

Reviewer A

This study evaluates the anti-inflammatory effect of a traditional Chinese medicine, Yanghepingchuan granule (YHPCG), on an asthma mouse model. The study discovered that the potential anti-inflammatory mechanism of YHPCG involves the inhibition of autophagy through the miRNA 328-3p/HMGB1/TLR4/NF-kB signaling pathway. Research investigating standardized herbal or phytopharmaceutical products is necessary to elucidate the pathways involved and provide substantial evidence to the community regarding the benefits of these widely utilized remedies.

MAJOR COMMENTS

1. In Figures 1A and 1B, it is not clear that YHPCG1, TAK 242, and the combination of YHPCG1 and TAK242 inhibited airway inflammation, hyperplasia of airway goblet cells and mucus secretion. Is it possible to quantify the histological findings in these experiments?

Reply1: Thank you very much for your valuable input on our study. It was an oversight on our part not to give the quantitative results of the images shown, and we apologize for the inconvenience in reading them. We have updated Figures 1A and 1B to include the corresponding graphs and statistics, as well as to clearly indicate the number and significance level of each group. A more detailed discussion explaining the significance of these quantitative data has been included in the results section of the paper, as well as in the figure legends, and the revised figures have been substituted for the original ones. These improvements will help communicate our findings more clearly and improve the reproducibility and interpretability of our experiments. Thank you again for your valuable comments and professional review.

Changes in the text: We add some data (see Page19,figure1) .

MINOR COMMENTS

1. The author stated that YHPCG had been extensively used as a traditional Chinese medicine (TCM) to treat various inflammatory diseases in China (lines 58-59). Additionally, asthma is currently treated with effective drugs, including TCM (lines 355-356). However, the discussion section regarding the practical clinical use of YHPCG in China still needs to be expanded. We recommend enhancing the discussion with literature that addresses the practical usage of YHPCG in China.

Reply1: First of all, I would like to express my gratitude for your careful review and valuable suggestions on my article. The part of the discussion you mentioned about the actual clinical use of YHPCG in China really needs to be further expanded. The lack of

discussion on this part is an oversight and we apologize for it. I have re-reviewed the relevant literature, especially those that specifically address the actual use of YHPCG in China, to add to the discussion section of the article (Refs. 32-35). Thank you again for your valuable suggestions that can make my article more complete and in-depth. Changes in the text: We have modified our text as advised (see Page12, line373-386) .

2. The writing consistency for YHPCG. The author has made the abbreviation Yanghepingchuan granule with YHPCG (introduction; line 125); however, in the discussion, it is still written Yanghepingchuan (i.e., line 388, line 390, line 403).

Reply2: Thank you for carefully reviewing my paper and providing useful feedback. I greatly appreciate your correction regarding the consistency of the abbreviation "Yanghepingchuan granule". The consistency issue in the article is indeed an important issue that needs to be corrected. I have taken steps to address this issue by ensuring that the abbreviation "YHPCG" is used wherever "Yanghepingchuan granule" is mentioned in the Discussion section of the paper. Changes have been made in the relevant places in the Discussion section to ensure that they are consistent. The conclusion of the article has also been revised to use the full name + abbreviation in the first reference, followed by the agreement to use the abbreviation "YHPCG" in all subsequent references. Thank you again for your valuable suggestions, which are very helpful for the accuracy of the article.

Changes in the text: We have modified our text as advised (see Page12, line387,388,390,403 and Page13,line413,417,421,423) .

3. We recommend changing "Yang" (line 276) to "YHPCG."

Reply3: Thank you for your valuable suggestions on our paper. We have made corresponding changes in the revised draft. Changing "Yang" (line 276) to "YHPCG" will help to express our point more clearly and ensure the accuracy of the paper.

Changes in the text: We have modified our text as advised (see Page9, line276) .

4. Please explain what TAK242 is in the introduction and the method sections. While in line 276, the authors describe TAK242 as a TLR4 inhibitor.

