Author's response to reviews

Title: Pingchuan Formula Improves Asthma via Restoration of the Th17/Treg Balance in a Mouse Model

Authors:

Fei Liu (liufeicindy@163.com)
Jianer Yu (jianeryu@126.com)
Li Bai (1248@szy.sh.cn)
Zheng Xue (xz695@163.com)
Xinguang Zhang (1110@szy.sh.cn)

Version: 3
Date: 15 June 2015

Author's response to reviews: see over
Dear Prof Mainen Moshi and Mr James Prozenko,

We thank you for your careful consideration of our manuscript. We appreciate your response and overall positive initial feedback, and have made modifications to improve the manuscript. After carefully reviewing the comments made by the Reviewers, we have modified the manuscript to improve the presentation of our results and their discussion, therefore providing a more complete context for the research that may be of interest to your readers.

We hope that you will find the revised paper suitable for publication, and we look forward to contributing to your journal. Please do not hesitate to contact us with other questions or concerns regarding the manuscript.

Best regards,
Response to Reviewers’ comments

#1 Reviewer's report

Title: Pingchuan Formula Improves Asthma via Restoration of the Th17/Treg Balance in a Mouse Model

Version: 2 Date: 8 February 2015

Reviewer1 : ZhenQu

Reviewer's report:

The author well demonstrated that the PingChuan Formula (PCF) could ameliorate the asthma mice model as good as dexamethasone, via regulate the Th17/Treg imbalance which could decrease the IL-6, IL-17, IL-23, TGF-β and ROR-γt expression while increase Foxp3 expression. Furthermore, PCF showed less side effects, as like body weight loss, compared with dexamethasone.

There are a few minor essential revisions:

1. In method section, there should be more detailed prescription of PCF, how much grams of each components and how to prepare the drugs. (line 107-109)

Response: Thank you for the reviewer’s suggestion. We have added a detailed prescription of PCF in method section.

2. A few typing errors: line 80 progression#progression; line 109 regulat#regulate.

Response: Thank you for pointing these out we have edited the text to correct the typing errors.

3. Line 225, there was no figure 4 in the manuscript. Is it should be figure 3?

Response: Thank you for pointing out our mistake we have edited the text to cite the correct figure number.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:

I declare that I have no competing interests.

Response: Thank you for taking the time to review our manuscript. The document has now been edited by a native English speaker to correct the language before publication.

#2 Reviewer's report

Title: Pingchuan Formula Improves Asthma via Restoration of the Th17/Treg Balance in a Mouse Model

Version: 2 Date: 18 March 2015

Reviewer 2: Yomna Mahmoud

Reviewer's report:

This manuscript reports on the effects of a traditional Chinese recipe (Pingchuan Formula, PCF) on pathological changes in the lungs of asthmatic mice. Results presented here demonstrate that asthmatic mice showed bronchial deformation, narrow in diameter, wall structure damage and a large number of inflammatory exudates. BALF inspection showed high levels of IL-6, IL-17, IL-23 and TGF-beta, and eosinophils and neutrophils. The authors mentioned that treatment with PCF downregulates RORt, elevates Foxp3 expression, reduces IL-6, IL-17, IL-23 and TGF-beta in BALF, thus restoring Th17/Treg balance, improving airway inflammation and reducing asthma symptoms.

Overall, this manuscript is easy to read; however, the results and discussion are not well presented and some of the data are not convincing. The manuscript needs to define many questions, and needs major compulsory revisions.

Major comments are as below:

1. Introduction- It is well constructed; however, details about the tested formula should be mentioned. When was it introduced to market? What are the chemical constituents of this formula?

Response: The Pingchuan Formula (PCF), was first proposed by Professor Yu Jianer in 1999 and then was applied in treating pediatric asthma clinically. We have included the details of the composition and the calculated components of
each ingredient of PCF in the methods section of the manuscript.

2. Materials and methods- What are the animal housing conditions. This should be described.

**Response:** thank you for the reviewer’s suggestion. We have added this information in the method section as “The animals were housed in a temperature-controlled room at 21–23°C and maintained on a 12 h light : 12 h dark cycle.”

3. The authors claim that the animals were weighed daily! Why? Daily weights are not reported in the results. If examining the weight gain/loss, the initial and final weights or even weekly changes should be observed

**Response:** Since the amount estimation of drugs and intragastric administration were based on the weight, the mice were weighed everyday but the weights were only periodically recorded and those results are presented in the results section.

<table>
<thead>
<tr>
<th>Comparison of mouse weight changes between groups</th>
<th>( ( \bar{x} \pm s ) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>group</td>
<td>n.</td>
</tr>
<tr>
<td>Control Group</td>
<td>20</td>
</tr>
<tr>
<td>Model Group</td>
<td>20</td>
</tr>
<tr>
<td>Dexamethasone Group</td>
<td>20</td>
</tr>
<tr>
<td>Pingchuan Decoction Group</td>
<td>20</td>
</tr>
</tbody>
</table>

*compared with CON group, \( P<0.05 \); # compared with MDL group, \( P<0.05 \); \( \Delta \) compared with DEX group, \( P<0.05 \).

4. The authors should include a PCF control group in their study. What is the effect of this formula on normal lung?

**Response:** Thank you for this suggestion. We agree that this might have added important details on the action of PCF but unfortunately we did not include this group in our study. As PCF is a clinical prescription applied in treating patients with cough and asthma that has been used since 1999. The aim of this study was to explore the mechanisms of PCF in treating asthma model rats, therefore the PCF control group was omitted. We have now added this to the limitations of the study in the discussion and as a suggestion for future study.

