



PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology

Manuscript NO: 39674

Title: Herb-partitioned moxibustion alleviates colon injuries in ulcerative colitis rats: cytokine expression profiling

Reviewer's code: 02510206

Reviewer's country: Austria

Science editor: Xue-Jiao Wang

Date sent for review: 2018-06-07

Date reviewed: 2018-06-07

Review time: 13 Hours

SCIENTIFIC QUALITY	LANGUAGE QUALITY	CONCLUSION	PEER-REVIEWER STATEMENTS
<input type="checkbox"/> Grade A: Excellent	<input checked="" type="checkbox"/> Grade A: Priority publishing	<input type="checkbox"/> Accept	Peer-Review:
<input checked="" type="checkbox"/> Grade B: Very good	<input type="checkbox"/> Grade B: Minor language polishing	(High priority)	<input checked="" type="checkbox"/> Anonymous
<input type="checkbox"/> Grade C: Good		<input checked="" type="checkbox"/> Accept	<input type="checkbox"/> Onymous
<input type="checkbox"/> Grade D: Fair	<input type="checkbox"/> Grade C: A great deal of language polishing	(General priority)	Peer-reviewer's expertise on the topic of the manuscript:
<input type="checkbox"/> Grade E: Do not publish	<input type="checkbox"/> Grade D: Rejection	<input type="checkbox"/> Minor revision	<input checked="" type="checkbox"/> Advanced
		<input type="checkbox"/> Major revision	<input type="checkbox"/> General
		<input type="checkbox"/> Rejection	<input type="checkbox"/> No expertise
			Conflicts-of-Interest:
			<input type="checkbox"/> Yes
			<input checked="" type="checkbox"/> No

SPECIFIC COMMENTS TO AUTHORS

the authors provide a proof of principle study which shows chnges in cytokine profiles in treated UC rats by an alternative medicine approach. This is a novel study conducted in an appropriate manner, presnetation of findigs is adequate and the paper well



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written. Some minor adjustments /corrections should be made as indicated in pdf-comments fields. - start from top and go to the final end (including figs and legends). -- Please go through and give also details in methods (protein array processing and ELISA - volumes, conditions, etc).

We are grateful to the reviewer for the precious and inspiring comments and questions which are significant in helping us improve the display of our study. We have rigorously made adjustments and supplements according to each comment. The details are shown as follow.

Question 1: Some minor adjustments /corrections should be made as indicated in pdf-comments fields (including figs and legends).

Answer: Adjustments/corrections have been made properly based on the comments by the reviewer. Please see the revised version for the details (changed parts are marked by red color). Furthermore, we have especially replaced the heatmap of 90 cytokines with the one displaying all samples.

Question 2: Please go through and give also details in methods (protein array processing and ELISA - volumes, conditions, etc).

Answer: More details of protein array processing and ELISA such as volumes, incubated time and temperature have been added.

Question 3: Why do you have only 8 of the 9 individuals? clarify/explain

Answer: There were 9 rats in each group (normal group, model group, HPM group) at first. After modeling, 3 rats (1 rat from each group) were sacrificed for UC model verification. So there were only 8 rats remained for intervention and indicator assessment.



PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology

Manuscript NO: 39674

Title: Herb-partitioned moxibustion alleviates colon injuries in ulcerative colitis rats: cytokine expression profiling

Reviewer's code: 00069774

Reviewer's country: Thailand

Science editor: Xue-Jiao Wang

Date sent for review: 2018-05-25

Date reviewed: 2018-06-08

Review time: 13 Days

SCIENTIFIC QUALITY	LANGUAGE QUALITY	CONCLUSION	PEER-REVIEWER STATEMENTS
<input type="checkbox"/> Grade A: Excellent	<input type="checkbox"/> Grade A: Priority publishing	<input type="checkbox"/> Accept	Peer-Review:
<input type="checkbox"/> Grade B: Very good	<input type="checkbox"/> Grade B: Minor language	(High priority)	<input type="checkbox"/> Anonymous
<input type="checkbox"/> Grade C: Good	polishing	<input type="checkbox"/> Accept	<input type="checkbox"/> Onymous
<input type="checkbox"/> Grade D: Fair	<input type="checkbox"/> Grade C: A great deal of	(General priority)	Peer-reviewer's expertise on the
<input type="checkbox"/> Grade E: Do not	language polishing	<input type="checkbox"/> Minor revision	topic of the manuscript:
publish	<input type="checkbox"/> Grade D: Rejection	<input type="checkbox"/> Major revision	<input type="checkbox"/> Advanced
		<input type="checkbox"/> Rejection	<input type="checkbox"/> General
			<input type="checkbox"/> No expertise
			Conflicts-of-Interest:
			<input type="checkbox"/> Yes
			<input type="checkbox"/> No

