Author’s response to reviews

Title: The modified Si-Jun-Zi decoction attenuates colon cancer liver metastasis by increasing macrophage cells

Authors:

Jin-Yong Zhou (jinyongzhou@126.com)
Min Chen (cm1211@163.com)
Cun-En Wu (714494480@qq.com)
Yu-Wen Zhuang (zhuangyuwen21@126.com)
Yu-Gen Chen (chenyg666@126.com)
Shen-Lin Liu (jsszyylsl@163.com)

Version: 2 Date: 19 Feb 2019

Author’s response to reviews:
February 19, 2019

Deepa Nath, PhD
BMC Complementary and Alternative Medicine

Re: Manuscript BCAM-D-17-01248R1

Dear Editor,

Thank you for forwarding the reviewer’ comments on our manuscript. According to their comments, we have added some references and changed the order of the references. We have also prepared a point-by-point response enclosed in this revised submission. In addition, we have included the information on the more funding support.

We would like to thank the reviewers again for their constructive comments. These comments have allowed us to include additional data and clarification that have significantly strengthened this study. We hope the revised manuscript is now suitable for publication.

Thank you.

Your sincerely,

Jin-Yong Zhou, MD, PhD
Gökhan Sadi (Reviewer 1):

"Dear Editor,

In their submitted study, the authors aim to investigate the effect of modified Si-Jun-Zi (SJZ) decoction which as an herb used in Chinese traditional medicine on metastasis of colorectal cancer cell metastasis to the liver tissues. They used a nude mice model of spleen-transplanted colon cancer cells to observe its liver metastasis. This model could partly mimic the clinic process of colon cancer, including cancer cells detaching from colon cancer tissue and entering the liver by portal venous system. In that sound, the subject matter of the manuscript is in the scope of the Journal. Experiments, statistics, and other analyses are performed to a high technical standard and are described in sufficient detail. The article is presented in an intelligible fashion and is written in good English. Following minor comments should be addressed prior to publication.

A: We thank the reviewer for the appreciation of the quality and clarity of our study.

Minor comments,

Q 1: In the methods section, the protocols for the time and dose studies should be given in "animal groups and treatment" section instead of flow cytometric analysis.
A: We have moved the protocols for the time and dose studies to the "animal groups and treatment" section (P 6, Line 20-22 and P 7, Line 19-22).

"Q 2: The results of time and dose response studies should be discussed more in discussion."
A: We have added more discussion on the time and dose response studies in P 10, Line 14-19.

"Q 3: In the first paragraph of discussion, open name of TCM should be given for the first time."
A: The full name of TCM has been spelled out at the first appearance in the manuscript (P 3, Line 13-14).

Kottayath Govindan Nevin (Reviewer 2):

"The paper entitled, The modified Si-Jun-Zi decoction attenuates colon cancer liver metastasis by increasing macrophage cells by Zhou et al, studied the mechanism of anticancer activity of the Chinese decoction, though the work is interesting, there are few minor concerns. We thank the reviewer for the interest and enthusiasm in our study.

Q 1: In the introduction some more information regarding the Si-Jun-Zi decoction should be included"
A: We have added more information and references on the Si-Jun-Zi decoction in the second paragraph of the introduction (P 3, Line 15-18).

"Q 2: In the animal studies how the HCT cells were intrasplenically transplanted? Procedure should be detailed or give the reference?"
A: We have included a reference for the procedure in the revised manuscript (P 6, Line 7-8): Giavazzi R, Garofalo A, Damia G, et al. Response to flavone acetic acid (NSC 347512) of primary and metastatic human colorectal carcinoma xenografts [J]. British journal of
"Q 3: Why MSJZ treatment was restricted to one group with 45mg/kg. What is the reason for selecting the dose? Do the authors conduct any preliminary experiments to determine the effective concentration?"

A: Firstly, we want to explain the concentration of herbs in our manuscript. Routinely, we extracted the working materials by boiling the herbs in water at a w/w ratio of 1:10. Following concentration, the extract was given to the mice. We used the weight of the raw herbs vs. animal body weight to indicate the dose. Therefore, 45 g/kg means the extract from 45 g raw herbs was given to mice per kilogram body weight. In our experiments, the herbal extract was concentrated to 3 g/ml and every mouse was given 0.15 ml twice a day.

(1) We calculated the dose for mice according to the clinical dose for patients according to the FDA guideline: https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM078932.pdf
The dose of this prescription for human is 165 g/kg daily. When it comes to mice, it will be (165 g / 60 kg)*12.3 (conversion coefficient)=33.825 g/kg. We chose 1.33 folds as the high dose (33.825*1.33=44.987 g/kg).

(2) Before the experiments, we searched the literature for the maximum safe dose of SJZ for mice. One paper in Chinese Journal of Veterinary Medicine (2009) showed that 48000 mg/kg was applied for the mice to test the acute toxicity, which gave no visceral abnormality or overall disorder. According to the criterion of acute toxicity classification, this is a non-toxic dose (Attachment: SJZ acute toxicity.pdf). This paper is in Chinese, but we can provide the English translation if needed.

Based on well-accepted Chinese Medicine theory, the SJZ decoction falls in the class of tonic prescription and is safe for human when used in a high dose. We chose the high dose at first, and the subsequent results of nude mice without tumor showed consistency.

