Author's response to reviews

Title: Long-term effect of three Tiao-Bu Fei-Shen therapies on NF-kappaB/TGF-beta1/smads2 signaling in rats with chronic obstructive pulmonary disease

Authors:

Ya Li (yuanfengtc@126.com)
Jiansheng Li (li_is8@163.com)
Weiwei Li (13526736192@139.com)
Suyun Li (lisuyun2000@126.com)
Yange Tian (yange0910@126.com)
Xiaofan Lu (fannaleo.09010322@163.com)
Suli Jiang (jiangsuli1007@163.com)
Ying Wang (yinghuaifei0001@126.com)

Version: 6 Date: 27 November 2013

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

Please give our heartfelt appreciation to the reviewers. It is obvious that they read critically but sympathetically and with a genuine empathy for the MS. I agree with your criticisms in almost all cases. I’m certainly ready to expend the effort and time needed to get the MS to publishable standard.

Reviewer's report

Title: Long-term effect of three Tiao-Bu Fei-Shen therapies on NF-kappaB/TGF-beta1/smad2 signaling in rats with chronic obstructive pulmonary disease

Version: 4 Date: 6 October 2013

Reviewer: Azadeh Manayi

Reviewer's report:

The manuscript entitled “Long-term effect of three Tiao-Bu Fei-Shen therapies on NF-κB/TGF-β1/smad2 signaling in rats with chronic obstructive pulmonary disease” tested the effects of some Chinese traditional medicines against COPD and calculated some biological factors and lung morphology in rats. In the review of the manuscript some points need to attend for improvement of the manuscript which are mentioned as following.

Major Compulsory Revisions

1- Dose of plants and aminophylline administered to animals did not describe how obtained? Why lower or higher dose did not administered?

RE: Thank you for your comments. It is a good question. The doses of herbs and aminophylline have been described in the Administration paragraph in the manuscript as follows:

The prescriptions the granules is as follows: Bufei Jianpi granules (For lung-spleen qi deficiency syndrome): Huang Qi (Astragalus propinquus) 15 grams (g), Dang Shen (Codonopsis pilosula) 15 g, Bai Zhu (Atractylodes macrocephala) 12 g, Fu Ling (Poria Cocos) 12 g. Bufei Yishen granules (For lung-kidney qi deficiency syndrome): Ren Shen (Radix Ginseng) 9 g, Huang Qi (Astragalus propinquus) 15 g. Shan Zhu Yu (Cornus Officinalis) 12 g, and Yin Yang Huo (Herba Epimedii) 9 g. Yiqi Zishen granule (For lung-kidney qi and yin deficiency syndrome): Ren Shen (Radix Ginseng) 9 g, Huang Jing (Polygonatum Kingianum) 15 g, Shu Di Huang (Radix Rehmanniae Praeparata) 15 g. Mai Dong (Ophiopogon japonicus) 15 g and Wu wei zi (Schisandra Chinensis) 9 g. The granules were prepared into fluidextractum according to the standard operating procedure established by Department of Pharmaceutics in the Hospital. The dosages of the three TCM prescriptions and aminophylline were...
calculated by: 

\[ D_{\text{rat}} = D_{\text{human}} \times \left( \frac{\text{HI}_{\text{rat}}}{\text{HI}_{\text{human}}} \times \frac{\text{BW}_{\text{human}}}{\text{BW}_{\text{rat}}} \right)^{2/3}. \]

D: dose; HI: habius index; BW: body weight.

Because the equivalent doses were calculated from their clinical usage, which has been proved having marked clinical efficacy, so we designed the study only with the medium doses.

2- It is not clearly described in the manuscript that all animals received bacterial solution in addition to tobacco smoke. Please clarify this subject in the methods part.

RE: All the cigarette smoke-exposed rats had received bacterial solution in this study. This has been clarified in the methods part as follows:

Rats were randomized into control, model, Bufei Jianpi, Bufei Yishen, Yiqi Zishen and aminophylline groups (10 male and 10 female rats in each group). COPD Rats were induced by tobacco smoke (Hongqi Canal® Filter tip cigarette, tobacco type, tar: 10 mg, nicotine content: 1.0 mg, carbon monoxide: 12 mg, Henan Tobacco Industry, Henan) of 8 cigarettes per treatment twice a day during the first two weeks, and then fifteen cigarettes per treatment, three times a day, from week 3 through 12. The rats were placed in a sealed box connected to smoke source, so as to receive two or three 30-minute-exposure per day with a three-hour interval between them. 0.1mL of Klebsiella pneumonia suspension (6 × 10^8 CFU/mL) was slowly dropped into nasal cavities alternately in all cigarette smoke-exposed rats, every 5 days, from week 1 through 8^{[11-15]}. Thank you.

Minor Essential Revisions

1- Abstract, line 3 of method part “was” have to replace with “were”.