Reply4: Thank you very much for your valuable suggestions on our paper. Not stating the meaning of TAK242 for the first time in the introduction was an oversight on our part and we apologize for that, we have explained TAK242 in the introduction and in the methods section to improve the quality of the paper. Ensure that our paper communicates our research more clearly. In the introduction, TAK242 is introduced as a small molecule compound drug that is a selective inhibitor of Toll-like receptor 4 (TLR4), a receptor in the immune system involved in recognizing and responding to signals from exogenous pathogens, such as bacteria and viruses. TAK-242 works by

reducing inflammation and immune responses by inhibiting signaling from TLR4. In the Methods section, we have added that TAK242 was studied as a TLR4 inhibitor. These revisions will help readers better understand the important role of TAK242 in our study and its relationship with TLR4. Thank you again for your review and suggestions.

Changes in the text: We have modified our text as advised (see Page3, line32 and Page4, line113) .

5. In lines 262 and 265, what do “tube” or “tube walls” mean?

Reply5: Thank you for your helpful suggestion. It was our fault for not explaining it in the article, and we have explained "tube walls" in the article. In lines 262 and 265, this paragraph describes a study on the protective effect of YHPCG against pathological damage in asthmatic rats. tube walls" refers to the walls of the bronchial tubes. When it says "tube walls from the normal group did not thicken", it means that the bronchial walls did not thicken in the normal group. However, in the model group, "tube walls were thicker regarding smooth muscles", which means that the bronchial walls of these rats became thicker due to the presence of smooth muscles, and a large infiltration of inflammatory cells was found. tube walls" refers specifically to the bronchial walls of the rats. Thank you again for your review.

Changes in the text: We have modified our text as advised (see Page9, line274) .

6. In line 287, the authors described, “In the early stage, collecting samples from asthma patients.” It is not clear the meaning of “early.” Are these results from human subjects? If so, please describe the details of the experiments in the method section.

Reply6: Thank you for your useful suggestion. In the passage in the article, line 287 "in the early stage" means in the early preliminary stage of the study. We used RT-qPCR to confirm the presence of HMGB1 in asthma patients. Plasma samples were collected from asthma patients in the early stage of the experiment and high-throughput sequencing was used to screen for differentially expressed miRNAs. miR-328-3p was the up-regulated miRNA, which was differentially significant and its expression was negatively correlated with HMGB1. Therefore, this section describes an early step in the study, a step that played a key role in subsequent analyses that led to further studies of specific miRNAs, such as miR-328-3p.

Changes in the text: No changes in the text.

7. In line 421, it is unclear that YHPCG promotes the expression of inflammatory factors.

Reply7: Thank you for your careful review and valuable comments on our paper. Regarding the content of line 421 that you mentioned, we intended to state that the

results showed that Yang and Pingchuan granules may increase the expression of miRNA328-3p, inhibit the protein levels of MyD88, HMGB1, TLR4, and NF- κ B, and promote the expression of the inflammatory factors IL-13, IL-4, and IL-5, and the secretion of cuprocytes, which can alleviate the airway inflammation of asthma. It was our oversight that we did not express ourselves clearly enough to make the results section questionable to you. We apologize for this. After reviewing the paper again, we realized that our presentation may indeed cause misunderstanding. We have revised the results section to more clearly communicate our point. Thank you again for your suggestions, which are very helpful in improving our paper.

Changes in the text: We have modified our text as advised (see Page14, line444-451) .

8. We recommend reviewing sentences in lines 301-302: In conclusion, miR-328-3p negatively regulates HMGB1 via targeting.

Reply8: Thank you very much for your valuable suggestions. I have re-examined the content of lines 301-302. Regarding the sentence "In conclusion, miR-328-3p negatively regulates HMGB1 via targeting", I realize that the original sentence may be too short and not clear enough to express the content of the study, which is an oversight in our writing. negligence in writing. I apologize for this. I have revised this part of the sentence to describe the study more clearly. The revised sentence reads, "In conclusion, HMGB1 expression was higher in asthmatics compared to normal controls, and an inverse correlation was found between HMGB1 and upregulated miR-328-3p."

Changes in the text: We have modified our text as advised (see Page10, line313-315) .