"Q 4: The grouping and treatment of animals should be clearly written viz Gp1...Gr...2...Gp3...Gp4 etc"
A: We have changed the names of the groups according to the reviewer’s suggestion.

For intrasplenically transplanted cancer cells in nude mice:
G1: Control, G2: 5-Fu, G3: Modified SJZ, G4: Combined

The corresponding part in figures and legends has also been revised (P6, Line 13-14 and P31, Line 2-8, 15-18).

"Q 5: What the authors mean by 3-week half-dose treatment group?"
A: We wanted to provide readers with more information about the time- and dose- response of this decoction. A half-dose treatment indicated the dose-effect.

Chakrabhavi Dhananjaya Mohan (Reviewer 3):

"The manuscript entitled "The modified Si-Jun-Zi decoction attenuates colon cancer liver metastasis by increasing macrophage cells" describes the effect of modified SJZ on CRC liver
metastasis in preclinical models. I have some concerns about the manuscript.

Q 1. Though the results are interesting, the basis of this work is the usage of SJZ in traditional Chinese medicine and there are no concrete scientific evidences to prove, which are bioactive principles are contributing for its antimetastatic activity. Since SJZ is a decoction, it is difficult to understand which is the bioactive component?

A: We want to thank the reviewer for the interest in our study. We performed this experiment to demonstrate the effect of this decoction, and focused on the change of the innate immune system. We tried to clarify some possible mechanisms in this manuscript, and feel that, in order to keep the study focused, the investigation of the bioactive components would fall beyond the current manuscript and instead be the focus of a future manuscript.

"Q 2. Moreover, why did authors hypothesize that it may possess antimetastatic activity and why in vitro studies were not carried out in the relevant models to evaluate its anticancer and antimetastatic activity?"

A: Empirical Chinese medicine knowledge has indicated that certain herbs in this decoction have anti-metastasis function. Contemporary literature also indicates that the components in our decoction have anti-metastasis ability. For example, Lu et al. reviewed the anti-metastasis function of Curcumae [Journal of ethnopharmacology, 2012,143(2):406-411]; Xia et al. also found that patrinia has anti-metastasis ability against colorectal cancer [Biochemical and biophysical research communications, 2018,503(3):2152-2159]. To our knowledge, the process of metastasis is complex and includes many steps. More importantly, it is related to the body’s defense ability. Therefore, when the drugs need to work through activating other cells (i.e. the immune system) to exterminate cancer cells, it is difficult to find a simple cell model to mimic the actual process in the whole body. So, in this case, we did not do experiments in vitro.

"Q 3. I feel, the amount of SJZ used in the study is voluminous (45g/kg body weight) and clinically not relevant. How was the dose decided? What is the maximum tolerable dose?"

A: In fact, the amount of modified SJZ used in the study was chosen according to the guidance of FDA, which was relevant to clinical applications. This dose was decided for the following reasons:

(1) We want to explain the concentration of herbs in our manuscript. Routinely, we extracted the working materials by boiling the herbs in water at a w/w ratio of 1:10. Following concentration, the extract was given to the mice. We used the weight of the raw herbs vs. animal body weight to indicate the dose. Therefore, 45 g/kg means the extract from 45 g raw herbs was given to mice per kilogram body weight. In our experiments, the herbal extract was concentrated to 3 g/ml and every mouse was given 0.15 ml twice a day.

(2) We calculated the dose for mice according to the clinical dose for patients according to the FDA guideline: https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM078932.pdf

The dose of this prescription for human is 165 g/kg daily. When it comes to mice, it will be (165 g / 60 kg)*12.3 (conversion coefficient)=33.825 g/kg. We chose 1.33 folds as the high dose (33.825*1.33=44.987 g/kg).

(3) Before the experiments, we searched the literature for the maximum safe dose of SJZ for mice. One paper in Chinese Journal of Veterinary Medicine (2009) showed that 48000 mg/kg
was applied for the mice to test the acute toxicity, which gave no visceral abnormality or overall disorder. According to the criterion of acute toxicity classification, this is a non-toxic dose (Attachment: SJZ acute toxicity.pdf). This paper is in Chinese, but we can provide the English translation if needed. Based on well-accepted Chinese Medicine theory, the SJZ decoction falls in the class of tonic prescription and is safe for human when used in a high dose. We chose the high dose at first, and the subsequent results of nude mice without tumor showed consistency.

"Q 4. Please provide the reference for in vivo experiments as you have started the treatment from the second day of transplantation?"

A : We checked the original experimental records and found that the treatment commenced 3 days after the transplantation. We apologize for this mistake and have the correction accordingly (P6, Line16).

The reference has also been added in the revised manuscript (P6, Line16).

The reason why we did not apply the drugs after the metastasis had occurred is that we wanted to explore the preventive function of this decoction.

"Q 5. Please provide the figure which shows change in the body weight from day 1 till the end of experiment for each group. Also, indicate the beginning of drug administration in the graph."

A: We have changed the figure showing the animal body weight at the reviewer’s suggestion (Fig 1A).

"Q 6. The manuscript needs language editing."

A: We have polished the writing of the revised manuscript (shown in red).

P 2, Line 2-5, 7, 9, 11-15, 17-18;
P 3, Line 2, 4-5, 8-10;
P 4, Line 8-10;
P 8, Line 11, 15-16, 18;
P 9, Line 10, 18-19;
P11, Line 1-2, 4, 11-12;
P12, Line 9, 13;
P13, Line 3, 6-11.