Peer Review File

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Reviewer A:

Comment 1: The authors mentioned that they added a space (Page 15, line 8), but they did not. It should be corrected before publication.

Reply 1: Thank you for your comment. We are sorry that we made such a careless mistake, we have carefully checked our manuscript to prevent similar mistakes from appearing again.

Changes in the text: see Page 16, line 4.

Reviewer B:

Reply: Thank you for your comment. We would like to express our appreciation to you for your valuable time and effort in reviewing and improving our work.

Comment 1: Page 7, lines 10-11 “Only Huayang Li was aware of the group allocation”.

The authors have added the above information. However, Huayang Li has participated in collecting and assembling data as stated in the “Author contributions” on page 21, line 17. This may cause bias in the data collection. Please explain: how such allocation (NOT blinded) still works as a randomized, blinded animal study?

Reply 1: Thank you for your comment. We are sorry for using a wrong statement. Indeed, one day after injection of MCT or equal volume of saline, each rat was tagged with an ear tag to identify it and the MCT-injected rats were randomly divided into the MCT+Veh group and the MCT+Cel group. Then the number of the ear tag was recorded in the EXCEL table to clarify the grouping information. only Huayang Li had the table, but would not open it in advance. The grouping would not be announced until all data had been collected, which meant that our study was indeed a blinded animal study. We have modified our text to better express our experimental information.

Changes in the text: see Page 7, line 10-19.

Comment 2: The authors have added the exact sample size for the three groups. However, they still have not explained HOW the sample size was reached.

Reply 2: Thank you for your comment. Monocrotaline rat pulmonary atrial hypertension (PAH) model is a widely used experimental PAH model. It has high stability and can efficiently reproduce the pathophysiology of clinical PAH (1). After reading the studies on rat PAH induced by monocrotaline, we found that most studies used 5-11 rats per group (2-4). Considering that the rats may die from PAH during the observation period, and in order to improve the power of test, we finally used the current sample size: the Normal group (n=10), the MCT+Vel group (n=13), the MCT+Cel group (n=12).

References:

1. Gomez-Arroyo JG, Farkas L, Alhussaini AA, et al. The monocrotaline model of pulmonary hypertension in perspective. Am J Physiol Lung Cell Mol Physiol 2012;302:L363-9.
2. Dai Y, Chen X, Song X, et al. Immunotherapy of Endothelin-1 Receptor Type A for Pulmonary Arterial Hypertension. J Am Coll Cardiol 2019;73:2567-80.
3. Cai Z, Li J, Zhuang Q, et al. MiR-125a-5p ameliorates monocrotaline-induced pulmonary arterial hypertension by targeting the TGF-β1 and IL-6/STAT3 signaling pathways. Exp Mol Med 2018;50:1-11.
4. Bordenave J, Thuillet R, Tu L, et al. Neutralization of CXCL12 attenuates established pulmonary hypertension in rats. Cardiovasc Res 2020;116:686-97.

Reviewer C:

A very interesting article. Please see my suggestions below.

Reply: Thank you for your comment. We would like to express our appreciation to you for your valuable time and effort in reviewing and improving our work.

Comment 1: Animal model: Better draw a flowchart regarding how the authors established the animal model, including the methods and details. Key details should be introduced in the methods too: inclusion criteria-not just weight but also other criteria like days of birth, any animals not included or excluded in the analysis and why.

Reply 1: Thank you for your comment. We have drawn a figure, and added this figure into our text as advised.

Changes in the text: see Page 27, line 2-7.

