Author's response to reviews

Title: I(f) Current Channel Inhibitor (Ivabradine) Deserves Cardioprotective Effect via Down-Regulating the Expression of Matrix Metalloproteinase (MMP)-2 and attenuating apoptosis in Diabetic mice

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Author's response to reviews: see over
Dear Editor-In-Chief

We appreciate for the efforts by your distinguished Reviewers commented on our manuscript entitled by “1567198821141050 - I(f) Current Channel Inhibitor (Ivabradine) Deserves Cardioprotective Effect via Down-Regulating the Expression of Matrix Metalloproteinase (MMP)-2 and attenuating apoptosis in Diabetic mice”.

We have been very carefully to answer all questions raised from Reviewers, as following:

Reviewer 1—minor issues:

1. Normal distribution of included variables should be evaluated
   
   **Answer:** Thanks! We have re-described the distribution of data in the Statistical Analysis section which is highlighted by blue color in the 1st paragraph in the Page 14

2. If possible diastolic dysfunction should be added and its effect by ivabradine should be added
   
   **Answer:** Thanks! We are very sorry for being unable to provide data of diastolic dysfunction, because cardiac echo was performed in such small animals. Our next study is ongoing to analyze the cardiac function including diastolic dysfunction by right heart catheter

Reviewer 2—minor essential revisions

1. Although not focused on patients with diabetes, major randomized trials regarding ivabradine (e.g. BEAUTIFUL, SHIFT)
should nevertheless be mentioned in the introduction or in the
discussion section

**Answer:** Thank you very much! We have added these two studies as Reference 38 and Reference 39 in red color.

2. A "limit section" should be added in the paper (include for example the small sample size)

**Answer:** Thanks! We have added Limitation section in the last paragraph in Page 21 and first paragraph in Page 22 as highlighted in red color.

3. What does this study add in clinical practice? What studies are desirable in the future?

**Answer:** Thank you very much! We have added the clinical relevance and future study in our freshly added Conclusion section in red color.

4. Add the “conclusions “ in the main paper

**Answer:** Thanks! We have added the Conclusion section in the last paragraph in red color in Page 22.

Our manuscript entitled by “I(f) Current Channel Inhibitor (Ivabradine) Deserves Cardioprotective Effect via Down-Regulating the Expression of Matrix Metalloproteinase
Ivabradine, a specific heart rate-lowering drug, has been extensively used for treatment of angina and congestive heart failure, without any alteration of blood pressure. Its main molecular mechanism was reported to anti-oxidation via NADPH pathway. However, effect of Ivabradine on cardiac function and the underlying mechanisms in diabetic animals were not fully studied.

Twenty diabetic mice were randomly assigned as IVBD (ivabradine was added in the diet at a dosage of 10 mg/kg/day, for 3-month) and Control (ivabradine was replaced by saline, for 3-month) groups. After 3-month treatment, microarray was performed to identify the differentially expressed genes, and cardiac function was measured by echocardiography. Immunohistochemistry analysis of ventricular myocardium was used to compare the difference in staining up-regulated gene(s) between two groups.

At 3-month, cardiac functional indexes in IVBD group were improved significantly, as shown by increment of left ventricular (LV) eject fraction, fraction of shortness, wall thickness at the end-systole, and by reduction of LV cavity. Ivabradine treatment attenuated the expression and staining score of matrix metalloproteinase (MMP)-2. Expression of several genes participated in the modulation of
inflammation and apoptosis was up-regulated, as such demonstrated by the increased expression and activity of 6Ckine, ACE/CD143, ALK-1, CT-1, CD27, Endoglin, IL-17E/F, IL-1ra/IL-1F3, and IL-2 Rα, macrophage inflammation protein-3 β (MIP-3β), Epigen. Ivabradine induced the dephosphorylation of Caspase 3, BAX and MMP-2, but enhanced the phosphorylation of NF-kB.

We thus feel that your readers would benefit from our findings and respectfully submit the revised manuscript for publication in your prestigious journal.

We certify that: 1) the paper is not under consideration elsewhere; 2) none of the paper or contents have been previously published; 3) all authors have read and approved the manuscript; and 4) the full disclosure of any potential relationship with industry.

Again, the authors are indebted to the Editorial Board and Reviewers for their expert input and

Sincerely yours,

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