Modified Huang-Lian-Jie-Du-Tang and its combination with memantine for Alzheimer disease: an in vivo study (abridged secondary publication)

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KEY MESSAGES
1. Huang-Lian-Jie-Du-Tang (HLJDT) is composed of Rhizoma coptidis, Radix scutellariae, Cortex phellodendri, and Fructus gardenia at the ratio of 3:2:2:3. It is a famous traditional Chinese medicine. We found that HLJDT increased the amyloid-β (Aβ) load in an Alzheimer disease mouse model owing to the APP-increasing effect of baicalein, which is the main pure compound of a constituent herb, Radix scutellariae.

2. In contrast, the modified HLJDT is composed of Rhizoma coptidis, Cortex phellodendri, and Fructus gardenia in the ratio of 4:2:4, and showed both memory-enhancing effect and Aβ-reducing effects in an Alzheimer disease mouse model.

3. Upon confirming the modified HLJDT’s efficacy and safety profile in clinical trials, the modified HLJDT may be prescribed for treating Alzheimer disease.

Introduction
Alzheimer disease (AD) is a persistently progressing neurodegenerative disorder. One AD pathogenesis is the accumulation of the amyloid-β peptide (Aβ). We have identified a novel function of modified Huang-Lian-Jie-Du-Tang (HLJDT-M) in treating AD through in vitro studies. In this project, we validated the in vivo efficacy of both HLJDT and HLJDT-M in a triple transgenic mouse model of AD (3XTg-AD). We have also investigated the combinational effect of HLJDT-M with anti-AD drug memantine for the clearance of the Aβ plaques and memory improvement in a mouse model of AD. HLJDT, HLJDT-M, and HLJDT + memantine were used to treat 3XTg-AD mice for 6 months, and the memory retention and decrease in the load of Aβ plaques were evaluated.

Methods
Memory retention and Aβ plaque load were assessed by the Morris water maze and immunohistochemical analysis, respectively, as described previously. ELISA was used to measure the levels of Aβ1-40 and Aβ1-42. The underlying mechanism of the clearance of the Aβ products was explored by Western blotting analysis. Chromatographic and mass spectroscopic techniques were implemented for analysing the qualities of the extracts.

Results
Berberine was the highest content of the active component present in the aqueous extracts of the HLJDT-M, followed by geniposide and palmatine (Fig.1). During Morris water maze, the spatial reference memory significantly enhanced only after prolonged treatment of HLJDT-M, compared with vehicle or HLJDT (Fig. 2). After oral administration of HLJDT-M for 7 months, the travel distance to locate the hidden platform significantly decreased, and the spatial learning tasks significantly improved after 4 to 5 days, compared with no significant improvement in HLJDT- or vehicle-treated 3xTg-AD mice. These results suggest that spatial learning of 3XTg-AD mice was not enhanced in the HLJDT-treated group but was ameliorated in the HLJDT-M-treated group. Combination of HLJDT-M with memantine did not show further improvement in memory.

Immunohistochemical analysis showed that the load of Aβ plaques was significantly decreased in HLJDT-M-treated mice, compared with the vehicle-treated group, whereas HLJDT treatment further increased the load. These results were also confirmed in ELISA. HLJDT-M-treated mice had significantly reduced soluble levels of Aβ1-42 by 29% and insoluble levels of Aβ1-42 by 34%. In contrast, the soluble and insoluble levels of Aβ1-42 were significantly increased in the HLJDT-treated mice (Fig. 3).
FIG 1. LC-ESI-Q/TOF chromatograms of (a) Huang-Lian-Jie-Du-Tang (HLJDT) and (b) modified HLJDT. (c) Chemical structure of representative compounds of HLJDT: phellodendrine, geniposide, genipin, epiberberine, jaterorhizine, coptisine, baicalin, berbeine, palmatine, wogonoside, baicalein, and wogonin.

FIG 2. Behaviour study of 3xTg-AD mice after treatment of Huang-Lian-Jie-Du-Tang (HLJDT) and modified HLJDT (HLJDT-M): (a) and (b) results of Morris water maze test for HLJDT, HLJDT-M-, and vehicle-treated 3xTg-AD mice. After visible platform training, mice are trained with 4 trials per day for another 6 days to learn the location of a hidden platform. Each point represents the mean length values of 4 trials per day (n=10). (c) In the probe trial, the HLJDT-M-treated mice exhibit the highest duration than the vehicle-treated mice in the target quadrant in the probe trial. (d) Routes of 3xTg-AD mice in Morris water maze test.
To understand the mechanism of Aβ clearance, the sodium dodecyl-sulfate fraction of brain lysate were analysed for Fl-APP, CTFs, and pAPPThr668. Results from the Western blotting showed that the levels of APP and CTF were significantly decreased after treatment of HLJDT-M. Regarding the levels of pAPP and pCTFs, the decrease was 39% and 50%, respectively, in the 1g/kg of the HLJDT-M treatment group, and was 43% and 44%, respectively, in the 2g/kg of the HLJDT treatment group, compared with vehicle control group. In contrast, all metabolic products of APP significantly were increased to an even greater extent in 1g/kg than 2g/kg of the HLJDT treatment group.

Discussion
HLJDT-M treatment by oral gavage reduced the Aβ plaque deposition and gliosis, and improved spatial learning and memory retention deficits in 3xTg-AD mice. Further, HLJDT-M treatment significantly decreased all the APP metabolic products including Aβ. HLJDT-M has profound effect on the clearance of Aβ1-42 and on decreasing the levels of pAPP/pCTFs. These data suggest that HLJDT-M has neuroprotective effects in AD mice via decreasing all metabolites of APP and by improving the memory retention.

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Disclosure
The results of this research have been previously published in:

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FIG 3. Modified Huang-Lian-Jie-Du-Tang (HLJDT-M) reduces amyloid-β (Aβ) peptide levels in TgCRND8 mice: (a) sodium dodecyl-sulfate-soluble and (b) formic acid-soluble levels of Aβ40 and Aβ42 from the right brain hemisphere measured by sandwich ELISA. Both Aβ40 and Aβ42 levels were decreased in the brains of HLJDT-M-treated mice (P<0.01). Compared with the HLJDT-M treatment groups, HLJDT significantly increased both sodium dodecyl-sulfate and formic acid-soluble levels of Aβ40 and Aβ42.

References