9. The mutation site design (Figure 3C) was clearly explained in the results section (lines 294-297). However, the subsequent sentences (lines 297-301) still need to be clarified as to which figure describes this statement. We recommend that the author describe Figure 3C at the end of line 307. In addition, we also recommend that the author give an explanation of Figure 3D in the result section

Reply9: We thank the reviewer for their detailed feedback. It was an oversight on our part that we did not add the labeling of the corresponding figure when we explained the results of the figure. We apologize for the inconvenience. We have explicitly referenced Figure 3C at the end of line 301 to ensure that the reader has a clearer connection between the description of the section and the corresponding figure. For Figure 3D the lack of a corresponding descriptive explanation in our article was an error on our part, and we have added a detailed description of Figure 3D to the results section. Hopefully, these changes will make the presentation of the article clearer and easier to understand. Thanks again to the reviewers for their suggestions and corrections, which are very valuable to us.

Changes in the text: We have modified our text as advised (see Page10, line308 and Page10, line314-320) .

Reviewer B

The paper titled “Yanghepingchuan granule improves airway inflammation by inhibiting autophagy via miRNA328-3p/High Mobility Group Box 1/Toll-like receptor 4 targeting of the pathway of signaling in rat models of asthma” is interesting. Asthma airway inflammation can be treated with Yanghepingchuan granules by inhibiting autophagy via miRNA328-3p/HMGB1/TLR4/NF- κ B signaling pathways. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) What is the potential application value of YHPCG in clinical practice? What is the basis for selecting the concentration of YHPCG in this study? Is the dosage safe in clinical practice? Please provide literature support.

Reply1: Thank you for your helpful suggestions. Yang He Ping Chuan Granules (YHPCG) is a hospital preparation developed by the First Affiliated Hospital of Anhui University of Traditional Chinese Medicine. YHPCG activates the kidneys, revitalizes the blood, resolves phlegm as well as relieves cough and asthma. It has been used in the clinical treatment of bronchial asthma (BA) for decades with significant efficacy (Refs. 32, 33). The YHPCG concentration of 7.74 g/kg (YHPCG group) was chosen because of the previous studies in the literature: Mechanism of Yanghe Pingchuan granules treatment for airway remodeling in asthma. and Yanghe Pingchuan Granules Alleviate Airway Inflammation in Bronchial Asthma and Inhibit Pyroptosis by Blocking the TLR4/NF- κ B/NRLP3 Signaling Pathway, the dosage is safe and effective in clinical practice. safe and effective. Therefore, this treatment concentration was chosen for our study.

Changes in the text: We have modified our text as advised (see Page12, line381-394) .

2) The position and format of the scale bars in Figure 1 are not consistent. Please carefully check and make corrections.

Reply2: Thank you very much for your valuable comments on our paper. We apologize for the inconvenience caused to you regarding the location and format of the scale in Figure 1, and we have taken appropriate steps to improve this. The position and format of the scale bars in Figure 1 have been carefully checked to ensure that they are consistent throughout the image. Ensure that the location of the scale bar and its formatting is in accordance with the specification. Secondly, we have ensured that the

quality of Figure 1 has been improved by providing an updated version of Figure 1 in the revision as well as replacing the original figure with the revised one.

Changes in the text: We have modified our figure as advised (see Page19, figure1) .

3) Is airway remodeling a secondary event to inflammation? How do you view the relationship between the two? What role do YHPCG play in this? It is recommended to add relevant content.

Reply3: Thank you for your helpful suggestions. The relationship between airway remodeling and inflammation has been thoroughly explored in numerous literatures. Typically, airway remodeling is viewed as a long-term consequence of chronic airway inflammation that may be triggered by smoking, allergic reactions, or other irritants. When inflammation occurs, cytokines and growth factors may be released, which in turn affect smooth muscle cells, fibroblasts, and other cell types, contributing to changes in tissue structure. However, the relationship between airway remodeling and inflammation is not entirely linear; they may occur simultaneously and, in addition to inflammation, airway remodeling may be associated with other mechanisms such as cell proliferation, apoptotic imbalance, and matrix remodeling. YHPCG can be used to treat asthma through blocking the PI3K/PKB pathway, inhibiting the abnormal proliferation of ASMCS, and ameliorating kidney Yang deficiency (Reference 34).

Changes in the text: We have modified our text as advised (see Page12, line370-380) .

4) The immunofluorescence results of this study are not very clear. Please improve the image resolution or replace the image.

