Author's response to reviews

Title: Inhibitory effects of Stemona tuberosa on lung inflammation in a subacute cigarette smoke-induced mouse model

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Author's response to reviews: see over
Dear Editor,

We thank you for your valuable comments and providing us with the opportunity to revise our manuscript. We have corrected the manuscript according to editor’s comments as follows.

Reviewer #2
1. The part of the background need to be concentrated.

According to your advice, some part of the background was revised to be concentrated.

2. In the part of “2.2. Preparation of Stemona tuberosa and phytochemical analysis”, how get the result “final ratio of water extracts vs starch is 7:3”. In addition, please provide the reference.

“Final of water extracts vs starch is 7:3” was written in the product label to help researchers know what proportion of this product. We add production batch reference number in the manuscript as “Batch No: 110410”.

3. In the part of “2.3. A mouse model of subacute CS-induced lung inflammation and drug treatment”, the author need to explain how to confirm the mouse model was successful.

As shown in the Results sections and figure 3, 4, and 5, we found that CS caused significant increase of inflammatory cells (Fig. 3), cytokines and chemokine (Fig. 4), and neutrophil accumulation and airspace enlargement (Fig. 5) in BALF and lung tissues. For mentioning this procedure, we revised the sentence at the end of “2.3. A mouse model of subacute CS-induced lung inflammation and drug treatment” as “On day 13, the mice were sacrificed to obtain BALF and lung tissues for confirming this mouse model is successful and analyzing the results.”

4. In the sentence “Specifically, the levels of TNF-# and IL-6 in the ROFL group were significantly decreased compared with levels in the CS group, but the level of KC only
had the tendency to be decreased, as was observed in previous studies [29]”, what does “ROFL” refer to?

Thank you for your advice. We forgot to describe the meaning of “ROFL group”. With your kindness, we found that explaining the meaning of “CS group” was also missed. The explanation of the exact meaning of “ROFL group” and “CS group” as “the group of mice treated with roflumilast (ROFL group)” and “The group of mice exposed to CS (CS group)”.

5. In the sentence “PAS stain method has been used to distinguish the epithelial thickness of bronchiole in other studies [49-50]”, what does “PAS” refer to?

The full name of “PAS” is “periodic acid Schiff” as mentioned at the end of the paragraph “2.6. Histological examination of lung tissues”

6. In the part of discussion, the first paragraph need to be concentrated.

According to your advice, the first paragraph in the part of discussion was revised to be concentrated.

7. The part of discussion need to be modified. The part of discussion should has more discussion and promotion.

Thank you for your advice, On the purpose of more discussion and promotion, we added some sentences in the part of discussion as “We could find a few limitations in this mice model to evaluate the effect of ST. First, we had a difficulty in finding the increased number of goblet cells or fibrotic changes in the bronchial epithelium of CS group. To evaluate the effect of ST with these characteristics, researchers should perform this experimental study with other strains of mice, or guinea pig (Wright et al 2008, Bartalesi et al 2005) Secondly, this study was designed to examine the effects of CS exposure in regard to inflammation only. Recent studies, however, examine the effects of CS exposure in the aspect of DNA damage as well as inflammation (Itoh et al 2014) Furthermore, one study researched the effect of a therapeutic herb, Zataria multiflora, on systemic inflammation of experimental animal model of COPD. They used biochemical parameters such as measurement of malondialdehyde and differential cell count in the blood sample (Boskabady et al 2014). According to these recent studies, other biomarkers that can evaluate the effect of ST on targeting the treatment of COPD should be performed for the further studies.”

8. The part of conclusion need to be strengthened.

To strengthen the part of conclusion, some sentences are added and revised as “We found the significant changes of lung inflammation in a subacute cigarette smoke-induced mouse model. With this mouse model, we discovered that ST extract treatment decreased a variety
of inflammatory cells of the lung such as macrophages, neutrophils, and lymphocytes and reduced the level of cytokines (TNF-α, IL-6) and a chemokine (KC) induced by CS exposure.”

9. The manuscript appeared many languages errors, the author need to check carefully and modify them, for example:

We indentified some language errors and corrected it carefully.

“ST was observed to significantly decrease the numbers of total cells, macrophages, neutrophils, and lymphocytes in the BALF of mice that were exposed to CS.”This sentence needs to be rewritten.

We corrected this sentence as “ST significantly decreased the numbers of total cells, macrophages, neutrophils, and lymphocytes in the BALF of mice that were exposed to CS.”

“The effects of ST on total cell counts, differential cell counts, and ELISA were similar to those of roflumilast, a recently approved drug for the treatment of antiinflammatory lung diseases such as COPD.” This sentence needs to be rewritten.

