

Dear Editors

World Journal of Gastroenterology

Revision of the manuscript: "Effect of electroacupuncture and moxibustion on immune and oxidative phosphorylation-associated protein expression profiles in DSS-induced ulcerative colitis rats" (Manuscript NO.: 70196, Basic Study)

On behalf of all my colleagues, I would like to thank you and the reviewers for critiquing the manuscript. We found that the comments from the reviewers are constructive and helpful, and have addressed the questions and carefully revised our manuscript accordingly. The reviewer and editor's suggestions have been adopted and we hope that the revised manuscript entitled "Mechanism of electroacupuncture and herb-partitioned moxibustion on ulcerative colitis animal model: a study based on proteomics" can be considered for publication in "World Journal of Gastroenterology". **Below are the point-by-point responses.**

Reviewer #1:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

Specific Comments to Authors: The manuscript entitled "Effect of electroacupuncture and moxibustion on immune and oxidative phosphorylation-associated protein expression profiles in DSS-induced ulcerative colitis rats" by Qi et al. aims to study molecular mechanisms underlying the effects of electroacupuncture and moxibustion in DSS-induced ulcerative colitis (UC) in modeled rats. As the authors stated in the Introduction "acupuncture and moxibustion have significant efficacy and advantages in the treatment of UC". However, there is not obvious because there is no strong evidence of the efficacy of these two procedures alone because in the Refs that the authors provided, they are mostly, used in combination with medication and herbs. Moreover, in Materials and Methods section an herb cake-based

moxibustion was also used, therefore its effects can be at least in part attributed to the influence of herbal components.

Response:

Thanks for your comments. Clinically, some UC patients need to take western medicine for a long time to control the progression and recurrence of the disease. On the basis of western medicine, moxibustion can reduce disease activity, maintain remission, and reduce the recurrence rate. After a period of moxibustion treatment later, some patients were able to reduce the amount of western medicine or even not use western medicine. In addition to the combined use of acupuncture and moxibustion therapy combined with drug treatment, there are also acupuncture or moxibustion used alone, such as the Ref6 (herbal cake-partitioned moxibustion or ginger-partitioned moxibustion, *J Acupunct Tuina Sci* 2016; 14: 231-241) and Ref7 (*World J Clin Cases* 2020; 8: 1515-1524). The moxibustion therapy we used in this article is actually herb-partitioned moxibustion (also call “herbal cake-partitioned moxibustion”), which is a critical component of moxibustion that combines the advantages of heat, herbal, and acupoints stimulation. In order to avoid misunderstanding, we have changed the “moxibustion” in the title to “herb-partitioned moxibustion” and marked in red.

Other major concerns:

1. Title of the manuscript should be changed because little evidence on the involvement of immune response and oxidative phosphorylation is provided (see below).

Response:

Thanks for the advice. We have changed the title of the article to “Mechanism of electroacupuncture and herb-partitioned moxibustion on ulcerative colitis animal model: a study based on proteomics” and marked in red.

2. In the Results and Discussion sections, GO and KEGG functional enrichment analyses require more deep and detailed functional analysis, instead of just

listing of biological processes without interpretation. For example, the term “binding” has a wide range of meanings, while, the terms “catalytic activity” and “metabolic process” mean the same functions, while “transporter activity” indicates functioning as a transporter protein, and “structural activity” means a role of a structural part of cells and organelles.

Response:

Thanks for your comments. We have added the content of this part accordingly in the manuscript. The detailed information has been added to the Results and Discussion sections of the manuscript and marked in red.

3. Additionally, Atp5o, Atp5l, Atp5f, Atp5h are ATP synthase (complex V) subunits, while Cox4i1 is a subunit of cytochrome c oxidase (complex IV) that is a component of electron transportation chain (ETC). Strictly speaking, these proteins cannot be attributed to oxidative phosphorylation because OXPHOS is coupling of ETC functioning with ATP synthesis; and there can be uncoupling of these two processes.

