Author's response to reviews

Title: Kihi-to, a herbal traditional medicine, improves Abeta(25-35)-induced memory impairment and losses of neurites and synapses

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Author's response to reviews: see over
Dear Editor;
Editorial Office of *BMC Complementary and Alternative Medicine*

Thank you for sending reviewer’s reports for our paper (MS No. 2142707661194422) entitled “Kihi-to, a herbal traditional medicine, improves Aβ(25-35)-induced memory impairment and loss of neurites and synapses”. We considered again reviewer’s comments, and added several data and descriptions. Details revised are shown in next page.

We hope that the 2nd revision is now sufficient for acceptance.

Yours sincerely,

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Reply comments to Dr. Raymond Chang,

Thank you for nice and useful suggestions for improvement of contents. We considered your opinions and revised several points.

1) To show that Kihi-to gives influences to expressions of calpain and calpastatin in the brain, new Fig. 7 was added. These data show tendencies of long-lasting expressional changes of calpain and calpastatin by Aβ(25-35) injection (26 days after Aβ injection), and inhibitory effects of post-applied Kihi-to to them.

2) As you suggested, it is not appropriate at the present to conclude by force that the mechanism of Kihi-to is inhibition of calpain system. Therefore, we deleted the last sentence in the previous Abstract. (Effects of Kihi-to may be related to at least in part calpain system inhibition.)

In addition, the second sentence in Conclusion part was replaced as below.

   Effects of Kihi-to may be mediated at least in part by the calpain system inhibition.

   Dysregulation of expression levels of calpain and calpastatin by Aβ(25-35) were also attenuated by Kihi-to.

3) There are some contradictions in previous discussions as you pointed out. Especially, interpretations of in vivo data using in vitro data were little bit overstated. We reorganized logic of the story. For example, we discussed this time that inhibition of Ca\textsuperscript{2+} entry by Kihi-to will be related to neuroprotective effect of Kihi-to with new reference ([36]). Because application timing of Kihi-to was similar in both experiments (Kihi-to was applied to cells simultaneously with Aβ(25-35)). The latter half of Discussion part was rewritten and reorganized (P.23, L.5 – P.24, L.17).
Reply comments to Dr. Chandishwar Nath,

Thank you for nice and useful suggestions for improvement of contents. We considered your opinions and revised several points.

1) We previously confirmed that mice injected by $A\beta$(35-25) showed similar memory activities to saline-injected mice (see below) under same experimental conditions. At present study, we used $A\beta$(35-25) for making a control group to indicate that $A\beta$(25-35)-induced memory deficits were sequence-dependent phenomena. We added these sentences in Methods (P.9, L.3 – L.6).

2) Generally speaking, traditional medicines are sometimes used for prevention of disease as you kindly pointed out. Although we don’t deny a possibility of preventive effects of Kihi-to on dementia, we would like to verify those aspects in a separate study.