

Response Letter

September 9, 2017

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 35425-manuscript).

Title: Dachaihu decoction ameliorates pancreatic fibrosis by inhibiting macrophage infiltration in chronic pancreatitis

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Thanks for your response and reviewers comments on our manuscript. We have modified the paper according to the comments of reviewer and editor. Our detailed responses are to be found below. Our reply to the reviewers is in italic and bold.

I. Review's comments

[Reviewer #1]

Reviewer #1: This is an interesting paper regarding the role of DCHD and macrophages in a model of fibrosis and chronic pancreatitis in mice. Could the authors please respond to the following questions/comments:

1) How was the arginine dose and frequency determined?

Answers: Our research group has long been engaged in the animal experimental research of acute and chronic pancreatitis. In the previous experiments, we induced acute pancreatitis mice model with 20% L-arginine in a dosage of 2g/kg, 3g/kg, 4g/kg/time, twice in 1h interval. 72h after modeling, the different degree of pancreatic injury was observed among the above three groups. We found that the mice in the group of 4g/kg L-arginine had a mortality rate of 40%, but

the mouse pancreas injury was too mild to find the significant pathologic morphological change in the dose of 2g/kg group. While the pancreas injury in 3g/kg group could be found with edema, necrosis and a lot of inflammatory cells infiltration. Therefore, we finally select the L-arginine dose of 3g/kg for the most stable model of acute pancreatitis. (see our paper “Effect of Qingyi decoction on p-STAT3 expression in pancreas of mice with severe acute pancreatitis induced by L-arginine, Chinese Journal of Pathophysiology 2011,27(11): 2175-2179”)

As we known, except for the pancreatic fibrosis, the necrosis and inflammation which is consistent with the pathology of acute pancreatitis, can be seen in the progression of chronic pancreatitis. So we injected the 20% L-arginine in a dose of 3g/kg/time, twice in 1h interval each week to induce chronic pancreatitis. 2 weeks after modeling, morphological signs of CP were found, including inflammatory cells infiltration, acinar cell necrosis, and a small amount of connective tissue deposition. At 4 and 6 weeks, loss of acinar cells, collagen deposition, and aggravated fibrosis in the pancreas were observed.

Why was DCHD administered intragastrically? Have other modes of administration been studied?

Answers: For most of the traditional Chinese medicine formula, the basic use of medication is oral administration for patients. Furthermore, oral administration has been confirmed to be the safest mode of administration until now. As a traditional Chinese medicine formula, DaChaihu decoction (DCHD, major radix bupleuri decotion) is available to administer intragastrically in mice. So we use the method of intragastric administration to the mouse in this experiment.

At present, because of the complex composition, a few of traditional Chinese medicine formula has been developed to

intravenous forms, such as Qingkailing injection. Due to the technical limitation, DCHD has not been developed to intravenous forms.

Arginine is involved in the NO pathway? Have the authors studied aspects and molecules involved in this pathway with their model?

Answers: L-arginine as a precursor of NO, high-dose administration can cause pancreatic hypoperfusion, leading to blood stasis and local inflammation spread, further induced pancreatic inflammatory response, it was originally used to replicate acute pancreatitis model.

We also detected some of the indicators related to oxidation, such as malondialdehyde (MDA), superoxide dismutase (SOD), etc. and found some changes in these two indicators. This experiment aims to discuss the role of macrophages and inflammatory factors in CP, there is no exhibition of indicators related to oxidation. The next step, we will explore the mechanism of CP involved by L-arginine to clarify the role of NO pathway in CP progression.

Did the histological improvement that the authors observed correlate with clinical improvement, ie laboratory wise?

Answers: In this study, we also observed that with the reduction of histological damage, the general status of the experimental animals in the treatment group was improved, such as increased diets, increased body weight, more active, etc. In clinic, the following signs including a reduction of abdominal pain as well as the recovery of digestive function have been adopted as the criteria for assessing the efficacy of treatment for CP. Thus, histological improvements in animal experiments are consistent with clinical CP improvements. Our research group used DCHD to treat patients with CP in department of Gastroenterology of our affiliated hospital, and we found the clinical symptoms, such as abdominal pain, abdominal distension and inappetence were all significantly improved.