Reply4: Thank you for your helpful suggestion. We've reimproved the quality of the Figure 5A immunofluorescence image by increasing the resolution. And we have replaced added and provided a separate revised figure in the revised manuscript to ensure the accuracy and clarity of the results. This will help to show the immunofluorescence results more clearly. Thank you again for your valuable comments.

Changes in the text: We have modified our figure as advised (see Page23, figure5A) .

5) What are the toxic side effects of different administration methods of YHPCG in the treatment of asthma? Will the synergy with TAK242 produce new toxic side effects? It is recommended to add relevant content.

Reply5: Thank you for your valuable questions and suggestions regarding our paper. Yanghe Pingchuan granules (YHPCG) are a medication prepared at the First Affiliated Hospital, Anhui University of Chinese Medicine. YHPCG are composed of Ephedra sinica Stapf., Rehmannia glutinosa (Gaertn.) DC, Inula japonica Thunb. Schisandra chinensis (Turcz.) Baill., Sinapis alba L., Draba nemorosa L., Angelica

sinensis (Oliv.) Diels, and Platycodon grandiflorus (Jacq.) A.DC. YHPCG are known to invigorate the kidney, activate the blood, resolve phlegm, and relieve cough and asthma. YHPCG have also been used in the clinical treatment of BA for many years with clear curative effects. Because of the scarcity of studies on this drug, it is not known what toxic side effects are associated with the different methods of administration of YHPCG in the treatment of asthma and whether synergism with TAK242 may produce new toxic side effects, which is a limitation of our study. However, the suggestions you have given us have expanded the content of our future research and provided us with new directions. Thank you again for your suggestions.

Changes in the text: No changes in the text.

6) There are many detection methods for autophagy and inflammation. If multiple methods are used, the results may be more reliable. It is suggested to add test results of other methods.

Reply6: Thank you very much for your valuable comments on our study. Your suggestion of using multiple methods to detect autophagy and inflammation does help to improve the reliability and scientific value of the paper. However, our limited research resources restrict the number and types of experiments we can perform. In addition, the focus of our research is to concentrate on specific assays to answer specific scientific questions, so adding multiple other methods to validate the same experimental results may deviate from our research focus. Although we cannot provide additional experimental data, we can provide other information that supports the reliability of our research. We have implemented a series of rigorous experimental designs and controls to ensure the accuracy and reproducibility of our data. We have also conducted a detailed literature study to validate our results and compare them with other studies. Thank you again for your feedback and suggestions, which we will carefully consider and strive to improve the quality of our paper.

Changes in the text: No changes in the text.

7) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “IRAK-M knockout promotes allergic airway inflammation, but not airway hyperresponsiveness, in house dust mite-induced experimental asthma model, J Thorac Dis, PMID: 33841934”. It is recommended to quote the article.

Reply7: Thank you for your valuable comments on our paper. We appreciate your feedback and apologize for the shortcomings you mentioned regarding the introduction section and the lack of citations to relevant literature, and have taken your suggestions on board. In the revised version, we have expanded the introduction section to ensure a more comprehensive introduction to our research topic. We also cite the article you

mentioned, "IRAK-M knockout promotes allergic airway inflammation, but not airway hyperresponsiveness, in house dust mite-induced experimental asthma model, J Thorac Dis, PMID: 33841934" to ensure that our paper is more complete and authoritative. This article is closely related to our research and will provide readers with more in-depth background information and literature support. Thank you again for your suggestions and we will do our best to ensure the quality and completeness of our paper. Changes in the text: We have modified our text as advised (see Page4, line109-112).

8) In addition to the pathway in this study, which signaling pathways may this effect be closely related to? It is recommended to add relevant content.

Reply8: Thank you for your valuable suggestions. In this study, we have explored the effects of the HMGB1/TLR4/NF- κ B pathway specific pathway, but your reminder guided us to think about other signaling pathways that are closely related to this effect. Following your suggestion, we have cited reference 34 in the text to add accordingly to increase the comprehensiveness of the study. This effect is also correlated with the phosphoinositide 3-kinase (PI3K) signaling pathway. These not only help to increase the comprehensiveness of our study, but also help to better understand the effects and possible mechanisms in our study. We believe that these modifications will help improve the quality of our study and thank you for your guidance. Once again, thank you for your valuable comments.

Changes in the text: We have modified our text as advised (see Page12, line394-399).