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Effects of baicalein on β-amyloid peptide-(25–35)-induced amnesia in mice

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Abstract

Baicalein may act on the benzodiazepine binding sites to exert an anxiolytic-like effect in mice. Since many benzodiazepine drugs have amnesic side-effect and baicalein can protect cultured cortical neurons from β-amyloid peptide-(25–35)-induced toxicity, this study examined the amnesic effect of baicalein and its effects on β-amyloid peptide-(25–35) (3 nmol/mouse, i.c.v.)-induced amnesia in mice. Using the step-through passive avoidance test, the results showed that baicalein (10–100 mg/kg, i.p.), unlike the benzodiazepine drug chlordiazepoxide (10 mg/kg, i.p.), had no significant amnesic effect. Baicalein (10–50 mg/kg, i.p.) also had no facilitating effect on the learning and memory. However, one dosage pretreatment, but not post-treatment, of baicalein (5 or 10 mg/kg, i.p.) attenuated β-amyloid peptide-(25–35)-induced amnesia. Interestingly, post-treatment for 7 or 13 days of baicalein (10–15 mg/kg/day, i.p.), like melatonin (10 mg/kg/day, i.p.), also attenuated β-amyloid peptide-(25–35)-induced amnesia. Therefore, this study demonstrated that baicalein has protective effect on β-amyloid peptide-(25–35)-induced amnesia.

Keywords: Baicalein; Melatonin; Anti-amnesic effect; β-Amyloid peptide-(25–35); Passive avoidance test

1. Introduction

Previous study using a benzodiazepine binding assay-directed separation led to the identification of three benzodiazepine binding site-interactive flavones (baicalein, oroxylin A, and skullcapflavone II with a K_i value of 13.1, 14.6, and 0.36 μM, respectively) from the water extract of Huangqin (Scutellariae radix), the dry root of *Scutellaria baicalensis* Georgi (Labiatae) (Liao et al., 1998). Further study demonstrated that baicalein (10 mg/kg, i.p.), like a typical benzodiazepine drug, chlordiazepoxide, has an anxiolytic effect in the Vogel conflict test in ICR mice (Liao et al., 2003). Since chlordiazepoxide has amnesic side-effect (Tohyama et al., 1991; Venault et al., 1986), it is interesting to examine whether baicalein has amnesic effect using the step-through passive avoidance test in mice.

It is well known that β-amyloid peptide is the major constituent of senile plaque, which is one of the pathological hallmarks of Alzheimer's disease and represents the underlying cause of the cognitive deficits observed in Alzheimer's disease (Cummings et al., 1996). It has been substantiated that the 11-amino-acid sequence (25–35) of β-amyloid peptide is neurotoxic for primary neurons (Pike et al., 1993; Yankner et al., 1990). Intracerebroventricular (i.c.v.) administration of the aggregated form of β-amyloid peptide-(25–35) significantly induces neuronal loss and amyloid deposits in brain and impaired cognitive performance in Y-maze, passive avoidance, and water maze tasks in mice (Maurice et al., 1996a). Since baicalein has been reported to protect the primary cortical neurons from β-amyloid peptide-induced neurotoxicity in vitro (Lebeau et al., 2001), it is also interesting to examine the effects of baicalein on β-amyloid peptide-induced amnesia in mice.

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It is well known that the cognitive deficits of patients with Alzheimer's disease are closely associated with dysfunction of central cholinergic neurotransmission (Mountjoy et al., 1984; Whitehouse et al., 1981). Therefore, one major current pharmacological therapy for Alzheimer's disease is to increase the level of the central cholinergic neurotransmitter, acetylcholine, with cholinesterase inhibitors (Knopman, 1998). Our previous study showed that a cholinesterase inhibitor, physostigmine, can facilitate cognitive functions in the acquisition, consolidation, and retrieval stages of learning and memory processes either in the scopolamine- or β-amyloid peptide-(25–35)-induced amnesia model in mice (Wang et al., 2001). Therefore, the present study also examined whether baicalein has such effects.

2. Materials and methods

2.1. Animals

Male Institute of Cancer Research (ICR) mice (25–30 g) were obtained from the Animal Center of National Taiwan University. They were maintained on a 12-h light and 12-h dark cycle (light on between 7:00 and 19:00) with food and tap water ad libitum. All experimental protocols in this study comply with international guidelines and were approved by Institutional Animal Care and Use Committee (IACUC) of National Yang-Ming University.

2.2. Drugs

Baicalein was purchased from Aldrich Chemical (Milwaukee, WI, USA); Tween 80, (−)-scopolamine hydrochloride, amyloid β-protein fragment 25–35 (β-amyloid peptide-(25–35)), and melatonin were purchased from Sigma (St. Louis, MO, USA); sodium chloride was obtained from ICN Biomedicals (Cleveland, USA). Baicalein was dissolved in normal saline containing 0.6% Tween 80, and (−)-scopolamine and melatonin were dissolved in twice-filtered water. These drugs were i.p. administered in a dosage of 0.1 ml per 10 g of body weight. β-Amyloid peptide-(25–35) was dissolved in sterile twice-filtered water and aggregated by incubation at 37 °C for 7 days before use.

