Peer Review File

Article Information: http://dx.doi.org/10.21037/cdt-20-201

Responds to the reviewer's comments:

Reviewer #1:

Comment 1: Please highlight the strengths in clinical practice.

Reply 1: We have added relevant contents in the text. For details, please refer to the revised version.

Changes in the text: Page14, line 3-6

Comment 2: It is suggested that some up-to-date references should be added.

Reply 2: We are grateful for the suggestion. The references have been added and the specific changes are marked in red in the revised manuscript.

Changes in the text: Page 17, line 4-5

Comment 3: The English writing needs to be embellished for the manuscript.

Reply 3: Thank you for underlining this deficiency. We had asked a native English speaker to modify the whole article.

Reviewer #2:

Major Issues:

Comment 1: Please note that the shortened explanation you are giving here might be misleading and can cause some misunderstanding in the context you described, since reference (7) is not giving clear evidence to proof your point. I think you might rather refer to your reference (3), which describes a theory of mitochondrial calcium overload in heart failure as the result of a dysregulated feedback loop between sarcoplasmatic reticulum and mitochondria.

There is very strong evidence that Ca2+ released from the sarcoplasmatic reticulum contributes for the most part to the cytosolic Ca2+ concentration after myocardial depolarization. There is no indication that the mitochondrial matrix significantly alters the cytosolic Ca2+ concentration at least under physiological conditions (Boyman et al., 2014). In case this significantly changes in the setting of heart failure, please provide sufficient supporting evidence including citations and expand on this in your explanation on the underlying mechanisms for a better understanding of our readers.

Reply 1: Thank you for underlining this deficiency. The references (7) have been replaced to reference (3) and the specific changes are marked in red in the revised manuscript.

Changes in the text: Page 3, line 6-8

Comment 2: Your results indicate an increase in the pressure development rate of the left ventricle, not in the rate of the ventricular contraction which would be the heart rate. These terms should be carefully distinguished.

Reply 2: Thank you for your suggestions. After careful check, we found that there was a deviation in the translation process, which has been corrected in the revised version.

Changes in the text: Page 12, line 3

Comment 3: Unfortunately this is not correct. The excitation-contraction coupling in cardiac muscle cells is described slightly differently. Extracellular Ca2+ influx induces intracellular Ca2+ release from the sarcoplasmatic reticulum (Keyword: Calcium-Induced Calcium Release) through the ryanodine receptor (Positive Feedback Loop) causing myocardial contraction.

Reply 3: Thank you very much for your advice. I'm very sorry for the mistake of expression caused by the problem of language translation. Your description is more professional and accurate, which is worthy of our careful study. We have made corrections in the revised manuscript.

Changes in the text: Page 12, line 16-18

Comment 4: Please note that on the basis of your presented data I cannot fully agree that you have sufficient evidence to proof a direct interaction between the HNO donor Angeli’s salt and the SERCA2a protein activity. Further investigations to proof this point would be necessary and have already been carried out by Tocchetti et al. (2007). But your in vivo observations nicely correlate and approve the theories that have already been published. Very similar in vivo investigations on the effect of HNO in a different heart failure model have already been made by Paolocci et al. (2003). I kindly ask you to emphasize this by giving a reference and clearly communicate the circumstances to our readers in order to pay credit to the investigations that have already been made. I would appreciate if you could emphasize the novel aspects of your own study as to better differentiate it from similar research.

Reply 4: Due to our improper use of words, the translation is wrong. We can't draw this conclusion directly. We have made modifications in the text. The novelty of this study has been described in the conclusion.

Changes in the text: Page 13, line 20-24

Minor Issues:

Comment 5: I would suggest to add the keywords in vivo and myocardial infarction to your title to make a clear distinction between the studies that have already been published in this field and your data. Especially (Tocchetti et al., 2007) already described the effect of Nitrosyl Hydrogen on the activity of SERCA2a in the setting of heart failure in vitro. I would therefore consider your contribution as a confirmatory proof of concept study that gives further valuable information in an in vivo disease setting. "In vivo Effects of Nitrosyl Hydrogen on Cardiac Function and Sarcoplasmic Reticulum Calcium Pump (SERCA2a) in Rats with Heart Failure after Myocardial Infarction"

Reply 5: Your suggestion is very constructive. After staidness consider, we think your title is more suitable for the content of the paper, and we have replaced the title in the revision.

Changes in the text: Page 1, line 1-3

Comment 6: I would recommend to rename the AS group to HF+AS group in order to make clear that both groups, HF and HF+AS, underwent the same surgical intervention causing heart failure. Also I would suggest to adjust the study group number to 26 male Wistar rats, since this is the actual number of animals that has been statistically analyzed as demonstrated on page 5 ll. 10-12.

Reply 6：Thank you again for your advice, we have renamed the AS group to HF+AS group and adjust the study group number to 26 male Wistar rats.

