Peer Review File

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Reviewer A

1. Introduction, it should be noted more about the known effects of CRP on the heart.
Response: We thank the reviewer for this suggestion. We have searched the literature related to the therapeutic effect of CRP itself on heart, however few studies were found. We have supplemented known function of main bioactive compositions of CRP on heart in the revised manuscript, see page 5, line 109 to line 124

2. Material and Methods, additional information should be included the composition of the CRP or the main active substance, %, What is this substance was dissolved?
Response: Based on the reviewer's suggestion, we have modified this part in the revised manuscript, please see page 6, line 147 to line 149. CRP was dissolved in sterile saline and was orally administrated after ISO infusion to the mice according to our previous studies on QLQX.

3. Why did you choose this dose and duration of CRP? It is necessary to give a reference.
Response: We thank the reviewer for this suggestion, in the revised manuscript, we have added the reference, see page 6 and page 7, line 149 to 151. CRP is one herb in QLQX, we chose the dose （0.5g/Kg/d） and duration (3 weeks) of CRP according to our previous study on traditional Chinese medicine QLQX (https://pubmed.ncbi.nlm.nih.gov/28647730; https://pubmed.ncbi.nlm.nih.gov/29862242/)

4. Indicate the duration of incubation of CRP in ISO + CRP group NRVMs. Line 175 - 24 hours, Line 186 - 48 hours. Indicate n= in each group.
Response: We thank the reviewer for this suggestion. Before ISO and CRP treatment NRVM were cultured in starvation medium for 24 hours to synchronize the cells and then treated with ISO and/or CRP for another 48 hours. We have modified this part, see page 8, line 190 to 192, line194 and line 198.

5. Line 240 Specify that used to load control.
Response: We thank the reviewer for this suggestion., we have added this information in the revised manuscript, see page 11, line 251 to 253.

Figures.
6. How the CRP can recover performance almost to the control level in ISO + CRP group? Fig.1 A, B, D.
Response: We thank the reviewer for raising this important question.
Comparing of left ventricular ejection fraction (EF) and fractional shortening (FS) between control mice and ISO+CRP treated mice (figure 1A), there is no significantly difference, indicating CRP protects against cardiac dysfunction induced by ISO infusion. However, the size of the cardiomyocytes (figure 1B) in ISO+CRP treated mice is significantly increased compared to control mice ($P<0.05$), although no obvious changes of the size of NRVMs were found between ISO+CRP group and control group（figure 1D）, suggesting CRP could attenuate cardiac dysfunction but could not totally reverse ISO-induced cardiac remodeling.

7. Fig. 1, 2. figures and captions are not clear, very small sign.
Response: We thank the reviewer for this suggestion, in the revised manuscript, we have changed the figures and captions.
8. Correct all the legends to figures, write the same in all figures according to indicators.
Response: We thank the reviewer for this suggestion, in the revised manuscript, we have corrected all the figures and figure legends.

Reviewer B

Major Concerns:
1) Although the authors claim that a PPARγ inhibitor blunts the protective effects of CRP, they are missing one control cohort which is vital to support this claim, namely an ISO + PPARγ inhibitor cohort. Without this control, one could hypothesize that the CRP and PPARγ inhibitor are simply acting in opposition to one another (CRP blunts ISO response, PPARγ inhibitor exacerbates the response) instead of CRP acting through PPARγ
Response: We thank the reviewer for raising this important question.
PPARγ has been found to play critical roles in inhibition of cardiac hypertrophy *in vitro* and *in vivo* (https://pubmed.ncbi.nlm.nih.gov/11889020/). We measured the expression level of PPARγ and found downregulation of PPARγ in both ISO-infused mice and ISO-treated NRVMs (figure 3A &3B). CRP treatment significantly increased PPARγ expression in both ISO-infused mice and ISO-treated NRVMs (figure 3A &3B). Based on these findings, we hypothesized PPARγ upregulation mediated by CRP treatment maybe essential for CRP to exert its protective effects against ISO-induced cardiac remodeling and dysfunction. To investigate whether CRP attenuates heart failure through PPARγ activation, we applied PPARγ inhibitor to ISO- and CRP-treated mice; and found that with the application of PPARγ inhibitor, CRP treatment failed to upregulate the expression of PPARγ (figure 4A) and could not attenuate cardiac dysfunction (figure 4B) and cardiac remodeling (figure 5A) of ISO-treated mice, suggesting PPARγ upregulation is necessary for CRP's beneficial effects in attenuating ISO-induced cardiac remodeling and dysfunction.