The authors correctly discuss TGF- β as a marker of fibrosis. Have they looked

at that in their model?

Answers: It has been recognized that TGF- β is an important indicator of pancreatic fibrosis, which has also been further confirmed in our previous related experiments. We also detected the expression changes of TGF- β and its receptors in this study, but in this paper, we didn't show the results about TGF- β , we used MASSON staining, combined with FN and α -SMA which are also sensitive index of pancreatic fibrosis, so these parameters are sufficient to reflect the degree of pancreatic fibrosis.

The authors should include in the discussion a paragraph about potential limitations of their study.

Answers: We have already added some of the potential limitations of our study in the discussion.

[Reviewer #2]

1. The component of DCHD most strongly associated with the inhibition of macrophage infiltration should be investigated. Since the Chinese herbal medicine is not used nor available in other countries, readers may not be interested in the use of DCHD itself.

Answers: Chinese medicine has made great contributions for Chinese health in the past 5,000 years, and its efficacy is obvious to all. In recent centuries, Chinese medicine has been spread to other Asian countries around China, for example, Japan, South Korea, and so on. In the future, we should strive to study the mechanism of Chinese medicine, so that more and more people around the world may understand and accept Chinese medicine.

2. English is poor. The manuscript needs English proof-reading by the natives.

Answers: We have invited experts of natives to modify the language, and provided the English proof-reading.

[Reviewer #3]

The authors have carried out an interesting study using a Chinese 'herbal'

remedy in a mouse model of chronic pancreatitis. The results suggest that in this mouse model the remedy (DCHD), which is made up of several potentially active compounds, reduces the role of macrophages in the development of chronic fibrosis.

1. The study is interesting and might be valuable in the further study of chronic inflammation in chronic pancreatitis but this is a long way from being useful in human disease.

Answers: DCHD is a common clinical recipe and has been widely used in the treatment of acute pancreatitis. In recent years, we have begun use DCHD to treat chronic pancreatitis patients in our affiliated hospital, and have found its preliminary effects. After treatment with DCHD, for most patients, the clinical symptoms including abdominal pain, abdominal distension and inappetence were all significantly improved. At the same time, our study also provides experimental basis for further promotion of DCHD in clinical practice

2. There are a large number of typographical errors scattered throughout the manuscript. there are also some grammatical errors that need correcting.

Answers: We checked the program and spelling in English, and corrected some mistakes.

3. The final 2 sentences of the discussion need changing & the last sentence removing. The manuscript is not set out in the manner that one would usually use for submission.

Answers: The final 2 sentences of the discussion have been modified and the last sentence has been removed. According to the reviewer's suggestions and submission rules, we have checked and modified manuscript to satisfy the submission manner.

II. Edit manuscript

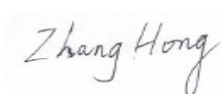
Answers:

1) We have revised our manuscript according to each of the reviewers' comments.

- 2) We have updated the manuscript according to the Guidelines and Requirements for Manuscript Revision-Basic Study.**
- 3) We have provided the Scientific Research Process write-up.**
- 4) We have provided an Audio Core Tip.**
- 5) We have subjected the final title of your manuscript to a Google Scholar search, and store screenshot images of the results.**
- 6) We have provided all files related to the Academic Rules and Norms, including Institutional Review Board statement, Institutional Animal Care and Use Committee statement, Animal Care and Use statement, Biostatistics statement, Conflict-of-Interest statement, and Data Sharing statement.**
- 7) We have provided the approved grant application forms and funding agency copy of any approval documents**
- 8) We have revised the language of our manuscript, and provided a language editing certificate which verified that the language of the manuscript has reached Grade A**
- 9) We have signed the Copyright Assignment form.**
- 10) We have submitted the revised manuscript and all related documents.**

Thank you again for publishing our manuscript in the World Journal of Gastroenterology.

Sincerely yours,



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