5. Which lung was used for histology? How was it fixed?
Response: The upper lobe of right lung was put into the cryovial. 10% Formalin was added to the tube to fix the lung and prepare for biopsy section. We have now included these details in the methods section under the subheading: Hematoxylin and Eosin (H&E) staining of lung tissue.

6. Results- What are the clinical signs observed on asthmatic mice? At day 28 post OVA challenge, they should have breathing difficulties. This is not mentioned.

Response: As clinical symptoms were only one of the criteria when estimating whether the model was successful or not. The symptoms were not observed for a long time like day 28. 7 days after excitation, the symptoms of asthma were significant. The clinical signs of mice at day 7 after challenge included panic and easy irritation, cold aversion and tendency to clump together, abdominal muscle spasm, ruffled hair, oral and nasal cyanosis, and shortness of breath. We have now added this to the methods.

7. What does the data in Table 1 represent? The absolute or relative body weight loss? This needs clarification.

Response: The data in table 1 represents the absolute body weights of animals in the four groups. The name of the table was renamed to avoid confusion.

8. Line 202: What are "other symptoms"?
Response: we have changed the text to” The mice in MDL group showed different degrees of anxiety, muscle twitching, urinary and fecal incontinence, hair fluffing, activity decrease, appetite reduction, oronasal cyanosis and shortness of breath.”

9. Quantification of the symptoms in different groups is suggested.
Response: Thank you for this suggestion we have attempted to quantify the symptoms in the mice, which included panic and easy irritation, cold aversion and tendency to clump together, abdominal muscle spasm, ruffled hair, oral and nasal cyanosis, and shortness of breath. The mice were scored with 1 or 0 according to whether they had a certain symptom or not. This method has now been described in the methods section and the results are presented in the table below but are presented in the text in the manuscript.

### Symptom score of BALB/c mice of different groups

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Sym. Score</th>
<th>X̄</th>
<th>±</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>20</td>
<td>0.40</td>
<td>0.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDL</td>
<td>20</td>
<td>5.15</td>
<td>0.59*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DEX</td>
<td>20</td>
<td>4.40</td>
<td>0.82*#</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCF</td>
<td>20</td>
<td>4.05</td>
<td>0.76*#</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
*compared with CON group, P<0.05; # compared with MDL group, P<0.05; Δ compared with DEX group, P<0.05.

10. The authors did not mention the histological features of the specimens. Bronchial epithelium has very characteristic features that should be mentioned. In addition, the specific pathological changes associated with asthma are well known, and are obvious in the figures, but not presented by the authors.

**Response:** thank you for the reviewer’s suggestion and we have added description of histological features of the specimens in our manuscript.

“In the CON group, bronchial epithelial cells were neatly arranged. The thickness of the bronchus was normal. There was no inflammatory infiltration around the bronchus. In the MDL group, bronchial epithelial cells were disordered; goblet cell were hyperplastic and thickened; the bronchus cavity had a chrysanthemum-shaped change. There was infiltration of inflammatory cells around the bronchus, such as eosinophils / neutrophils. The overall condition in the DEX and PCF groups improved slightly compared with the MDL group.” We have integrated these descriptions into the results section.

11. Types of inflammatory cells at 7 and 28 d should be mentioned and linked to cell differentiated in BALF. What is the characteristic cell type seen at acute and chronic stages. This should be clearly referred to.

**Response:** Eosinophils were the major type of cell in BALF (bronchoalveolar lavage fluid) in acute and subacute stages. (day 7 and day 28). We have now added this information to the results section.

12. The abbreviations, number of specimens in each group and significance value should be provided at the footnotes of the tables.

**Response:** Thank you for the reviewer’s suggestion. We have edited the footnotes of the tables to improve the clarity of the tables.

13. Discussion- This is a general discussion with a few number of references. The results of the study were not compared to other work. It would have helped the authors if they reported the chemical constituents of the formula and discuss their effect on similar models.

**Response:** Thank you for the reviewer’s suggestion. We have edited the discussion according to your suggestion. Additional references:


14. Figures- Though the histological figures are very clear, they were not well described in the results nor discussed in the discussion.

Response: thank you for the reviewer’s suggestion and we have added description of histological features of the specimens in the results section of our manuscript. As described in the section above in response to point 10. These describe the typical features of The MDL group and how they were subtly improved by treatment we hope this is now clearer in the discussion.

15. Figure 3 Panel A- FOXp3 immunoreactivity for MDL group. The histology of this tissue appears normal!!! ..... Same figure Panel E- FOXp3 immunoreactivity for DEX and PCF groups. The histology of these tissues appears comparable to control asthmatic tissues (MDL group)!!! This is contradictory to what has been mentioned in the histology section.

Response: Thank you for pointing this out. We repeated the experiments and have provided updated images for these results. The images are attached (Immunohistochemistry assay of Foxp3 in lung tissues of CON/MDL/DEX/PCF groups after 7 d(top) and 28d of excitation and treatment (n=10 for each group))
16. There are other papers reporting the effect of the same formula in asthmatic mice. The authors did not mention many of them in the introduction or compare the data of those mentioned in the introduction with the current data in the discussion.

**Response:** Thank you for the reviewer’s suggestion. We have tried to reference the most relevant studies in our introduction. We have now added some additional discussion of these papers in the discussion section of the manuscript and have increased the number of studies we have cited.

Additional references:


Minor:

1. Abstract- Too many abbreviations were used in the abstract.

   **Response:** Thank you for the reviewer’s suggestion we have edited the abstract to reduce the number of abbreviations.

2. Many typos. See the attached file.

   **Response:** Thank you for taking the time to review our manuscript and for pointing these errors out we have edited the text to correct them.