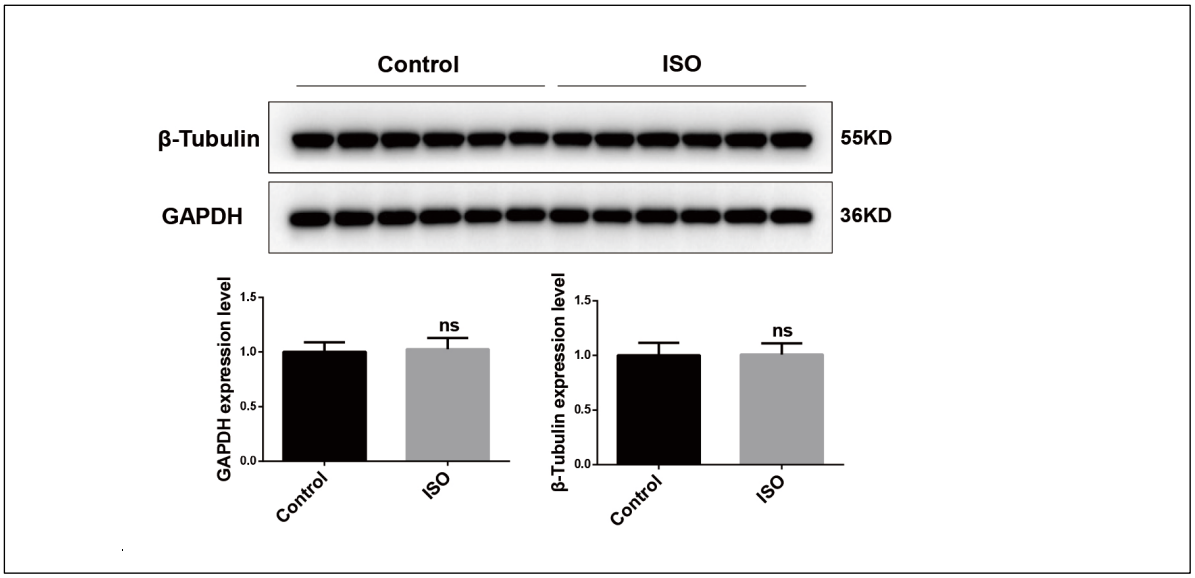
PPARγ agonists have been found to suppress the hypertrophy of cultured NRVMs (https://pubmed.ncbi.nlm.nih.gov/11581147/). We applied PPARγ agonist to ISO- and CRP-treated NRVMs and found PPARγ agonist could not further enhance the beneficial effects of CRP against ISO-induced hypertrophy in cultured NRVMs (figure 4E), indicating CRP and PPARγ agonist work in the same pathway to inhibit the hypertrophy of cultured NRVMs.

However, other mechanism might also be applied to the therapeutic effects of CRP on heart, which requires further investigation. Nonetheless, we really appreciate the reviewer's suggestion and this will greatly benefit our future studies.

2) Although GAPDH is a good reference gene in many cases, in rats it has been shown to vary during cardiac hypertrophy and failure. (https://pubmed.ncbi.nlm.nih.gov/20331858/) The authors may wish to consider using an alternative reference gene in the NRVMs.
Response: (1) We thank the reviewer for raising this important question.

To determine the protein expression level in NRVMs, we harvested the cells and extracted the total protein with the lysis buffer, then we quantified the concentration of protein of each sample using standard BCA protein assay. The same amount of each protein sample was loaded for SDS-PAGE and western analysis, and we found GAPDH was stable in different treatment groups, so we chose GAPDH as a loading control in our previous studies and in this study.
Based on the reviewer's suggestion, we also compared the expression of β-tubulin and GAPDH in both control and ISO-treated NRVMs (n=6:6) and found there is no significant difference.

Minor Concerns:
1) ISO injection vs osmotic pump infusion have different effects on the organism even though both will result in eventual hypertrophy and failure. The authors may wish to discuss this briefly as infusion is both the more common and more physiologically relevant means to induce HF. (see https://pubmed.ncbi.nlm.nih.gov/15791293/).
Response: We thank the reviewer for this suggestion, we have added this part in the revised



manuscript, see page 14 and page 15, line 345 to line 353.

2) Labels on 1B, 2A and 4C should be in black. White color is very hard to see
Response: We thank the reviewer for this suggestion, in the revised manuscript, we have changed the colors of the captions in these figures.

3) Figures in general should be higher resolution... labels were very hard to make out.
Response: We thank the reviewer for this suggestion, in the revised manuscript, we have uploaded higher resolution figures.