RE: Thank you for your kindly detailed revisions and comments. The entire manuscript has been revised carefully.

2- Abstract, line 5 of methods please check that “8” stands for what? Better to replace it with “IL-8”.

RE: IL-8 has been deleted in this manuscript, because it has been reported in another article. We are sorry, but unfortunately, we must have made a mistake, rat IL-8 gene or protein cannot be found in the database on PUBMED website.

3- Abstract, line 4 of result part replace “significant” with “significantly”.

RE: It has been corrected in the manuscript, and also in the entire article.

4- The words “qi” and “yin” in the manuscript stand for what words? It is
better to explain them.

**RE:** The words “qi” and “yin” in the manuscript are special terms in traditional Chinese medicine. Qi is the vital life force or energy in the body, and it is also invisible fundamental element to form substances in traditional Chinese philosophy and medicine theory. In Chinese philosophy, yin stands for the feminine, latent, corporeality and passive principle (characterized by dark, cold, wetness, passivity, disintegration, etc.). It’s the material base of qi, such as blood, flesh, bones, ect. They have been explained in the manuscript as follows:

- qi (vital energy in life body)
- qi-yin (vital energy in life body and its material base, such as blood, flesh, bones, ect.)

5- in the first paragraph of introduction the sentence “inflammatory injury and repair and airway remodeling in COPD by activating Smad2” better to replace with “inflammatory injury and repair as well as airway remodeling in COPD by activating Smad2”.

**RE:** Thank you very much for your kindly detailed revisions and comments.

The word “and” has been replaced.

5- The name of the bacteria should be in italic form: Klebsiella pneumonia.

**RE:** Thank you very much for your kindly detailed revisions and comments. It has been changed into italics.

7- In the methods part, preparation of COPD: “All rats arrived at the animal facility of the laboratory seven days before the experiment, were housed in the individually...” replace with “All rats arrived at the animal facility of the laboratory seven days before the experiment, they were housed in the individually...”.

**RE:** Thank you very much. It has been changed and decrypted in animal part as follows:

All rats arrived at the animal facility of the laboratory seven days before the experiment, they were housed in the individually ventilated cages (CA25, Fengshi, Suzhou, China), and given sterile food and water ad libitum.

8- The country of the tobacco smoke have to mention in the manuscript.

**RE:** Thank you very much. Henan province has been replaced with Zhengzhou city, China.

9- Last sentence of the preparation of COPD in the methods part, sentence started with the number which have to be for example with “one hundred µL”
or rewrite in a different way.

RE: Thank you very much. It has been replaced with one hundred µL.

10- In the methods, administration part: concentration of the drugs have to correct for example “Bufei Jianpi granule (4.84 g•kg-1•d-1)” to “Bufei Jianpi granule (4.84 g/kg-1/d-1)”.

RE: Thank you very much. The units g•kg-1•d-1 and mg•kg-1•d-1 have been corrected into g/kg/d and mg/kg/d. But I think “-1” shouldn’t be there.

11- All the plant scientific names should be in italic form in the manuscript, for example: “Astragalus propinquus”.

RE: Thank you very much. This part has been rewrote as follows:

The prescriptions the granules is as follows: Bufei Jianpi granules (For lung-spleen qi deficiency syndrome): Huang Qi (Astragalus propinquus) 15 g, Dang Shen (Codonopsis pilosula) 15 g, Bai Zhu (Atractylodes macrocephala) 12 g, Fu Ling (Poria Cocos) 12 g. Bufei Yishen granules (For lung-kidney qi deficiency syndrome): Ren Shen (Radix Ginseng) 9 g, Huang Qi (Astragalus propinquus) 15 g, Shan Zhu Yu (Cornus Officinalis) 12 g, and Yin Yang Huo (Herba Epimedii) 9 g. Yiqi Zishen granule (For lung-kidney qi and yin deficiency syndrome): Ren Shen (Radix Ginseng) 9 g, Huang Jing (Polygonatum Kingianum) 15 g, Shu Di Huang (Radix Rehmanniae Praeparata) 15 g, Mai Dong (Ophiopogon japonicus) 15 g and Wu wei zi (Schisandra Chinensis) 9 g.

12- The city of the apparatus which used in the study have to mention in the manuscript as well as their company and country.

RE: Thank you very much. All the companies, cities and countries have been described in the manuscript.

13- Last sentence of morphology part in methods started with number which have to replace with this one “Four bronchial wall thickness were measured in each slice and averaged”.

RE: Thank you very much. It’s described as follows:

Four bronchial wall thickness were measured in each slice and averaged.

14- Please explain “PBS” and “GAPDH” abbreviations in the text when they appear for the first time.

RE: Thank you very much. It’s described as follows:

phosphate buffer solution (PBS) and
glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

15- In the result part, cytokines, second paragraph after “At week 32” following word “The” is in capital that should be corrected. In this sentence
two p value reported which are not clear related to what parameters.

