CHAPTER V

DISCUSSION & CONCLUSION

The β-amyloid protein is thought to be critical in the pathogenesis of AD. The two major forms of Aβ peptide 1-40 or 1-42 have been well described as the prominent components of the senile plaques in AD. Accumulation of the peptides is associated with the progressive neuronal death, impairment of learning and memory. An intracerebroventricular injection of nanomoles doses of $A\beta_{25-35}$ (Maurice et al., 1996) or $A\beta_{1-28}$ (Flood et al., 1991, Maurice et al., 1996) impairs avoidance behavior and Y-maze alternation behavior in mice. Similarly, continuous intracerebroventricular infusion of $A\beta_{25-35}$ (Olariu et al., 2001), $A\beta_{1-40}$ (Nitta et al., 1994) and $A\beta_{1-42}$ (Yamada et al., 1999) induces learning and memory impairment in rats. The $A\beta_{25-35}$ is a subset of $A\beta_{1-42}$ located at the C-terminal end in the hydrophobic domain. This short peptide has been proposed to be a functional domain of β-amyloid responsible for its neurotoxic properties. Previous studies (Maurice, 1996; Olariu et al., 2001; Yamaguchi and Kawashima, 2001) had confirmed neurotoxic effects of $A\beta_{25-35}$ and its induction of learning and memory impairments. Therefore, in the present study, mice centrally treated with $A\beta_{25-35}$ were used as an experimental animal model of neurodegeneration for investigating potential neuroprotective effects of asiaticoside.

In this study, a single intracerebroventricular injection of 9 nmoles aggregated $A\beta_{25-35}$ caused learning and memory deficits, as assessed by spontaneous behavior in Y-maze, Morris water maze, but not in passive avoidance. On the other hand, locomotor activity in all the β-amyloid protein-injected mice did not differ from that in control mice. Furthermore, behavioral abnormalities correlated with an increase in lipid peroxidation and a decrease in total GSH content in the brain.

Spontaneous alternation behavior in the Y-maze test is a measure of the short-term memory. The spontaneous alternation may have a component of spatial working memory since the animals should recall the previous memory of explored arm in order to explore another arm in consecutive choices.

The data showed that spontaneous alternation behavior in the Y-maze task was significantly decreased in mice treated with $A\beta_{25-35}$. This implies that $A\beta_{25-35}$ may cause neuronal damages and subsequent short-term memory impairment. However, daily oral administration of asiaticoside at doses of 5-25 mg/kg/day effectively prevented the memory deficit in $A\beta_{25-35}$ treated mice. It seems unlikely that changes in the spontaneous alternation were due to changes in exploratory behavior in the Y-maze since the total number of arm entries did not significantly differ among groups. Furthermore, these results exclude a possibility of motor deficit induced by i.c.v. administration with β-amyloid. All of $A\beta_{25-35}$ treated groups displayed the same exploratory behavior in new environments (activity cage and Y-maze) as control groups. Therefore, it is suggestive that higher alternation in the Y-maze test after treatment with asiaticoside at dose 5-25 mg/kg/day may be due to the disruption of short-term memory, but not a deficit in locomotion or exploration.

On the other hand, spatial working memory was investigated in water maze by changing the platform location during training course. Hence, animals would use the visible cues to find the unfixed hidden platform in each day. The data showed that working memory in water maze task was not significantly different among groups. On one hand, this implies that administration of asiaticoside at doses of 5-25 mg/kg/day asiaticoside did not improve short-term memory. On the other hand, this may be due to interferences from previous reference memory trials. In this study, working memory test was carried out on a next day after finishing reference memory test. Therefore, it is quite possible that it was not a new learning and memory task for these mice. After repeated trials of reference memory task, mice had already recognized the position of visible cues and it was easily recalled during trials of working memory task as evidenced by short escape latencies in every treatment groups even on the first day of working memory test. To reduce interactions between two spatial memory tasks, they should be performed a few days apart in order to eliminate the recalled memory.

