Author's response to reviews

Title: Elevated expression of periostin in diabetic cardiomyopathy and the effect of valsartan

Authors:

jun guan (18300271751@139.com)
mingqing xing (375816589@qq.com)
yue shi (1156162434@qq.com)
xueying tan (13012413468@126.com)
changqing jiang (liuwenqibaobei@126.com)
wenqi liu (laoshuaig@163.com)
hongyan dai (daihy9@163.com)

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Author's response to reviews: see over
Dear Editors:

We would like to submit the enclosed manuscript entitled “Elevated expression of periostin in diabetic cardiomyopathy and the effect of valsartan”, which we wish to be considered for publication in this journal. No conflict of interest exits in the submission of this manuscript, and manuscript is approved by all authors for publication. I would like to declare on behalf of my co-author that the work described was original research that has not been published previously, and not under consideration for publication elsewhere, in whole or in part. All the authors listed have approved the manuscript that is enclosed. In this work, we evaluated periostin may play a crucial role in cardiac remodeling and myocardial interstitial fibrosis process of DCM and it could be one of the important mechanisms for valsartan to improve the ventricular remodeling of DCM. For two reviewer's comments, we have made some detailed explanation and revision (the revised place shown into blue font). If you have any queries, please don’t hesitate to contact me at the address below.

Thank you and best regards.

Your sincerely,

Corresponding author: Dai Hong-yan

E-mail: laoshuaiqi@163.com

505621988@qq.com

Thanks for the comments and recommendations given by two reviewers.
Here are the detailed explanations for the comments from two reviewers.

**Answer to the first reviewer's report:**

1. **The authors need to be aware of the lack of the study and should mention them in the paper.**

Answer: In our study, blood glucose levels were too high, which resulted in some rats of ketoacidosis, infection, or even death. In future experiments, preventing acidosis caused by high blood sugar, keeping the environment clean and treating infections as well as other complications are very important in this kind of trials

**Answer to the second reviewer's report:**

**Major Compulsory Revisions**

1. **Methods line 14: could the authors explain what means "by lavage", how was valsartan given to the animals?**

   Answer: We want to write “by gavage”, "by lavage" is just a slip of the pen. In our study, rats in diabetes plus valsartan group were given valsartan by gavage using a suitable intubation cannula at 30mg/kg/day dose levels to soluble in 5 mL distilled water, whereas rats in the diabetes group were administered with the same amount of distilled water each day.

2. **Methods line 20: what was the distribution of glucose values in diabetic rats? How were multiple test taken into account?**
Answer: We are very sorry that we can not understand this question accurately. In our understanding “the distribution of glucose values” means the changed distribution of blood glucose levels of diabetic rats during the experiment, which has been reported in Figure 1. And we don’t know the meaning of “How were multiple test taken into account?” Could you please give me some more explanation?

3. Results: absolute p-values would be preferable.

Answer: We have added the absolute p-values in this paper.

4. Results: interpretation of results taking into account statistical aspects.

Answer: We have added a statistical analysis of results based on guidance. For example, the left ventricular coefficient of diabetic rats was higher than control rats (p=0.003), whereas it was lower in the valsartan-treated diabetic rats than the diabetic rats without valsartan (p=0.006), those differences have statistical significant.

5. Table 1. could be removed, since the same information is in the Methods.

Answer: We have removed Table 1.

6. Discussion: a significant proportion of diabetic rats died before analysis. Any comments on the possible effect of this on the results?

Answer: At the end of three weeks after STZ injection, the diabetic group rats were at a state of hyperglycemic, unfortunately, eight rats in diabetic
group were died due to diabetic ketoacidosis, infection, fighting each other or digestive complications. In addition, there was one rat, which blood glucose does not meet the model standard, removed. Then, the remaining 41 diabetic rats were randomly divided into valsartan treatment group and non-valsartan treatment group. The basic characters, such as Blood glucose and body weight, had no significant difference between these two groups. Specific blood glucose data: diabetic group (25.940±0.661) vs diabetic plus valsartan (27.551±0.839) p=0.207; Specific weight data: diabetic group (315.0±21.36) vs diabetic plus valsartan (323.3±28.15) p=0.825. So the proportion of diabetic rats died before grouping had no influence on the final results. Even though, preventing acidosis caused by high blood sugar, keeping the environment clean and treating infections as well as other complications are very important in this kind of trials.

**Minor Essential Revisions**

1. **Results line 10: left ventricular coefficient (left ventricular weight/body weight)-> mass index**

   Answer: Left ventricular coefficient, used to assess the extent of cardiac hypertrophy, which can calculate by comparing the left ventricular weight and body weight (See Reference [20]). However, Left ventricular mass index (LVMI) is Left ventricular mass (LVM)/ Body surface area (BSA). We have used the former one.

2. **Discussion: previous literature on the significance of periostin**
**could be in Introduction.**

Answer: we have discussed the importance of periostin in myocardial fibrosis and cardiac remodeling using previous literature in introduction (paragraph 2) and discussion.

**6. Legend to the Fig.1. Exact p-values would be preferable.**

Answer: Serum glucose levels of experimental animals.

The first week: control (7.513 ± 1.589) vs DCM (26.534 ± 0.684) #p=0.0002.

The third week: control (7.085 ± 0.527) vs DCM (25.940 ± 0.661) #p=0.0001.

The sixth week: control (7.703 ± 0.454) vs DCM (28.811 ± 0.448) #p=0.0001.

The ninth week: control (9.282 ± 0.167) vs DCM (29.441 ± 0.406) vs valsartan (27.303 ± 0.398) #p=0.0001, *p=0.0197.

The twelfth week: control (7.948 ± 0.242) vs DCM (29.129 ± 0.591) vs valsartan (26.887 ± 0.832) #p=0.0001, *p=0.0332.

The fifteenth week: control (8.949 ± 0.579) vs DCM (29.101 ± 0.599) vs valsartan (26.554 ± 0.542) #p=0.0001, *p=0.0347

**7. Legend to the Fig. 2. Exact p-values preferable.**

Answer: The degree of left ventricular hypertrophy of experimental animals.

control (1.43 ± 0.08) vs DCM (1.81 ± 0.8) vs valsartan (1.52 ± 0.55)
8. Figures 4 and 5. Arrows could be added showing the corresponding findings. The author can be trusted to make these. For example, missing labels on figures, the wrong use of a term, spelling mistakes.

Answer: Figure 4 has been coupled with arrows (see fig.4). Figure 5 is the result of western blot. We have not seen this kind of arrows added in western blot results in previous literature, so we do not know how to add arrows here.

**Discretionary Revisions**

**Discussion:** altered FFA-metabolism in diabetes has been shown to be one contributing factor to diabetic cardiomyopathy. The lipid metabolisms in rats differs from that in humans. This aspect could be discussed shortly.

Answer: In diabetic cardiomyopathy process, its myocardial cells metabolic disorders include glucose metabolism and lipid metabolism. The carbohydrate oxidation for energy is reduced and the use of lipid oxidation is increased as well as the material for the energy transferred from glucose to fatty acids, so the free fatty acid is elevated caused by lipid metabolism disorders has been widely accepted [5]. In addition, Carley et al [6] have shown that fatty acid, the endogenous ligand of Peroxisome Proliferator Activated Receptors α (PPARα), which uptake and utilization in the regulation of PPARα. Moreover, the energy
metabolism signaling pathway of PPARα-FFA in cardiomyocytes may joint with ventricular remodeling, which are important reasons for the pathogenesis of diabetic cardiomyopathy [7]. More study is needed to demonstrate if there is any relationship between FFA-metabolism and periostin on diabetic cardiomyopathy.