RE: Thank you very much. It has been corrected and described as follows:

As shown in Figure 3, at both week 20 and 32, the levels of TNF-α and IL-1β in the model group were significantly higher than those in control group \((P<0.01)\), at the meanwhile, they were much lower in the three TCM- and aminophylline-treated group groups \((all \ P<0.01)\). The three TCM-treatments showed much more benefit than aminophylline \((all \ P<0.01)\), especially Bufei Jianpi and Bufei Yishen granules.

At week 32, the expressions of TNF-α and IL-1β were higher in model group than that at week 20 \((P<0.01)\), while were lower in the three TCM-treated groups \((IL-1β, \ P<0.05; \ TNF-α, \ P<0.01)\).

16- Same as above in the results, Phosphorylation of Nuclear Factor κB and Inhibitor of κBα part, last sentence “The” is in capital and the p values are not clearly related to the parameters which better use “respectively” at the end of the sentence to clear that they are in the order. In the Expressions of TGF-β1 mRNA and Smad2 mRNA part also this happened which have to be corrected.

RE: Thank you very much. It has been corrected and described as follows:

At week 32, the expressions of p-NF-κB and p-IκBα were higher in model group than those at week 20 \((P<0.01 \ and \ P<0.05 \ respectively)\), while in three TCM-treated groups were lower \((P<0.05 \ or \ P<0.01)\).
Reviewer's report

Title: Long-term effect of three Tiao-Bu Fei-Shen therapies on NF-kappaB/TGF-beta1/smad2 signaling in rats with chronic obstructive pulmonary disease

Version: 4 Date: 11 October 2013
Reviewer: Mahdi Vazirian

Reviewer's report:

Review of the manuscript entitled "Long-term effect of three Tiao-Bu Fei-Shen therapies on NF-κB/TGF-β1/smad2 signaling in rats with chronic obstructive pulmonary disease" by Ya Li, et al.

The manuscript includes interesting experimental results which may be beneficial for patients with COPD disease in the future. However, before final consideration, answering some questions about the experiment and edition of presented text should be considered. The minor revisions and a suggested change in the manuscript are listed below:

Minor Essential Revisions

1. It seems that the manuscript includes some grammatical errors which should be checked and corrected before final consideration. There are also some spellings in the text (e.g. aminophylline instead of aminophyline and NaCL which must be NaCl).

RE: Thank you very much. We have revised the entire manuscript carefully with Dr. Wang's help, he has experiences to publish more than 30 articles in Circulation, Hypertension, Am J Physiol Regul Integr Comp Physiol and Mol Med journals.

2. Please explain how the dosages are chosen for the experiment (For three herbal formulations and aminophylline).

RE: Thank you for your comments. It is a good question. The dosages used in this study is equivalent doses, which were calculated from their clinical usage, they have been proved having marked clinical efficacy. So we designed the study only with the equivalent doses.

The doses of herbs and aminophylline have been described in the administration paragraph in the manuscript as follows:

The prescriptions the granules is as follows: Bufei Jianpi granules (For lung-spleen qi deficiency syndrome): Huang Qi (Astragalus propinquus) 15 grams (g), Dang Shen (Codonopsis pilosula) 15 g, Bai Zhu (Atractylodes macrocephala) 12 g, Fu Ling (Poria Cocos) 12 g. Bufei Yishen granules (For lung-kidney qi deficiency syndrome): Ren Shen (Radix Ginseng) 9 g, Huang Qi (Astragalus...
propinquus) 15 g, Shan Zhu Yu (Cornus Officinalis) 12 g, and Yin Yang Huo (Herba Epimedii) 9 g.
Yiqi Zishen granule (For lung-kidney qi and yin deficiency syndrome): Ren Shen (Radix Ginseng) 9 g,
Huang Jing (Polygonatum Kingianum) 15 g, Shu Di Huang (Radix Rehmanniae Praeparata) 15 g, Mai
Dong (Ophiopogon japonicus) 15 g and Wu wei zi (Schisandra Chinensis) 9 g. The granules were
prepared into fluidextractum according to the standard operating procedure established by Department
of Pharmaceutics in the Hospital. The dosages of the three TCM prescriptions and aminophylline were
calculated by: \( D_{rat} = D_{human} \times \left( \frac{HI_{rat}}{HI_{human}} \times \frac{BW_{human}}{BW_{rat}} \right)^{2/3} \). D: dose; HI: habius index; BW: body

3. Is there any reasonable clue for following the experiment for 32 weeks as the long term follow up?
RE: In the previous two clinical trials, the enrolled patients received a 6-month treatment and a further 12-month follow-up, and got marked clinical efficacy. But it is difficult to determine the treatment and follow-up periods in rats. In this study, the COPD rats received a 12-week treatment and a 12-week follow-up periods. However, a three-month period is as long as a 10-12 years period in human. It is a really long treatment.