Effects of asiaticoside on long-term memory deficit were investigated by using reference spatial memory performance in the water maze and a multiple trial passive avoidance test. Daily oral administration of asiaticoside at doses of 5-25 mg/kg/day markedly attenuated spatial memory deficit that induced by $A\beta_{25-35}$ as considered

from decreased escape latencies in platform trials and increased time spent in the platform quadrant in probe trials. It is apparent that oral administration of asiaticoside at a dose of 50 mg/kg/day showed modest preventive effect on $A\beta_{25-35}$ induced memory deficit. This may be due to a well established dose-dependent nature of pharmacological effects. Asiaticoside at higher doses may have other, yet undefined, effects on the CNS that counteract its effect on learning and memory. In mice, optimal effective oral doses of asiaticoside in attenuating memory deficits might be in a range of 5-25 mg/kg/day.

To confirm effects of asiaticoside on long-term memory, the multiple trial passive avoidance tests were conducted. Step-through latencies were determined at 24 hr after the acquisition trial. However, this task failed to illustrate any changes in memory performance in all treatment groups. Particularly, it was not able to demonstrate beta-amyloid induced memory impairments in a passive avoidance task as shown earlier in mice (Maurice et al., 1998) and rats (Olariu et al., 2001; Yamaguchi and Kawashima, 2001). The underlying reason may be the instrumental inefficiency. The experimental setup for passive avoidance test used in this study was made by the experimenter from local materials and equipments with limited standardization and calibration. In addition, the difference between the day of testing in this study (18 days after the injection of $A\beta_{25-35}$) and that in other studies (less than 10 days after the injection of Aβ) may be responsible for this unexpected result. This discrepancy is considerable with previous findings that memory impairments on passive avoidance task disappeared 21 days after injection of $A\beta_{25-35}$ (Giovannelli et al., 1995) or 14 days after stop infusion of $A\beta_{25-35}$ (Nitta et al., 1997)

In the present study, after finishing behavior tests, the brain tissues of mice were dissected out and cerebral oxidative stress was measured. It was apparent that $A\beta_{25-35}$ might elevate oxidative stress in mouse brains due to increased levels of lipid peroxidation and decreased antioxidant GSH contents. However, $A\beta_{25-35}$ induced elevation of brain oxidative stress was effectively prevented by oral administration of asiaticoside at doses of 5-25 mg/kg/day in a parallel fashion with its preventive effect on memory deficit. These findings are consistent with the recently reported property of asiaticoside as a strong antioxidant (Abdul Hamid et al., 2002; Gupta et al., 2003; Mook-Jung et al., 1999; Shukla et al., 1999b; Veerendra Kumar and Gupta, 2002).

Therefore, it is likely that neuroprotective effects of asiaticoside against $A\beta_{25-35}$ induced oxidative stress, neuronal damages and memory impairment, may be due to, at least partly, its antioxidant property.

Although the exact mechanisms underlying neurotoxicity induced by Aβ peptide remain unclear, the evidence so far suggests that the damage described in AD brains is consistent with some degree of oxidative stress induced by the Aβ peptide. In this connection, future directions on AD treatments may be focused on the use of antioxidants or pharmacological agents that provoke enhancement of the intracellular antioxidant mechanism and neuroprotection. Among a wide variety of natural products, asiaticoside, a triterpene constituent in *Centella asiatica*, may prove to be one of potential candidates for further preclinical and clinical studies as a therapeutic supplement in the treatment of neurodegenerative disorders.

In conclusion, the present study demonstrates that oral administration of asiaticoside at a dose range of 5-25 mg/kg/day could prevent learning and memory deficits in mice that induced by intracerebroventricular injection of $A\beta_{25-35}$. This neuroprotective effect may be mediated, at least partly, by the reduction in cerebral oxidative stress conferred by the remarked antioxidant property of asiaticoside.