2.3. Step-through passive avoidance test

This experiment was according to our previous established method (Wang et al., 2001). The experimental apparatus for the step-through passive avoidance test is an automated shuttle-box (Cat. 7551 Passive Avoidance Controller and Cat. 7553 Passive Avoidance Mouse Cage, UGO Basile, Italy), which is divided into an illuminated compartment and a dark compartment of the same size by a wall with a guillotine door. Each mouse was put through the adaptation trial by placing it gently in the illuminated compartment, facing away from the dark compartment. After 10 s, the door was opened and the mouse moved into the dark compartment freely. When the latency to leave the illuminated compartment was less than 30 s, the mouse was chosen for the training trial 2 h later. The training trial is similar to the adaptation trial except that the door is closed as soon as the mouse steps into the dark compartment and an inescapable foot shock (0.6 mA, 2 s) is delivered through the grid floor (Venault et al., 1986). The responses to the electric shock were observed and scored as follows: 0, no response; 1, flinch (movement of any part of the body); and 2, run (running or jumping) or vocalization (Riekkinen, 1994). The retention test was performed 24 h after the training trial in the similar manner without the electric shock and the step-through latency to the dark compartment was recorded. The maximal cut-off time for step-through latency was 300 s (Venault et al., 1986).

To examine the amnesic effect of baicalein or chlordiazepoxide, the test drug was administered 30 min before the training trial and then the retention test was performed 24 h after the training trial.

2.4. Amnesia models

Scopolamine- or β-amyloid peptide-(25–35)-induced amnesia model was used to examine the anti-amnesic effects of baicalein. In scopolamine-induced amnesia, scopolamine (1 mg/kg, i.p.) was administered 20 min prior to the training trial. In β-amyloid peptide-(25–35)-induced amnesia, the aggregated form of β-amyloid peptide-(25–35) (3 nmol) was administered i.c.v. using a microsyringe with a 28-gauge stainless-steel needle 3.0 mm long (Hamilton) according to Maurice et al. (1996a) and our previous report (Wang et al., 2001). In brief, the needle was inserted unilaterally 1 mm to the right of the midline point equidistant from each eye, at an equal distance between the eyes and the ears and perpendicular to the plane of the skull. Peptides or sterile twice-filtered water (3 μl) were delivered gradually within 3 s. Mice exhibited normal behavior within 1 min after injection. It has been shown by Maurice et al. (1996b,1998), that the scrambled β-amyloid peptide-(25–35) is without effect in mice, but decreases locomotor activity. Therefore, sterile twice-filtered water, but not scrambled β-amyloid peptide-(25–35), was used in the present study as the i.c.v. control. Neither insertion of the needle nor injection of the twice-filtered water had a significant influence on survival, behavioral responses, or cognitive function. After 14 days, the mice were put through the passive avoidance test (Maurice et al., 1996a). To examine the anti-amnesic effect of baicalein (especially in the acquisition, consolidation, and retrieval stages of learning and memory processes), baicalein was i.p. administered 30 min before or immediately after the training trial, or 30 min before the retention test (Matsuno et al., 1994). In contrast, baicalein was i.p. administered 30 min before i.c.v. treatment of β-amyloid peptide-(25–35) to examine the protective effect of baicalein.

In a previous pilot study on the progression of β-amyloid peptide-(25–35)-induced amnesia, it was found that the step-through latency in the retention test was progressively reduced when mice were trained for the step-through passive avoidance task 2, 7 or 14 days after i.c.v. treatment of β-amyloid peptide-(25–35) (data no shown). Therefore, to examine the effect of baicalein or other drug on the progression of β-amyloid peptide-(25–35)-induced amnesia, the test drug was i.p. administered once on day 3 or day 8, or once per day for 7 days (from day 2 to day 8) or 13 days (from day 2 to day 14) after i.c.v. treatment of β-amyloid peptide-(25–35) (as day 1).

2.5. Data analysis

The results of passive avoidance test were expressed as the medians and interquartile ranges and the data were analyzed by Kruskal–Wallis non-parametric one-way analysis of variance (ANOVA) and followed by Mann–Whitney rank sum test. The statistical significance level was set at $P<0.05$.

3. Results

Throughout the passive avoidance test, it was found that none of the treatments affected either the step-through latency in the adaptation and training trials or the sensitivity to electric shocks as compared with that of the control animals (data not shown).