SPECIFIC COMMENTS TO AUTHORS

Herb-partitioned moxibustion alleviates colon injuries in ulcerative colitis rats: cytokine expression profiling: By Dan Zhang et al The article reports an important study elucidating the mechanism of action of moxibustion in chronic inflammatory bowel



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disease. The work has been well designed and extensively carried out with worthy effort. Some questions may be benefit to some readers and this reviewer who have never involved in this traditional medication. Question such as how stimulating flow of blood by using moxa inducing changes in inflamed tissues. Authors may review briefly why position of CV6 and ST25 are selective for the treatment of UC. In this study, the work was carried out only at ST25? Authors should declare that the animal study has been approved by institutional animal ethics committee. How animals were restrained in the moxibustion process and process of euthanasia at the end. How authors assess spiritual state, what parameters were used in Fig.2B, what is the gross score? It is quite surprised to see that the down-regulated IFN-g, TNF-a, TLR4, CCR4 were involved with the development of UC? And on the other hand moxibustion resulted in up-regulated TNF-a, and IFN-g were associated with therapeutic effect?. Since anti-TNF antibody is one of main stream of treatment of IBD, authors have any comments on such contradictory result. As the analysis by KEGG pathway reported in the Manuscript is rather vogue and provide little information for further study, authors may comments and pick up the most likely ones for clarification. There is a number of typing errors in the manuscript that should be taken care of.

We are grateful to the reviewer for the precious and inspiring comments and questions which are significant in helping us improve the display of our study. We have rigorously made adjustments and supplements according to each comment. The details are shown as follow.

Question 1: How stimulating flow of blood by using moxa inducing changes in inflamed tissues.

Answer: Studies showed that moxibustion at acupoints can improve blood flow in internal organs, which is significant to its therapeutic effectiveness in treating the

corresponding organic diseases[1-4]. One recent study also reported that thermal stimulation to abdomen significantly regulated blood flow volume in the superior mesenteric artery and brachial artery[5]. Thermal effect is the most crucial factor for moxibustion to play its role[6]. Via the thermal stimulation to acupoints, moxibustion treats colitis possibly by influencing blood flow in intestinal arteries, increasing blood flow volume in intestinal mucosa, improving intestinal microcirculation and accelerating the repair of colonic mucosa injuries.

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5. Takayama S, Seki T, Watanabe M, Takashima S, Sugita N, Konno S, Takeda T, Arai H, Yambe T, Yaegashi N, Yoshizawa M, Maruyama S, Nitta S.. Changes of blood flow volume in the superior mesenteric artery and brachial artery with abdominal thermal stimulation. *Evid Based Complement Alternat Med*, 2011; 2011: 214089.
6. Kawakita K, Shinbara H, Imai K, Fukuda F, Yano T, Kuriyama K. How do acupuncture and moxibustion act? - Focusing on the progress in Japanese acupuncture research. *J Pharmacol Sci*, 2006; 100(5): 443-59.

Question 2: Authors may review briefly why position of CV6 and ST25 are selective for the treatment of UC. In this study, the work was carried out only at ST25?

Answer: Either ST25 alone[1-2] or used in combination with CV6[3-4] has been

proved effective in easing the intestinal inflammation in UC rats and repairing the mucosa injuries. The current study specifically chose to use ST25 alone to avoid the between-acupoint interactions brought by selection of multiple acupoints.

References

1. Shi Y, Qi L, Wang J, Xu MS, Zhang D, Wu LY, Wu HG. Moxibustion activates mast cell degranulation at the ST25 in rats with colitis. *World J Gastroenterol*. 2011, 28;17(32): 3733-8.
2. Huang Y, Ma Z, Cui YH, Dong HS, Zhao JM, Dou CZ, Liu HR, Li J, Wu HG. Effects of Herb-Partitioned Moxibustion on the miRNA Expression Profiles in Colon from Rats with DSS-Induced Ulcerative Colitis. *Evid Based Complement Alternat Med*, 2017;2017:1767301.
3. Wang X, Liu Y, Dong H, Wu L, Feng X, Zhou Z, Zhao C, Liu H, Wu H. Herb-Partitioned Moxibustion Regulates the TLR2/NF- κ B Signaling Pathway in a Rat Model of Ulcerative Colitis. *Evid Based Complement Alternat Med*, 2015;2015:949065.
4. Liu HR, Tan LY, Cui YH, Wu HG, Jiang B, Zhao TP, Wang XM. Regulating the secretion of insulin-like growth factor- I and transforming growth factor beta 1 of colonic fibroblasts by moxibustion at Tianshu and Qihai in rats. *Zhongguo Zuzhi Gongcheng Yanjiu Yu Linchuang Kangfu* 2007, 11(49): 9878-9881 [DOI:10.3321/j.issn:1673-8225.2007.49.012]

Question 3: Authors should declare that the animal study has been approved by institutional animal ethics committee.

