Author’s response to reviews

Title: Weipiling ameliorates gastric precancerous lesions in Atp4a/- mice

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Dear Esther Fagelson:

Thank you very much for your valuable feedback. The following is the answer to the questions.

1. On page 3 of the revised manuscript, we added the reasons and references for selecting animal model studies as:” Replication of disease models is an effective tool for studying disease pathogenesis and treatment. For the study of GPL, researchers used chemical drug induction and HP infection methods, but there are limitations in model replication time, large individual differences, and unstable models, thus affecting the study of GPL. As we reported before, with the development of genetic engineering technology, knockout mice are gradually being used to study precancerous lesions. The phenotypic progression to IM observed in the Atp4a/- mouse model is similar to the evolution of gastric cancer in humans, as initially described by Correa. So in this research, we choose the Atp4a/- mice to simulate GPL.”
On the page 3 to 4, We added the reason for choosing Weipiling to study GPL:” Weipiling (WPL) is based on an improved version of Sijunzi decoction. Weipixiao, another Chinese medicine formula, also based on the Sijunzi decoction, has shown a certain protective effect on precancerous lesions. Weipiling can be prepared from conventional decoction of eight Chinese medicinal herbs including Astragalus Membranaceus, Atractylodes macrocephala Koidz (Baizhu), Pseudostellaria Heterophylla, Poria cocos (Schw.) Wolf (Fu-ling), Curcuma zedoaria, Panax notoginseng (Burkill) F. H. Chen ex C. H, Hericium erinaceus (Rull ex F.) Pers and Hedyotis Diffusa Willd. These Chinese medicinal materials described above have pharmacological effects of replenishing qi to invigorate the spleen, and removing blood stasis and detoxify, however the Weipixiao is only the “Yi Qi Hua Yu” decoction. Experimentally, some WPL individuals exhibited potential anticarcinogenesis activities. Astragalus membranaceus induced by apoptosis of human gastric carcinoma via intrinsic mitochondrial pathways. Clinical research showed that Atractylodes macrocephala Koidz can be beneficial for treating cancer cachexia, Poria cocos (Schw.) Wolf (Fu-ling) arrest Cell-cycle and induce apoptosis of breast carcinoma. Curcuma zedoaria inhibited AGS human gastric cancer cell viability by activating caspase-8, caspase-9, caspase-3, and PARP, which contributed to apoptotic cell death in AGS human gastric cancer cells. Hedyotis Diffusa Willd. has been using to treat malignant tumors of gastric, colon, rectal, . Clinical and basic research shows that a WPL decoction can effectively relieve clinical symptoms and reverse precancerous lesions, but the specific mechanism governing this effect is unknown. The aim of this study was to investigate the effect of the WPL decoction on the GPL animal model and explore the molecular mechanism underlying its anti-malignant transformation of gastric mucosa and corresponding inhibition of gastric cancer.”

2. on the page 5, We have supplemented in detail the method of animal euthanasia, as:“At the end of the 10th week, these mice in the model group were humanely terminated with sodium pentobarbital (60mg/kg, injected intraperitoneally) diluted with phosphate-buffered saline (PBS). Each treatment was placed in a 1 ml syringe as dictated by the volume of injectate. A new 25 G 5/8” hypodermic needle was attached to each syringe for injection. Two-person injection technique used in the study, with one person holding the mice in dorsal recumbency (head down) and the second person gently restraining the right pelvic limb to facilitate intraperitoneal injection in to the right caudal quadrant. The left thoracic wall was then auscultated continuously with a stethoscope to identify cessation of heartbeat (CHB). Following CHB, each animal was carefully removed from the chamber and positioned in dorsal recumbency for necropsy examination.”
3. The present research and The article [https://bmccomplementalternmed.biomedcentral.com/articles/10.1186/s12906-018-2309-3](https://bmccomplementalternmed.biomedcentral.com/articles/10.1186/s12906-018-2309-3) both study the GPL, however, The animal model for studying gastric precancerous lesions is different that we use the Atp4a-/mice, and the Jinhao Zeng, et al. used the N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced GPL in male Sprague-Dawley rats. Besides, Jinhao Zeng, et al. elucidated the role of traditional Chinese medicine in the treatment of gastric precancerous lesions from the perspective of early angiogenesis and microvascular abnormalities, our results showed that WPL could ameliorate GPLs in Atp4a-/mice, this effect might be partly achieved by inhibiting the expression of transporters and suppressing the aberrant activation of mTOR/HIF-1α.

②[https://www.sciencedirect.com/science/article/pii/S0753332218323278?via%3Dihub](https://www.sciencedirect.com/science/article/pii/S0753332218323278?via%3Dihub), the article has studied Xiaoyao San, traditional Chinese medicine, ameliorates depressive-like behaviors by triggering autophagosome formation to alleviate neuronal apoptosis. As you know, We all clarify the mechanism by which Chinese medicine may exert its therapeutic effect from the perspective of apoptosis, however, Traditional Chinese medicines can be composed of different prescriptions according to different diseases. Weipiling is based on an improved version of Sijunzi decoction and have pharmacological effects of replenishing qi to invigorate the spleen, and removing blood stasis and detoxify, the tunel assay showed that it can induce apoptosis of gastric mucosal epithelial cells.


Xiao Tan He Wei Decoction is one of the prescriptions of phlegm, which is based on an improved version of DaoTan decoction, consisting of radix bupleuri, processed rhizome pinnelliae, poriacocos, coptischinensis, radix glycyrrhizae preparata, etc., and the Weipiling is based on an improved version of Sijunzi decoction. Weipiling can be prepared from conventional decoction of eight Chinese medicinal herbs including Astragalus Memranaceus, Atractylodes macrocephala Koidz (Baizhu), Pseudostellaria Heterophylla, Poria cocos (Schw.) Wolf (Fu-ling), Curcuma zedoaria, Panax notoginseng (Burkill) F. H. Chen ex C. H, Hericium erinaceus (Rull ex F.) Pers and Hedyotis Diffusa Willd. These Chinese medicinal materials described above have pharmacological effects of replenishing qi to invigorate the spleen, and removing blood stasis and detoxify. So, we use the different Chinese medicinal materials to study gastric precancerous lesions. In addition, we use different animal models to study gastric precancerous lesions.
Some of the same ideas contained in these publications, we added references as references in the writing process of the article. In the background, “Weipiling (WPL) is based on an improved version of Sijunzi decoction. Weipixiao, another Chinese medicine formula, also based on the Sijunzi decoction, has shown a certain protective effect on precancerous lesions .”,and “These Chinese medicinal materials described above have pharmacological effects of replenishing qi to invigorate the spleen, and removing blood stasis and detoxify, however, the Weipixiao is only the “Yi Qi Hua Yu” decoction.” In the Discussion” Impairment of apoptosis was observed in GPL and gastric cancer , .”.

4. The uncropped western blots have been added in the supplementary material.

5. When I use the Excell software to count the data of the Tunnel results, due to negligence, the order of data pasting is confusing, so I re-do the statistical results map, put the correct picture 9 in the article, please forgive me, I am very sorry for my mistake.

Best wishes

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