Author's response to reviews

Title: Protective effects of cardiotonic pills on damage induced in the white matter and hippocampus by chronic cerebral hypoperfusion

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Author's response to reviews: see over
Mikel Aickin
Section Editor
BMC Complementary & Alternative Medicine

Thank you for your letter, along with the comments from reviewers on our manuscript “Protective effects of cardiotonic pills on damage induced in the white matter and hippocampus by chronic cerebral hypoperfusion” co-authored by Ki Mo Lee, Ji Hye Bang, Jung-Soo Han, Bu Yeo Kim, In Sun Lee, Won Kyung Jeon (Manuscript ID: 4801935471048980)”. And we are so sorry that the revision is late. Additional experiment took longer than we expected.

Please find attached a revised manuscript in which we have attempted to fully address the reviewers’ comments. We would like to thank the reviewers and feel that these revisions have focused the report and strengthened the paper overall.

This manuscript has been edited by American Journal Experts, and Certificate is attached in last page.

Reviewer's report

Title: Protective effects of cardiotonic pills on damage induced in the white matter and hippocampus by chronic cerebral hypoperfusion

Version: 2 Date: 15 August 2013

Reviewer: zhong-xiang Yao

Reviewer's report:

Major Compulsory Revisions

The authors want to examine whether CP treatments ameliorate the brain damage induced by chronic cerebral hypoperfusion. By using histochemistry and western blotting, it is found that CP may ameliorate white matter and hippocampal damage induced via chronic cerebral hypoperfusion by attenuating the induction of white matter lesions and neuroinflammatory processes; furthermore, the
suppression by CP upon the inflammatory reaction might be involved in MAPK signaling. They suggested that CP may be a potential candidate for the prevention and treatment of AD and VaD.

The major revisions:

1. Please explain the relationship among the MAPK signals and inflammatory mediators at the whole hippocampal levels, and oligodendrocytes or microglia at cell levels.

   ; As your advice, the relationship among the MAPK signals and inflammatory mediators was explained in Discussion, paragraph 2 line 9-13.

2. Why do you choose this dose of CP and this period of CP administration?

   ; 1) We have referred the previous reports to determine the dose of CP in this study [1-3]. These reports have demonstrated the protective effects of CP using various doses (from 100 to 1000 mg/kg). In addition, in preliminary experiment to examine antithrombotic effect of CP in SD rat, various doses of CP (50-500 mg/kg) were treated to FeCl\textsubscript{3}-induced thrombosis model. We found that 200 mg/kg of CP shows the optimal antithrombotic effect. On the basis of the above findings, 200 mg/kg of CP was applied in this study.

   2) The period of CP administration in this study has modified the duration of treatment performed in the previous report [4]. Furthermore, we have previously demonstrated that administration of Fructus mume into BCCAo-induced rats for the same period attenuates the memory impairment [5].

References


3. The title should be more accurate on the effects of CP.

; We change the title as follows “Cardiotonic pill attenuates white matter and hippocampal damage via
decreasing inflammation and microglial activation in chronic cerebral hypoperfused rats”.

The minor revisions:

1. In abstract, methods line 1: “Wister rat” should be “Wistar rat”.

; Wister rat is corrected with Wistar rat in abstract, methods line 1 as your advice.

2. In abstract, conclusion line 2-3: “a trait that suggests therapeutic potential for the prevention of vascular dementia” is not suitable.

; This portion is corrected as follows “suggests that CP may be potentially useful in the treatment of vascular dementia”.

3. In background, paragraph 4 line 3-5: “CP treatments restored the reduced expression levels of myelin basic protein (MBP) and reduced microglial activation in the white matter” is confused.

; This portion is corrected as follows “CP treatments restored the myelin basic protein (MBP) degradation and microglial activation in the white matter and hippocampus of chronic BCCAo rats.”.

4. In methods, “Animal surgery and drug administration” paragraph 1 line 1: “using a mixture of 5% isoflurane and oxygen” is confused.

; This portion is corrected as follows “with 5% isoflurane in a 70% nitrous oxide and 30% oxygen mixture”.

5. In methods, “Animal surgery and drug administration” paragraph 1 line 6: “During hypoperfusion, approximately 4% of the rats”, the 4% should give an accurate number too.

; This portion is corrected as follows “two rats showed neurological symptoms”.

6. In methods, “Animal surgery and drug administration” paragraph 1 line 9: the accurate number of the “excluded” rat.

; This portion is corrected as follows “seven rats that lost 80% or more of their pre-surgical weight during drug or vehicle administration were excluded from the present study”.

7. In methods, “Animal surgery and drug administration” paragraph 2 line 7: “until the end of the experiment”, the accurate time of the experiment.

; This portion is corrected as follows “until the 41st day after first vehicle/drug treatment”.

8. in methods,” Animal surgery and drug administration” paragraph 2 line 7:” During drug administration, 8 rats were lost”, which group of these 8 rats belong to?

; This portion is corrected as follows “the vehicle and CP treatment groups each lost four rats due to the stress related to long-term oral feeding” in methods,” Animal surgery and drug administration” paragraph 2 line 8-9.

