If HF and HF+DMC has statistical difference we will place “#” to show the significance just like Fig 1C. Moreover, if we removed the stars the readers may misunderstand that HF+DMC and HF+ATV have no significant difference compared with control group.
So we keep the stars in Fig 1E. Considering the possible confusion, we made the figures in another way shown representatively as follows. We don’t think the figures look beautiful especially in Fig 2A, therefore we keep the initial way in figures’ presentation. We would be happy to do so if the Reviewer prefers the following way in figures.

Figure 1

Figure 2

4. In Fig4B, the damage of endothelial cells is not obvious and indicative. The authors should illustrate it using better and high-magnification images. This is the same as in Fig4C and 4D that the so-called recovery of endothelial cell is not indicative.
We are very sorry for the unsatisfactory histological presentation. We illustrated it using better images as shown in Fig 4. After HF diet the damage was only observed in vascular endothelium. The endothelium is a monolayer of flat cells and looks very thin under the microscope. In Fig4B, the borderline of endothelial cells is unclear and the nuclei are protrudent and even lost. As shown with arrows, no nucleus is found in continuous three or four endothelial cells. But in Fig4C and 4D, the protected endothelial cells arrange continuously and display nuclei continuously.