Answer: This study had been approved by the institutional animal ethics committee ever before it took place and the whole performance rigorously followed the International Guiding Principles for Biomedical Research Involving Animals recommended by World Health Organization. Please see the section of Materials and Methods in the revised version for detailed description and the ethic certification document is attached in the appendix.

Question 4: How animals were restrained in the moxibustion process and process of euthanasia at the end.



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Answer: During moxibustion, the rats were restrained by using rat fixator and a researcher used both of his hands to assist the rat to stay in a supine position and expose its abdomen where the herbal cake and moxa were placed. The assistance of researcher's hands can sooth the rats and the warm effect from moxibustion was also comfortable, both acting to reduce rat's struggle. In the end, euthanasia was carried out by intraperitoneal injection of pentobarbital sodium (150mg/kg) to inhibit the respiration. The information has been added properly into the section of Materials and Methods in the revised manuscript.

Question 5: How authors assess spiritual state, what parameters were used in Fig.2B, what is the gross score?

Answer: Rat's spiritual state was roughly assessed by observing the performance in eating, drinking and moving in this study by referring the previous studies[1]. The result revealed obvious decreases in eating and drinking frequencies in UC rats and they spent more time huddling with eyes closed rather than moving around, based on which we can conclude that the UC rats had a poor spiritual state. Since this was a subjective and simple indicator to help observe the general state of the rats, we didn't consider it necessary to perform a statistical analysis. The influence of HPM on UC rats was mainly judged according to the histopathological indicators of colon. The gross score in Fig2B majorly observed the adhesion, formation of ulcers and inflammation in colons[2] and the scoring standard is provided in Table1.

References

1. Wang X, Liu Y, Dong H, Wu L, Feng X, Zhou Z, Zhao C, Liu H, Wu H. Herb-Partitioned Moxibustion Regulates the TLR2/NF- κ B Signaling Pathway in a Rat Model of Ulcerative Colitis. *Evid Based Complement Alternat Med*, 2015;2015:949065.



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2. Wang XM, Lu Y, Wu LY, Yu SG, Zhao BX, Hu HY, Wu HG, Bao CH, Liu HR, Wang JH, Yao Y, Hua XG, Guo HY, Shen LR. Moxibustion inhibits interleukin-12 and tumor necrosis factor alpha and modulates intestinal flora in rat with ulcerative colitis. *World J Gastroenterol*, 2012;18(46):6819-28.

Question 6: It is quite surprised to see that the down-regulated IFN-g, TNF-a, TLR4, CCR4 were involved with the development of UC? And on the other hand moxibustion resulted in up-regulated TNF-a, and IFN-g were associated with therapeutic effect?.

Answer: Actually, it's also a surprise to our research group. Therefore, we searched relevant literatures and found that dysfunction of TNF-a could lead to exacerbation of colitis in early stage, which indicated that TNF-a protect colon by acting on different receptors[1-4]. Thus we suppose that colonic cytokines may present various changes and functions in different stages of colitis, and either insufficient or excessive expressions will possibly cause immune-related inflammatory damage in colon. This phenomenon is definitely worth further exploration. Shown in the current study, moxibustion up-regulated the decreased level of TNF-a, indicating that moxibustion can correct the abnormal expression of cytokines which can be considered as an evidence for the recovery of normal immune system.

Reference

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4. Naito Y, Takagi T, Handa O, Ishikawa T, Nakagawa S, Yamaguchi T, Yoshida N, Minami M, Kita M, Imanishi J, Yoshikawa T. Enhanced intestinal inflammation induced by dextran sulfate sodium in tumor necrosis factor- α deficient mice. *J Gastroenterol Hepatol*, 2003;18(5):560-9.

Question 7: As the analysis by KEGG pathway reported in the Manuscript is rather vogue and provide little information for further study, authors may comments and pick up the most likely ones for clarification.

Answer: Through functional cluster and KEGG pathway analyses, specific differential cytokines and plausibly-involved pathways were selected in this study. The analyses showed that the pathways interacting between the cytokines and their receptors should be closely associated with the action of moxibustion, and MAPK signaling pathway and JAK/STAT signaling pathway were most possibly involved in the anti-inflammation effect. We believe that further study in this field will help unveil the action mechanism of moxibustion in treating UC.