Changes in the text: Page 2, line7-9

Comment 7: Since there are different isoforms of SERCA and you only investigated SERCA2a, I would kindly ask you to emphasize this detail for further clarification.

Reply 7：We looked up a lot of references and found SERCA2a is more representative, so we tested this index. We are very sorry for our hasty conclusion. Our research suggested that Nitrosyl hydrogen could improve the cardiac function possibly by increasing protein activities of SERCA2a in rats, but whether this conclusion is applicable to all different isoforms of SERCA in rats needs further study in the future.

Changes in the text: Page 2, line 20

Comment 8: I would highly appreciate if you could add the name of the ECG Monitoring System you were using to make it easier for fellow researchers to

reproduce your study.

Reply 8: The name of the ECG Monitoring System we were using was BL-410 Bioassay System, it is a comprehensive monitoring system, which can monitor electrocardiogram and hemodynamics related indexes by selecting different modes.

Changes in the text: Page 4, line 7-8

Comment 9: Please give further information about the surgical intervention you were performing regarding the myocardial ischemia time (permanent ligation/ reperfusion time), the suture technique, suture material and analgetic medication adminestered. In case you have previously established a study demonstrating the equivalence of the Area at Risk (AaR) between HF and AS group, do not hesitate to add this information for the supplementary data.

Reply: In the surgical intervention, we used permanent ligation, the suture technique was simple interrupted suture, suture material was 7 / 0 non-invasive suture, and analgetic medication administered was 1% pentobarbital sodium. We have illustrated this information in the manuscript.

Comment 10: Please provide further information about the exact starting time point of the drug intervention. It would be more convenient if you could emphasize this information also in the results section. Also references showing the potency of the dosage applied in similar disease settings would be very useful.

Reply 10: At the 4th week after the establishment of heart failure model, drug intervention was started, and the experiment was finished 14 days after drug intervention.

Changes in the text: Page 8, line 19-20

Comment 11: In order to avoid any irritation or misunderstanding, please make sure to explain your endpoint criteria in detail.

Reply 11: By measuring the respiratory rate, urine volume, food intake and other indicators of the control group rats, we found the rest of the rats markedly reduced activity and respiratory rate, and the mortality of the control group rats increased, thus the experiment ended.

Comment 12: Please be more specific and describe the appropriate form of anesthesia administered to the animal during echocardiography.

Reply 12: Analgetic medication administered we used was 1% pentobarbital sodium 40 mg/kg. We have illustrated this information in the manuscript.

Changes in the text: Page 4, line 20-21

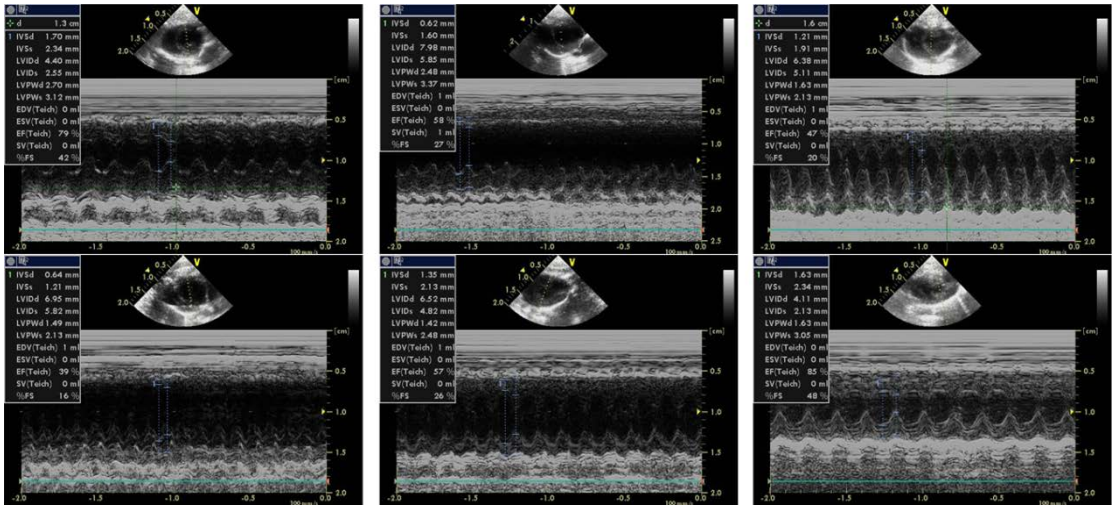
Comment 13: I am pretty sure you were measuring the left ventricular long axis and short axis section of the heart.

Reply 13: Your understanding is completely correct. We have made correction in the revised version.

Changes in the text: Page 6, line 4

Comment 14: Please provide further information if the investigators were blinded during image acquisition and analysis, since echocardiography is subject to individual perception. Also please describe the exact time points of the image acquisition. A graphic with a time line is a very convenient way to visualize this.