4. What means "phanic" in the text?
RE: It has been replace with “marked”.

5. According to figure captions we have no description for a, b, c, etc. in figure
RE: Thank you very much. The figure captions have been described as follows:

**Figure legends**

**Figure 1**
Pathological changes in the lungs and bronchiole in rats treated with Bufei Jianpi, Bufei Yishen, Yiqi Zishen granules or aminophylline at week 20 and 32 (H&E stained). a = control, b = model; c = Bufei Jianpi; d= Bufei Yishen; e = Yiqi Zishen; f = aminophylline. 1 = week 20; 2 = week 32. Amplification \( \times 200 \).

**Figure 2**
Bronchiole wall thickness in rats treated with Bufei Jianpi, Bufei Yishen, Yiqi Zishen granules or
aminophylline at week 20 and 32. a = control, b = model; c = Bufei Jianpi; d= Bufei Yishen; e = Yiqi
Zishen; f = aminophylline. \(^*P<0.05\), \(^{*}P<0.01\), vs model group; \(^*P<0.05\), **P<0.01, vs Bufei Jianpi
group; △P<0.05, △△P<0.01, vs Bufei Yishen group; ▲P<0.05, ▲▲P<0.01, vs Yiqi Zishen group; ◦P<0.05, ◦◦P<0.01, vs Week 20.

Figure 3

Levels of TNF-α (A) and IL-1β (B) in the lungs in rats treated with Bufei Jianpi, Bufei Yishen, Yiqi Zishen granules or aminophylline at week 20 and 32. a = control, b = model; c = Bufei Jianpi; d = Bufei Yishen; e = Yiqi Zishen; f = aminophylline. *P<0.05, **P<0.01, vs model group; †P<0.05, ††P<0.01, vs Bufei Jianpi group; ‡P<0.05, ‡‡P<0.01, vs Bufei Yishen group; §P<0.05, §§P<0.01, vs Yiqi Zishen group; ¶P<0.05, ¶¶P<0.01, vs Week 20.

Figure 4

Phosphorylation of NF-κB and IκBα of lung in rats treated with Bufei Jianpi, Bufei Yishen, Yiqi Zishen granules or aminophylline at week 20 and 32. a = control, b = model; c = Bufei Jianpi; d = Bufei Yishen; e = Yiqi Zishen; f = aminophylline. *P<0.05, **P<0.01, vs model group; †P<0.05, ††P<0.01, vs Bufei Jianpi group; ‡P<0.05, ‡‡P<0.01, vs Bufei Yishen group; §P<0.05, §§P<0.01, vs Yiqi Zishen group; ¶P<0.05, ¶¶P<0.01, vs Week 20.

Figure 5

Expressions of TGF-β1 and Smad2 mRNA in rats treated with Bufei Jianpi, Bufei Yishen, Yiqi Zishen granules or aminophylline at week 20 and 32. a = control, b = model; c = Bufei Jianpi; d = Bufei Yishen; e = Yiqi Zishen; f = aminophylline. *P<0.05, **P<0.01, vs model group; †P<0.05, ††P<0.01, vs Bufei Jianpi group; ‡P<0.05, ‡‡P<0.01, vs Bufei Yishen group; §P<0.05, §§P<0.01, vs Yiqi Zishen group; ¶P<0.05, ¶¶P<0.01, vs Week 20.

6. I didn't notice any difference between your control and model groups (Although we know that control group are not exposed to fungi and the smoke but it is better to explain that in the text).

RE: Thank you very much. It has been revised in the Preparation of COPD model in the method part as follows:

Rats were randomized into control, model, Bufei Jianpi, Bufei Yishen, Yiqi Zishen and aminophylline groups (10 male and 10 female rats in each group). COPD Rats (except control ones) were induced by tobacco smoke (Hongqi Canal® Filter tip cigarette, tobacco type, tar: 10 mg, nicotine
content: 1.0 mg, carbon monoxide: 12 mg, Henan Tobacco Industry, Zhengzhou, China) of 8 cigarettes per treatment twice a day during the first two weeks, and then fifteen cigarettes per treatment, three times a day, from week 3 through 12. The rats were placed in a sealed box connected to smoke source, so as to receive two or three 30-minute-exposure per day with a three-hour interval between them. One hundred µL of Klebsiella pneumonia suspension (6 × 10^8 CFU/mL) was slowly dropped into nasal cavities alternately in all cigarette smoke-exposed rats, every 5 days, from week 1 through 8\[11-15\].

Discretionary Revisions
1. You are recommended to move some parts in discussion section to introduction (e.g. The effects of NF-κB/TGF-β/Smads pathways are as follows:)
RE: Thank you very much for all your comments. We have move this section to introduction.