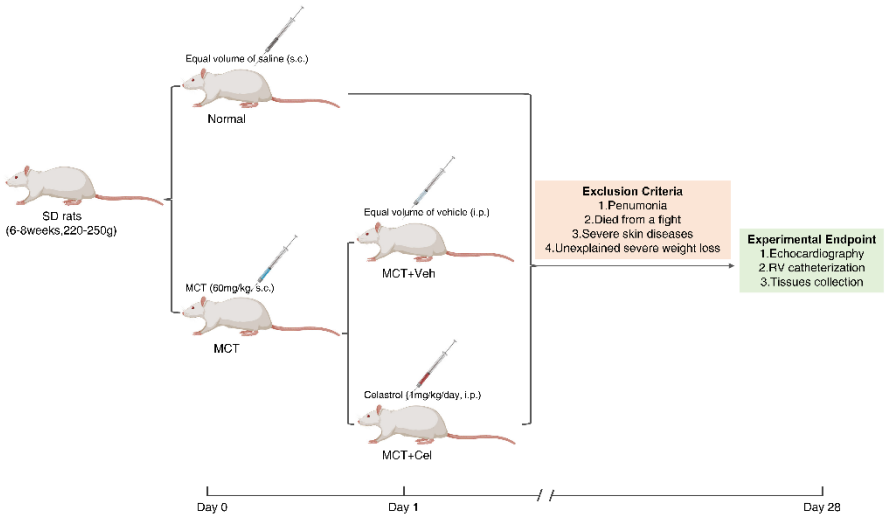

Fig.1 Study design Sprague-Dawley rats (6-8weeks, 220-250g) were subcutaneously injected MCT (60 mg/kg) or equal volume of saline at day 0. MCT-injected rats were randomly divided into the MCT+Veh and MCT+Cel groups at the day 1. The rats from MCT+Cel group received daily intraperitoneal injection of celastrol (1 mg/kg) for 4 weeks, and an equal volume of vehicle was administered to the MCT+Veh group. The four exclusion criteria were illustrated in the figure.

Comment 2: Animal model: what're the housing and husbandry conditions?

Reply 2: Thank you for your comment. We have added this information into our text as advised.

Changes in the text: Young male Sprague-Dawley rats (6-8weeks, 220-250g, purchased from Laboratory Animal Center of Sun Yat-sen University, Guangzhou, China) were housed in a specific pathogen free room with 12 h light/dark cycle and stable humidity and room temperature (40~70% and 20 ~ 25℃, respectively). 3 or 4 rats were housed in each cage. Sterile water and rat chow were provided ad libitum. (see Page 7, line 3-8)

Comment 3: Study protocol: Better register the study at Animal Study Registry (ASR): www.animalstudyregistry.org and name the register number in the article.

Reply 3: Thank you for your comment. We have registered the study at Animal Study Registry (DOI: 10.17590/asr.0000266).

Changes in the text: see Page 6, line 22; Page 7, line 1.

Comment 4: Randomisation: what's the method used to generate the randomisation sequence?

Reply 4: Thank you for your comment. We did not use randomization sequence to group. After modeling, each rat was identified by an ear tag with a number. All the MCT-injected rats were put into a cage, and then rats were randomly picked out and cross-assigned to the MCT+Veh group or the MCT+Cel group.

Changes in the text: see Page 7, line 10-11.

Comment 5: Blinding: please clarify who was aware of the group allocation, the conduct of the experiment, the outcome assessment and the data analysis.

Reply 5: Thank you for your comment. One day after injection of MCT or equal volume of saline, each rat was tagged with an ear tag to identify it and the MCT-injected rats were randomly divided into the vehicle group and the celastrol group. Then the number of the ear tag was recorded in the EXCEL table to clarify the grouping information and only Huayang Li had the table. The grouping would not be announced until all data had been collected, which meant that no one knew which rat belonged to which group until the data had been collected. Huayang Li, Quan Liu and Yuan Yue conducted the experiment and the outcome assessment. Shunjun Wang, Suiqin Huang, Lin Huang and Li Luo analyzed data.

Changes in the text: see Page 7, line 10-19; Page 23, line 10-13.

Comment 6: Confounders: did the authors take any strategy to minimise potential confounders? E.g. the order of measurements, animal/cage location.

Reply 6: Thank you for your comment. To minimize potential confounders, the injection sequence of the MCT+Veh and MCT+Cel groups was different every day and the order of measurements was also random.

Changes in the text: see Page 7, line 16-18.

Comment 7: Results: present the statistics with a measure of variability. E.g. mean and SD, median and range, the effect size with a confidence interval.

Reply 7: Thank you for your comment. We have modified our text as advised

Changes in the text: see Results/Para 1-6.

Comment 8: Abstract: add information on the animal species, strain and sex.

Reply 8: Thank you for your comment. We have modified our text as advised.

Changes in the text: see Page 3, line 8.

Thank you for your review and kind comments. We tried our best to improve the manuscript and made some changes in the manuscript. We appreciate for Editors/Reviewers' warm work earnestly, and hope that the correction will meet with approval. Once again, thank you very much for your comments and suggestions.

References:

1. Gomez-Arroyo JG, Farkas L, Alhussaini AA, et al. The monocrotaline model of pulmonary hypertension in perspective. Am J Physiol Lung Cell Mol Physiol 2012;302:L363-9.
2. Dai Y, Chen X, Song X, et al. Immunotherapy of Endothelin-1 Receptor Type A for Pulmonary Arterial Hypertension. J Am Coll Cardiol 2019;73:2567-80.
3. Cai Z, Li J, Zhuang Q, et al. MiR-125a-5p ameliorates monocrotaline-induced pulmonary arterial hypertension by targeting the TGF-β1 and IL-6/STAT3 signaling pathways. Exp Mol Med 2018;50:1-11.
4. Bordenave J, Thuillet R, Tu L, et al. Neutralization of CXCL12 attenuates established pulmonary hypertension in rats. Cardiovasc Res 2020;116:686-97.