Author’s response to reviews

Title: Protective Effect of Hydrogen-Rich Saline on Pressure Overload-induced Cardiac Hypertrophy in Rats: Possible Role of JAK-STAT Signaling

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Author’s response to reviews:

Dear editor and reviewers,

Thank you very much for your comments. The comments have been carefully taken into account and a new revised submission has been uploaded. We highlighted all the changes in red. And the responses are as follows,

Editor Comments:

Stylistic comments to address:

1) Abstract: Objective and Introduction should be changed to Background
Response: We have changed.

2) A Conclusion heading should be added after the Discussion
Response: We have added.
3) Author contributions: please use authors initials instead of full names.

Response: We have changed.

4) Ethical approval and consent to participate: a statement indicating that the study was performed following approval of the Institutional ethics committee/animal experimentation committee is required

Response: We have added.

5) Figure 4:

(i) the Western blot of JAK appears to be identical to the b-actin loading control. Please clarify.

Response: Each experiment has been done for 3 times and statistical analysis was performed. And we chose a better quality image as a representative image for publication. The image has been changed by a better quality one.

(ii) b-actin loading control: the same Western blot image has been used in all three panels in this Figure for the loading control. Please could you explain why the IL-6, JAK and STAT3 blots shown in the Figure do not appear to have been run on the same gels as the b-actin images provided. Ideally, the representative b-actin blot should be from the same Western blot as the target protein Western blot, i.e. the membrane probed with the anti-IL-6 antibody, the membrane stripped and then re-probed with the anti-b-actin antibody.

A similar instance also occurs in Figure 5. Furthermore, the b-actin blot is the same as in Figure 4.

Response: ANP, BNP, IL-6, JAK, STAT3 and beta-actin were run on a same gel/ membrane. It was possible that we then cut out the membrane with a scissors to get different strips and probed the different stripes with their corresponding primary antibody. We did pilot study first.

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Reviewer reports:

Jacques Couet (Reviewer 1): This is fairly well written manuscript although it lacks some important details.
In the introduction (line 15), it is written that hydrogen has been used in medical care. It is not the case since it has been assayed only in experimental settings in humans or animals.

Response: We agree with the review and made the change accordingly.

In the methods, only dosage of HRS is indicated. I suppose HRS is administered IP once a day but this should be indicated. Is the treatment initiated at the time of surgery, before or after?

Response: We have added the following sentence in Methods Section: HRS was injected into the peritoneal cavity once a day after surgery.

In the results section, a table should be added to include animal body weight, heart weight, LV weight, lungs (signs of heart failure) and other characteristics if available.

Response: In this study, we are very sorry that we did not weigh the lungs. We have made a Table 1 to show the records of all weights.

In Figure 2, fibrosis quantification should have been included. Include Figure 5 results with Figure 4.

Response: We added the fibrosis quantification in Figure 2. To our knowledge, image J is a widely used software for image quantification.

The main problem with this study is that we do not know if effects on the JAK-STAT pathway are direct or not. It is mentioned that IL-6 is produced by neutrophils and activated macrophages in the myocardium. It is likely the case but it was not demonstrated in their studies. This should be corrected in the Discussion or demonstrated in the Results section.

Response: We agree with the review’s comments, and have made changes in discussion accordingly.

Umberto Barbero (Reviewer 2): Fan et al performed an interesting work on the use of H2 to improve cardiac hypertrophy and evolution to HF in rats. I think the paper is really well written, statistics are correct and the conclusion interesting. Images are of quality and well described. The paper should be suitable for publication after some minor revisions.

Abstract, page 3 line 17-20: after they were divided in 4 group, authors should put a double dot and then the group in numerical order to make this point clearer (i.e. 1. sham-operated (sham), 2: AAC-model, 3: AAC + Low HRS (LHRS), 4: AAC + High HRS (HHRS))

Response: We have made the changes in both Abstract and Method Section.

Abstract, line 31: what (blue) means? If not pertinent in the abstract it should be removed.
Abstract line 31-37: please re-write the sentence "Apparent interstitium and collagen protein expression (blue) in left ventricle could be observed in AAC-model group, meanwhile, the levels of atrial natriuretic peptide (ANP) and brain natriuretic peptides (BNP), were also attenuated after HRS treatment" because it is not clear.

Response: We have re-written the sentence in Abstract.

Abstract line 45: please change "which may associate with decreasing JAK-STAT signaling pathway." To "which may be associated to a decreasing in JAK-STAT signaling pathway."

Response: Thank you for your comments. And we have made changes accordingly.