We corrected this sentence as “The effect of ST was similar to that of roflumilast, a recently approved drug for the treatment of anti-inflammatory lung diseases such as COPD, in terms of total cell counts, differential cell counts, and cytokine productions.”

“On the other hand, mice treated with roflumilast (ROFL group) and ST (ST group) in all doses showed significant decreases in the number of total inflammatory cells in their BALF.”This sentence needs to be rewritten.

We corrected this sentence as “On the other hand, roflumilast and ST in all doses showed significant decrease in the number of total inflammatory cells in BALF”

10. The crude drug Stemona tuberosa was used for the insecticide. In animal experiments, the security needs further consideration.

Thank you for your kind consideration. Indeed we carefully handle the ST extracts during the experiments and stored it in sealed isolated container.

Reviewer #3

Minor Essential Revisions
1. Whole manuscript are not uniform for abbreviation. Authors should manuscript conforms to the journal style. For example, what is the abbreviation of ‘KC’,
We uniformed the use of abbreviation according to your advice and the instruction for authors in this Journal.

2. Keywords are not uniform for example, COPD, tumor necrosis factor-alpha, interleukin-6.

We uniformed the keywords according to your advice and the instruction for authors in this Journal as “CS, Stemona tuberosa, BALF, COPD, TNF-α, IL-6.”

3. Which phyto-constituents are responsible for anti-COPD effects?

Thank you for your advice. ST is composed of several constituents such as neotuberostemonine, tuberostemonine, croominine, and stemoninine. Chemical structures and effects of each constituent were well discovered in the previous studies (Xu et al 2006, Zhou et al 2006, Jiang et al 2006). Based on these researches, further studies should be performed to find which constituents have the inhibitory effects on lung inflammation. We added the explanation in the Discussion section as “Also, further researches should be performed to discover which phyto-constituents of ST have crucial inhibitory effect on lung inflammation.”

4. Why author use roflumilast for positive control? It is used in clinical therapy?

Roflumilast is the only oral phosphodiesterase 4 inhibitor indicated for clinical use in the treatment of COPD. Several clinical trials have been performed and proved its effectiveness on COPD. Due to these reasons, we used roflumilast for positive control in this study. We added the explanation in Background section as “Roflumilast is generally used for treatment of COPD patients with reliable evidence of clinical trials (Rabe et al 2011, Zheng et al 2014).”

5. Why author estimate TNF-alpha, IL-6, and chemokine?

TNF-α, IL-6, and KC (chemokine) have been estimated in cigarette smoke-induced mice model of COPD in the previous studies. We added the sentence to explain as “These cytokines and chemokine have been generally used in CS-induced mice model, especially for COPD in previous studies (Brandsma et al 2008, McGrath-Morrow et al 2011, Jung et al 2013).”

6. Why authors did not checked fibrosis factor? For example, TGF-beta, Smad, and Chemokine….

We checked the chemokine (KC) in this study. The level of KC was significantly increased
in the CS group. However, the level of KC was not significantly changed in any ST groups, as was observed in the previous study (Fortin 2009). This result was described in Results section as “Specifically, the levels of TNF-α and IL-6 in the group of mice treated with roflumilast (ROFL group) were significantly decreased compared with levels in the CS group, but the level of KC only had the tendency to be decreased, as was observed in previous studies (Fortin et al 2009)”. In addition, fibrotic changes was not shown in CS-induced mouse model which use C57Bl/6 mice in the previous study (Bartalesi et al 2005). Accordingly, we will perform this experiment with other strains of mice, or guinea pig later. We added the explanation in Discussion section as “First, we had a difficulty in finding the increased number of goblet cells or fibrotic changes in the bronchial epithelium of CS group. To evaluate the effect of ST with these characteristics, researchers should perform this experimental study with other strains of mice, or guinea pig (Wright et al 2008, Bartalesi et al 2005).”

7. Authors should checked for fibrosis in lung therefore added MT staining figures.

Thank you for your advice. In the previous study, fibrosis was not observed in lung tissues of C57Bl/6 mice which used in our experiment (Bartalesi et al 2005). We will check the fibrosis in lung tissue in the same experimental model we performed, using other strains of mice, or guinea pig, based on other researches (Wright et al 2008, Bartalesi et al 2005). We described this explanation in Discussion section as “First, we had a difficulty in finding the increased number of goblet cells or fibrotic changes in the bronchial epithelium of CS group. To evaluate the effect of ST with these characteristics, researchers should perform this experimental study with other strains of mice, or guinea pig (Wright et al 2008, Bartalesi et al 2005).”

Sincerely,

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Hyunsu Bae M.D., Ph.D.