Traditional herbal medicines inhibiting amyloid β protein deposition as the therapeutic and preventive strategy of Alzheimer's disease

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ABSTRACT:

The deposition of amyloid β (Aβ) protein is a consistent pathological hallmark of Alzheimer’s disease (AD) brains; therefore, inhibition of Aβ fibril formation and destabilization of pre-formed Aβ fibrils is an attractive therapeutic and preventive strategy in the development of disease-modifying drugs for AD. To find novel therapeutic and preventive drugs from traditional herbal medicines, we introduce traditional medicinal herbs inhibiting Aβ deposition, mainly on our study. *Uncaria rhynchophylla* and *Paeonia suffruticosa* not only inhibited fibril formation of Aβ but it also destabilized pre-formed Aβ fibrils in a concentration-dependent manner in vitro. These herbs also improved long-term memory impairment and inhibited the accumulation of Aβ in the brain in Tg2576 mice. Moreover, Yokukansan, a traditional herbal medicine including *Uncaria rhynchophylla*, also improved the memory disturbance and abnormal social interaction such as increased aggressive behavior and decreased social behavior in Tg2576 mice. Furthermore, 1,2,3,4,6-penta-O-galloyl-β-D-glucopyranose (PGG), an active compound of *Paeonia suffruticosa*, inhibited Aβ fibril formation and destabilized pre-formed Aβ fibrils in vitro and in vivo, as observed with *Paeonia suffruticosa*. Our results suggest that some traditional medicinal herbs and traditional herbal medicines have strong inhibitory effects on formation of Aβ fibrils in vitro and in vivo. Traditional herbal medicines are likely to be a potent and novel therapeutic agent to prevent and/or treat AD, and these active compounds as well as PGG are also likely to be a safe and promising lead compound in the development of disease-modifying drugs.

KEYWORDS:
Amyloid β protein deposition, *Uncaria rhynchophylla*, *Paeonia suffruticosa*, Yokukansan, 1,2,3,4,6-penta-O-galloyl-β-D-glucopyranose (PGG)

Inhibitory effects of amyloid β protein deposition by traditional herbal medicines

Alzheimer’s disease (AD) is the most prevalent cause of dementia, characterized by loss of memory and cognition as well as behavioral and occupational instability in
old age. One of the pathological characteristics of AD is a progressive deposition of insoluble amyloid β protein (Aβ) in the form of senile plaques. The increasing evidence that formation of these aggregates causes primary neurodegeneration in AD has led to the amyloid hypothesis, which states that the accumulation of Aβ in the central nervous system is highly neurotoxic and leads to deterioration of synaptic functions. Moreover, several findings suggest that Aβ accumulation begins at relatively early stages in AD patients. Therefore, formation, deposition, and aggregation of Aβ in the brain are primary targets for complete amelioration of dementia. Currently, drugs available for dementia such as acetylcholinesterase inhibitors exert only a temporary benefit in cognitive dysfunction, and they do not prevent or reverse the formation of Aβ deposits. We believe that the essential requirement for a truly effective anti-dementia drug would be the prevention of Aβ fibril formation or destabilization of aggregated Aβ or a combination of both.

Herbal remedies are used worldwide and have a long history of use to alleviate a variety of symptoms of many different conditions and diseases. Recently, clinical trials in patients with AD have also shown that some traditional herbal medicines called kampo improved Mini-Mental State Examination scores (Iwasaki et al., 2004) and blood flow in the cerebral cortex (Maruyama et al., 2006). In the course of our survey of truly effective anti-dementia drugs from traditional herbal medicines, much attention has been given to traditional medicinal herbs inhibiting Aβ deposition.

We referred to old Chinese literature and selected some medicinal herbs, based on the reported benefits on memory and intelligence, and several extracts showed destabilizing activity of Aβ fibrils, such as Uncaria rhynchophylla and Paeonia suffruticosa.

Uncaria rhynchophylla and Yokukansan
Concerning the inhibitory effect of Uncaria rhynchophylla on Aβ fibril formation, concentration-dependencies were examined by using the thioflavin T method. We observed that extracts of Uncaria rhynchophylla induced a concentration-dependent decline in fluorescence intensity in both Aβ1-40 and Aβ1-42. In order to determine whether these extracts have a destabilizing activity on preformed Aβ fibrils, we performed further thioflavin-T experiments. Fluorescence derived from thioflavin T was decreased dose-dependently after the addition of each extract of Uncaria rhynchophylla to preformed Aβ fibrils in a similar extent as the inhibitory effects on Aβ aggregations.