Reply 14: The investigators were blinded during image acquisition and analysis and the graphics have been added in the revised manuscript. The following graphics are the result of our experiment (the left two pictures are HF group, the middle two pictures are HNO group and the right two pictures are sham group).



Comment 15: Please be more specific about the amount of blood you were collecting after the intervention. Also kindly provide further information about the washing procedures, the developer and exact standard used for the spectrophotometer in the following.

Reply 15: After 2 weeks of intervention, 1ml of blood was collected from the tail vein, and the serum was separated. The serum and standard sample were added to the enzyme plate coated with human NT-ProBNP antibody and incubated at 37 ℃ for 1 hour. The plates were washed for 5 times, and the biotin-labeled antibody 50ul was added to each reaction hole and incubated at 37 ℃ for 2 hours. After washing the plate for 5 times, 30min was incubated with horseradish peroxidase labeled antibody 50ul at 37 ℃. Then the plate was washed for 5 times and 8min was incubated at 37 ℃

with 50ul fluorescent substrate. Then the biotin-labeled antibody 50ul was added to each reaction hole and incubated at 37 ℃ for 2 hours. The plate was washed again for 5 times, and 30min was incubated at 37 ℃ with horseradish peroxidase labeled antibody 50ul. The plate was washed for 5 times and 8min was incubated at 37 ℃ with 50ul fluorescent substrate. The values of standard and sample were measured at 450nm wavelength, and the content of NT-ProBNP in the sample was calculated. Adding developer solution to develop for 3 minutes, then placing in fixative solution for 10 minutes, It is washed with clean water and dried.

Comment 16: Please provide the product name of the pressure transducer catheter. For visualization purposes, it would be nice if you could add representative graphs of the acquired pressure waves.

Reply 16: The pressure pipes and sensors we use are the relevant supporting devices of BL-410 biological detection system.

Comment 17: Please be more specific and describe the appropriate form of anesthesia administered to the animal during the hemodynamic measurement thoroughly.

Reply 17: Analgetic medication administered we used was 1% pentobarbital sodium 40 mg/kg. We have illustrated this information in the manuscript.

Comment 18: Please provide further information regarding the clone of the primary and secondary antibody to make it easier for fellow researchers to reproduce your study.

Reply 18: The antibodies we used are SERCA2a first antibody (Abnova company), sheep anti-mouse second antibody (Abnova company) and β-actin first antibody (Abnova company).

Comment 19: Here you should state the starting time point of the drug intervention.

Reply 19： We have added relevant information to revised manuscript.

Changes in the text: Page 8, line 19-20

Comment 20: Please provide an appropriate reference showing the anti-oxidative effect and significance of HNO for the myocardial remodeling process. Avoid general statements as much as possible and expand on the main possible advantages of HNO over Nitrates in the treatment of heart failure patients as discussed in the literature to give the readers a clearer impression of the clinical relevance.

Reply 20: We have added relevant contents in the text. For details, please refer to the revised version.

Changes in the text: Page 10, line 5-14

Comment 21: I think this paragraph would greatly benefit from a clearer description of the suspected underlying mechanisms and molecular pathways. Over the last years, extensive research has been done investigating the functional role of SERCA2a, Phospholamban and Ryanodine Receptor in this context of HNO treatment. Please elaborate on this to give our readers an overview about the current state of knowledge.

Reply 21: In this study, we preliminarily discussed the function of HNO in SERCA2a treatment, and the detailed potential mechanism and molecular pathway will be further explored in the follow-up study.

Comment 22: Please be more specific about the biological effects you are addressing here and very briefly give the explanation for this effect in a physiological setting in one or two sentences.

Reply 22: Because the vasodilation effect induced by HNO is 1500 times that of nitrite, and its vasodilation effect can be significantly weakened by HNO inhibitors, but not by NO inhibitors, AS exerts its vasodilation effect mainly through HNO and has a protective effect on heart failure rats.

Changes in the text: Page 11, line 1-3

Comment 23: Missing reference/ citations

Reply 23: According to your suggestion, we have added following references:

Changes in the text: Page 12, line 21-23

Comment 24: Could you please elaborate on your theory? In how far could the formation of a disulfide bond in the cysteine active thiol group of SERCA2a enhance its biological function?

Reply 24: In this experiment, we observed that the activity of SERCA2a protein increased in the AS treatment group, but there was no significant difference in protein expression between the SERCA2a treatment group and the control group. Combined with reference 19, we speculated that HNO may play a cardioprotective effect by oxidizing the cysteine active sulfhydryl group on SERCA2a.

Comment 25: Please correct the syntax. Unclear meaning.

Reply 25: We are very sorry for the mistake, we have made corrections in the revised version.

Changes in the text: Page 13, line 15-19

Comment 26: Figure 1: Please define the triangle in the labeling ; Figure 3: Please define the vertical axis of the graphs ; Table 3: Please define the abbreviation LVEDP

Reply 26: We are very sorry for the mistake, we have made corrections in the revised version.