9. in methods,” Western blot analysis” paragraph 1 line 1: “on day 63” should give the beginning time.

; This portion is corrected as follows “on day 63 after BCCAo surgery”.

10. in methods,” Western blot analysis” paragraph 1 line 2: “Tissue homogenates” will tell us the accurate region of sample.

; This portion is corrected as follows “Tissue homogenates of the hippocampus” in methods,” Western blot analysis” paragraph 1 line 3.

11. in methods,” immunohistology”, the subtitle: should be “immunohistochemistry”.

; Immunohistology is corrected with immunohistochemistry as your advice.

12.in results, “CP treatment increased chronic BCCAo-induced MBP reduction in the white matter and hippocampus”, paragraph 1 line 9: “including the corpus callosum, fimbria, fornix, and hippocampus” is easier to be confused.

; The paragraph 1 line 8: “including the corpus callosum, fimbria, fornix, and hippocampus” is excluded from this manuscript.

13. in results,” CP inhibited chronic BCCAo-induced microglial activation in the white matter and hippocampus” paragraph 1 line 10: “callosum, fimbria, fimbria, fornix, and hippocampal subregions”, there are two “fimbria”.

; The one of two “fimbria” is excluded from the paragraph.

14.in Figure legends, Figure 1 line 1: “the chronic BCCAo-induced”, sometimes it uses the “chronic”, sometimes not(such as in Figure 2 line 1).

; The “chronic” is added throughout this manuscript.

15. in Figure legends, Figure 2 line 3-4: “including the corpus callosum, fimbria, optic tracts, and the hippocampal subregions” is easier to be confused.

; Figure 2 line 3-4: “including the corpus callosum, fimbria, fornix, and hippocampus” is excluded
Reviewer’s report

Title: Protective effects of cardiotonic pills on damage induced in the white matter and hippocampus by chronic cerebral hypoperfusion

Version: 2 Date: 21 August 2013

Reviewer: Seong Yun Kim

Reviewer’s report:

I suggest major compulsory revisions.

In this manuscript, authors suggested that the cardiotonic pill (an herbal medicine composed of Salvia miltiorrhiza, Panax notoginseng, and Dryobalanops aromatica gaertners) may ameliorate the damage in the white matter and hippocampus induced by chronic cerebral hypoperfusion in rats. In general, this study was carefully designed and well executed in the part which evaluated effects of the cardiotonic pill on white matter damage induced by permanent bilateral common carotid artery occlusion (BCCAO). Nevertheless, there are some concerns which authors should address to improve the quality of the paper.

They are as follows:

I. This study was conducted to examine the effects of the cardiotonic pill (CP) on white matter and hippocampal damage by chronic cerebral hypoperfusion which was induced in male Wistar rats subjected to permanent BCCAO. It is well known that white matter damage is typically induced in an animal model used in this study. However, it is unclear that the hippocampal damage was induced in the animal used in this study. Authors argued the hippocampal damage evaluated by immunostaining for myelin basic protein (MBP) was significantly attenuated in the CP-treated group compared to the
vehicle-treated group (Fig 1). 1) In Fig 1, it is vague in which part of the hippocampus the level of MBP expression was evaluated. Are there some references in which the damage of the grey matter including the hippocampus was evaluated by immunostaining for MBP? Authors need to validate the evaluation of the hippocampal damage using immunostaining for MBP. 2) Further, it is not determined in this article whether neuronal death was induced in the hippocampus of Wistar rats subjected to BCCAO.

; 1) The part of the hippocampus the level of MBP expression was replaced with another figure, and 2) we determined neuronal death by counting NeuN-positive cells (neuronal antibody) in CA1, CA3, and DG subfields of the hippocampus of Sham-operated control, BCCAO+Vehicle group, and BCCAO+CP group. As a result, neuronal death was observed in rats with chronic BCCAO compared to Sham-operated control. Interestingly, this cell death was reduced in CP-treated chronic BCCAO rats, but this reduction was not statistically significant compared to BCCAO rats with vehicle treatment (Supplementary Fig. 2).

II. The authors need to better check English and to refine their writing: for examples,

1) Title: ~~ cardiotonic pills (?) on damage induced in the white matter and hippocampus by chronic cerebral hypoperfusion

; We change the title as follows “Cardiotonic pill attenuates white matter and hippocampal damage via decreasing inflammation and microglial activation in chronic cerebral hypoperfused rats”.

2) in lines 4-5 from bottom: ~~ rats that lost 80% or more of their presurgical weight during drug or vehicle administration were excluded from ~~;

; This portion is corrected as follows “seven rats that lost 80% or more of their pre-surgical weight during drug or vehicle administration were excluded from the present study”.

3) in the first line of Results section: CP treatment increased chronic BCCAO-induced MBP reduction in the white matter and hippocampus

; The first line of Results section is corrected as follows “CP restored the reduced expression of MBP in the white matter and hippocampus induced by chronic BCCAO”.

We’d like to thank the reviewers again for the helpful comments and we hope that this manuscript is now suitable for publication in BMC Complementary & Alternative Medicine. Please do not hesitate
to contact me for any additional information.

Sincerely

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