Response:

Thanks for the question. We agree with the reviewer's assessment. Oxidative phosphorylation is coupling of ETC functioning with ATP synthesis; and there can be uncoupling of these two processes. Mitochondrial oxidative phosphorylation is the process by which the ATP synthase conserves the energy released during the oxidation of different nutrients as ATP. ATP synthase and Cox4 are two types of key enzymes for oxidative phosphorylation energy metabolism. Atp5f1 is a nuclear gene responsible for encoding the F0 subunit of ATP synthase, which is closely related to energy metabolism. Cox4i1 is the main subunit of cytochrome c oxidase, a key enzyme in energy metabolism, and plays an important role in mitochondrial oxidative phosphorylation. Defects in oxidative phosphorylation leads to a decrease in cellular ATP production. Studies have reported that the activity of mitochondrial respiratory chain complexes in UC patients is reduced, and mucosal ATP is absent (*Mol Cell Biochem* 2010, **342**: 111-115; *Inflamm Bowel Dis*

2012, **18**: 2158-2168). In this study, we first analyzed the significantly differentially expressed proteins (DEPs) between groups, and then the KEGG pathway database was used for pathway enrichment analysis of DEPs. We found that the pathways mainly enriched in primary immunodeficiency, the NF- κ B signalling pathway, oxidative phosphorylation and so on, and the significantly differentially expressed proteins IDs involved in oxidative phosphorylation include Atp5h, Atpo, Cox41, Atp5l, Atp5f, etc., so we say that these differentially expressed proteins are oxidative phosphorylation pathway-related proteins. In order to avoid misunderstanding, we have changed the title of the article to “Mechanism of electroacupuncture and herb-partitioned moxibustion on ulcerative colitis animal model: a study based on proteomics”. In the future, we will focus on ETC and ATP synthase as well as the coupling process to in-depth study the regulation of acupuncture and moxibustion on oxidative phosphorylation in UC.

4. The term “immune system” was identified only in GO processes and KEGG pathways. However, no one protein involved in the immune response regulation was identified and analyzed in this study. Instead, in PPI network analysis, proteins involved in inflammation regulation such as serpins were identified. It would be good to discuss them in more details.

Response:

Thanks for your advice. For PPI network analysis, we found that proteins involved in inflammation regulation such as serpins were identified (serpinb6 in M/N comparisons, serpin1 in HM/M and EA/M comparisons). Serpins are the largest known family of serine proteinase inhibitors, which regulate innate immunity by inhibiting the serine proteinase cascades that initiate immune responses such as melanization and antimicrobial peptide production (*Immunology* 2020; 159: 109-120). Several human serpins have been shown to regulate serine proteases associated with processes such as inflammation and immune responses (*Methods Mol Biol* 2018; 1826: 1-7). And increased activity of serine proteases is demonstrated in IBD patients and may contribute to the

onset and the maintenance of the disease (*Int J Mol Sci* 2021; 22: 6088). This appears to be a novel finding that will require additional research. We have added the content of this part accordingly in the manuscript. The detailed information has been added to the discussion of the manuscript.

5. Additionally, functional analysis of proteins that were changed in their expression level in M, HM and EA (as shown by Western blotting) groups should be provided.

Response:

Thanks for your comments. We understand that functional analysis experiments supplementing of proteins that were changed in their expression level in M, HM and EA may better reveal the molecular mechanism of electroacupuncture and herb-partitioned moxibustion on ulcerative colitis. In the present study, the western blot analysis was to confirm the reliability of the iTRAQ data, and we think that proteomics analysis combined with western blot analysis may not be optimal, but should be sufficient to draw a conclusion that EA and HM could regulate the expression of Atp5l, Atp5f1, Cox4i1 that associated with oxidative phosphorylation. And due to time constraints, supplementary protein function experiments may not be possible, but in the future we will further supplement cell function experiments to in-depth study the mechanism of these differentially expressed proteins on UC. Just as the shortcomings mentioned at the end of the article, the underlying mechanisms may require further study through animal and cell experiments.

Minor concerns:

1. Figure 4 was missed in the text despite it presents among the manuscript Figures.

Response:

Thanks for your comments. Figure 4 mentioned in the first line of “iTRAQ quantification of differentially expressed proteins” in the results section.

2. In Materials and Methods section, scoring of the colon should be described. Also, “microscopic scoring of the colon was performed according to the Score Criteria of Colonic Histological Damage” stated. while in the Fig.3 legend tells “macroscopic scores”.