To determine the in vivo effects by Uncaria rhynchophylla and Yokukansan, a traditional herbal medicine including Uncaria rhynchophylla, memory function was examined using the passive-avoidance task followed by measurement of Aβ burden in the brains of Tg2576 mice. Step-through passive-avoidance tests were carried out on mice at 11–14 months of age. Memory retention tests were performed once a week for 78 days after the final acquisition trial. The latency time of the Tg(+) group was significantly shorter than that of the Tg(-) group, and the shorter latency was significantly prolonged by treatments with Uncaria rhynchophylla and Yokukansan.
in a dose-dependent manner. Next, to determine whether oral *Uncaria rhynchophylla* and Yokukansan treatment affected the accumulation of Aβ in cerebral cortex, the cortical Aβ1-40 and Aβ1-42 levels were measured. Large amounts of both forms of Aβ were detected in the cortex of Tg(+) mice, and both *Uncaria rhynchophylla* and Yokukansan inhibited Aβ1-42 accumulation in Tg(+) mice in a dose-dependent manner. The effects of *Uncaria rhynchophylla* and Yokukansan on aggressive behavior, social behavior, and motor activity were examined. The aggressive behavior in the Tg(+) group increased significantly more than that in the Tg(-) group. The increase was significantly inhibited by treatment with *Uncaria rhynchophylla* and Yokukansan. On the other hand, social behavior in the Tg(+) group decreased significantly more than that in the Tg(-) group. The decrease was significantly inhibited by treatment with *Uncaria rhynchophylla* and Yokukansan.

In conclusion, these studies demonstrated that *Uncaria rhynchophylla* and Yokukansan inhibited accumulation of Aβ fibrils in vitro and in vivo and improved not only memory deficits but also BPSD-like behaviors such as increased aggressive behavior and decreased social behavior in Tg2576 mice.

*Paeonia suffruticosa*

To examine the inhibitory effect of *Paeonia suffruticosa* on Aβ fibril formation, concentration-dependencies were examined by the thioflavin T method. We observed that fluorescence intensity in Aβ1–40 and Aβ1–42 declined in a concentration-dependent manner. In the analysis of fibril destabilization, fluorescence derived from thioflavin T was decreased in a dose-dependent manner after the addition of each of the extracts of *Paeonia suffruticosa* to pre-formed Aβ fibrils, and the degree of inhibition was similar to that observed on Aβ aggregation.

To determine whether oral *Paeonia suffruticosa* treatment affected memory loss, step-through passive-avoidance tests were carried out in Tg2576 mice. In retention trials, *Paeonia suffruticosa*-treated Tg mice were indistinguishable from non-transgenic littermates on days from 50 to 78 of testing. Next, we evaluated Aβ immunoreactivity in brain sections from these mice. The number of Aβ-positive spots in the cortex and hippocampus were obviously lower in the *Paeonia suffruticosa*-treated Tg2576 mice compared with the untreated Tg2576 mice. We next measured the levels of Aβ in brain tissue samples from Tg mice using a sensitive ELISA method. Consistent with the results of Aβ immunostaining, the Aβ1–42 concentration in the samples from *Paeonia suffruticosa*-treated Tg2576 mice was significantly lower than the concentration in untreated mice.

To determine the active compound of *Paeonia suffruticosa*, concentration dependence of the inhibitory effects of some compounds of *Paeonia suffruticosa* on Aβ fibril formation was examined. Only 1,2,3,4,6-penta-O-galloyl-β-D-glucopyranose (PGG) induced a concentration-dependent decline in the thioflavin T fluorescence intensity associated with Aβ1–42. To further characterize the breakdown products that accumulated in the presence of PGG, aliquots of Aβ1–42 oligomerization reaction mixtures in the presence or absence of PGG were assayed by flow cytometric analysis. Samples treated with PGG reduced the fluorescence...
intensity in a concentration-dependent manner, suggesting that PGG was a strong inhibitor of Aβ1–42 oligomerization.

To determine the effect of oral PGG treatment accumulation of Aβ in Tg type mice, we evaluated Aβ immunoreactivity in brain sections from untreated and PGG-treated mice. The number of Aβ-positive spots in the hippocampus was obviously lower in PGG-treated mice compared with untreated mice. We next measured the levels of Aβ1–40 and Aβ1–42 in brain samples from Tg mice by using a sensitive ELISA method. In the brains of Tg mice treated with PGG by repeated oral administration, the Aβ concentrations were significantly lower than those in PGG-untreated mice.

In conclusion, our study demonstrates that *Paeonia suffruticosa* and PGG not only inhibit Aβ fibril formation but also disassemble pre-formed Ab fibrils. As a result, it improved memory deficits in Tg2576 mice.

**CONCLUSION**

Traditional herbal medicines may be new class of therapeutic and preventive drugs for AD through regulation of the formation and the clearance of senile plaques.

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