Response:

Thanks for your comments. We have added the content of this part accordingly in the manuscript. The macroscopic scoring of the colon was performed according to the following parameters: hyperemia, wall thickening, ulceration, inflammation extension, and damage no damage, score 0; hyperemia without ulcers, score 1; hyperemia and wall thickening without ulcers, score 2; one ulceration site without wall thickening, score 3; two or more ulceration sites, score 4; 0.5 cm extension of inflammation or major damage, score 5; 1 cm extension of inflammation or severe damage, score 6 (*Theranostics* 2017; 7: 3446-3460). The microscopic scoring of the colon was performed according to the following parameters: damage/necrosis, inflammatory cell infiltration, submucosal edema, and hemorrhage of mucosa. Colonic gross damage scores were recorded according to the severity of changes: no change, score 0; mild, score 1; moderate, score 2; severe, score 3 (*J Tradit Chin Med* 2019; 39: 356-363). The detailed information has been added to the Materials and Methods of the manuscript. And “microscopic scoring of the colon was performed according to the Score Criteria of Colonic Histological Damage” was shown in Figure 3-B and 3-D. “macroscopic scoring of the colon was performed according to the Score Criteria of Colonic General Damage” was shown in Figure 3-A and 3-C.

3. In the Results section, the sentence “the cellular components mainly included cells, organelles, the cell membrane, macromolecular complexes, extracellular components, and the extracellular domain” should be removed because all existed compartments are listed and these data are uninformative.

Response:

Thanks for your advice. We have deleted the sentence “the cellular components mainly included cells, organelles, the cell membrane, macromolecular

complexes, extracellular components, and the extracellular domain”.

Reviewer #2:

Scientific Quality: Grade A (Excellent)

Language Quality: Grade B (Minor language polishing)

Conclusion: Accept (General priority)

Specific Comments to Authors: Title: Please do not use abbreviations and provide full description of DSS in the title. This is recommended to use the term “Animal Model” instead of “rats”.

Response:

Thanks for the advice. We have changed the title of the article to “Mechanism of electroacupuncture and herb-partitioned moxibustion on ulcerative colitis animal model: a study based on proteomics”.

Abstract: Please write full description for Sv2a, Ncbp1, Cps1, Cox4i1, Atp5f1, Dclk3.

Response:

Thanks for your comments. We have added the content of this part accordingly in the Abstract section.

Core tip: Please rephrase “Cox4i1 that associated with oxidative phosphorylation, then might” to “Cox4i1 that are associated with oxidative phosphorylation, that might”

Response:

Thanks for the advice. We have made corresponding modifications in Core tip section.

Figure 2 Legend: Please write full description of SD rat in A; Figure 3-9 Legends: Please provide full description of DSS and UC; Conclusions: Line 1: Please change “to provided” to “to provide”; It is suggested that please provide full

description of DSS and UC; MATERIALS AND METHODS DSS-induced UC
Model preparation: 2nd last Line: Please change “to evaluated” to “to evaluate”

Response:

Thanks for the comment. We have made corresponding modifications and marked in red.

As per ARRIVE guidelines please provide route of administration (mention oral), method of euthanasia if animal were suffering, time of the day for drug administration. Please mention if the animals were drug or test naïve, previous procedures, number of cage companions.

Response:

Thanks for the comment. We have added the content of this part accordingly in the MATERIALS AND METHODS section.

Grouping and interventions: Please enter a space between carthami, Salvia

Response:

Thanks for the comment. We have made corresponding modifications and marked in red.

Statistical analysis: Please full description of ANOVA and LSD. Also please mention the methods used for Proteomics data analysis.

Response:

Thanks for the comment. We have made corresponding modifications and marked in red. The methods used for proteomics data analysis was mentioned in “iTRAQ quantification and Bioinformatics analysis”.

Results: Protein-protein interactions (PPI) analysis: Please mention the STRING version and p value for PPI. Please rephrase the paragraph in a way that sentences do not start with a number.

Response:

Thanks for your comments. The STRING resource is available online at

<https://string-db.org/>. The STRING version was 11.0, and p value for PPI was < 0.05 . We have added the content of this part accordingly in the Results section.

REFERENCES: The references# 1-7 are up until 2017. This would be better if some of the articles from recent years could be replaced/added instead. Reference#16 is from 1990. It is suggested to replace reference from recent years.

Response:

Thanks for your advice. We have made corresponding modifications and added some references from recent years in References section.

Re-reviewer:

It is hard to assess the revised version of the manuscript because (i) the authors provided the lengthy responses and (ii) the corrections in the text of the manuscript were not highlighted in any way. I recommend the authors to resubmit the revised version of the manuscript with correction highlights.

Response:

Thanks for your comments. We have resubmitted the revised version of the manuscript with correction highlights, and the condensed answer is as follows.

We have carefully addressed all the questions above. Thank you and reviewers for providing the comments to improve our manuscript.

If you have any other questions, please do not hesitate to contact us. Thank you so much!

Best regards,

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