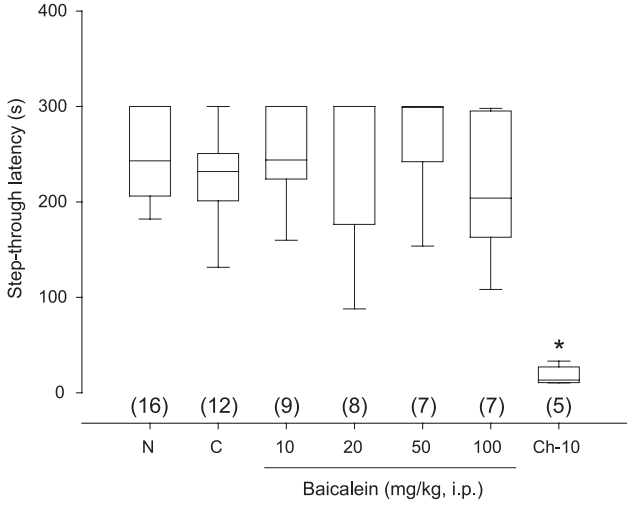

Fig. 1. Effects of baicalein and chlordiazepoxide on the step-through passive avoidance task in mice. Various doses of baicalein (10–100 mg/kg, i.p.) and chlordiazepoxide (10 mg/kg, i.p.; Ch-10) were administered 30 min before the training trail. The step-through latency was recorded in the retention test performed 24 h after the training trial. Data are expressed as medians (horizontal bar within the column), interquartile range (column), and 5th to 95th percentile range. The number of mice in each group is indicated in parentheses. N means the group of naïve mice (without any treatment), and C means the vehicle (saline containing 0.6% Tween 80) control group. *$P<0.05$, as compared with the control group (C) (Mann–Whitney rank sum test).

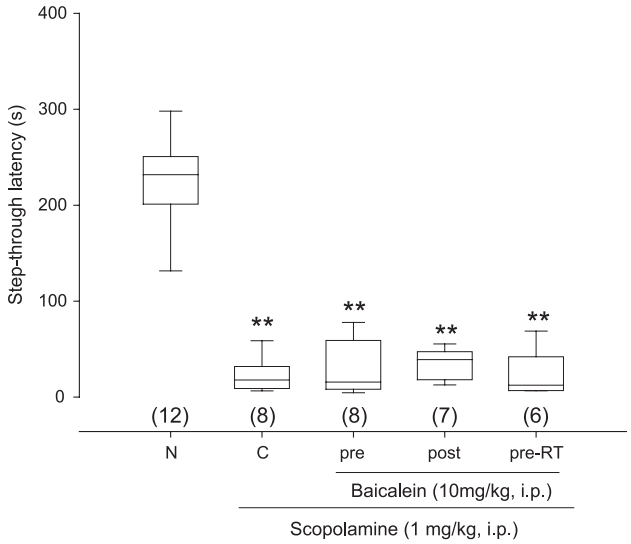

Fig. 2. Effects of baicalein on scopolamine-induced amnesia in the step-through passive avoidance test in mice. Scopolamine (1 mg/kg, i.p.) was administered 20 min before the training trial. Baicalein (10 mg/kg, i.p.) was administered 30 min before the training trial (pre), immediately after the training trial (post), or 30 min before the retention test (pre-RT). The step-through latency was recorded in the retention test performed 24 h after the training trial. Data are expressed as medians (horizontal bar within the column), interquartile range (column), and 5th to 95th percentile range. The number of mice in each group is indicated in parentheses. **$P<0.01$, as compared with the group of naïve mice (N).

3.1. Effect on the passive-avoidance test

As shown in Fig. 1, the step-through latency in the retention test was not significantly changed by baicalein treatment (10–100 mg/kg, i.p. administered 30 min before the training trial) ($H(5)=4.75$, $P>0.05$) but was markedly reduced by chlordiazepoxide treatment (10 mg/kg, i.p.) ($P<0.05$).

3.2. Effect on scopolamine-induced amnesia

As shown in Fig. 2, scopolamine (1 mg/kg, i.p.) markedly reduced the step-through latency in the retention test when administered 20 min before the training trial. Baicalein (10 mg/kg, i.p.) had no significant effects on scopolamine-induced amnesia when administered 30 min before the training trial, immediately after the training trial, or 30 min before the retention test.

3.3. Effects on β-amyloid peptide-(25–35)-induced amnesia

Fourteen days after i.c.v. administration of twice-filtered water (vehicle control) or the aggregated form of β-amyloid peptide-(25–35) (3 nmol), mice were trained for the step-through passive avoidance task. As shown in Fig. 3, the vehicle control group showed a step-through performance comparable to that of the non-treated group (i.e. naïve group), whereas the β-amyloid peptide-(25–35)-treated group showed a significant decrease in the latency (pre-training group: $H(2)=26$, $P<0.001$; post-training group: $H(2)=29.9$,

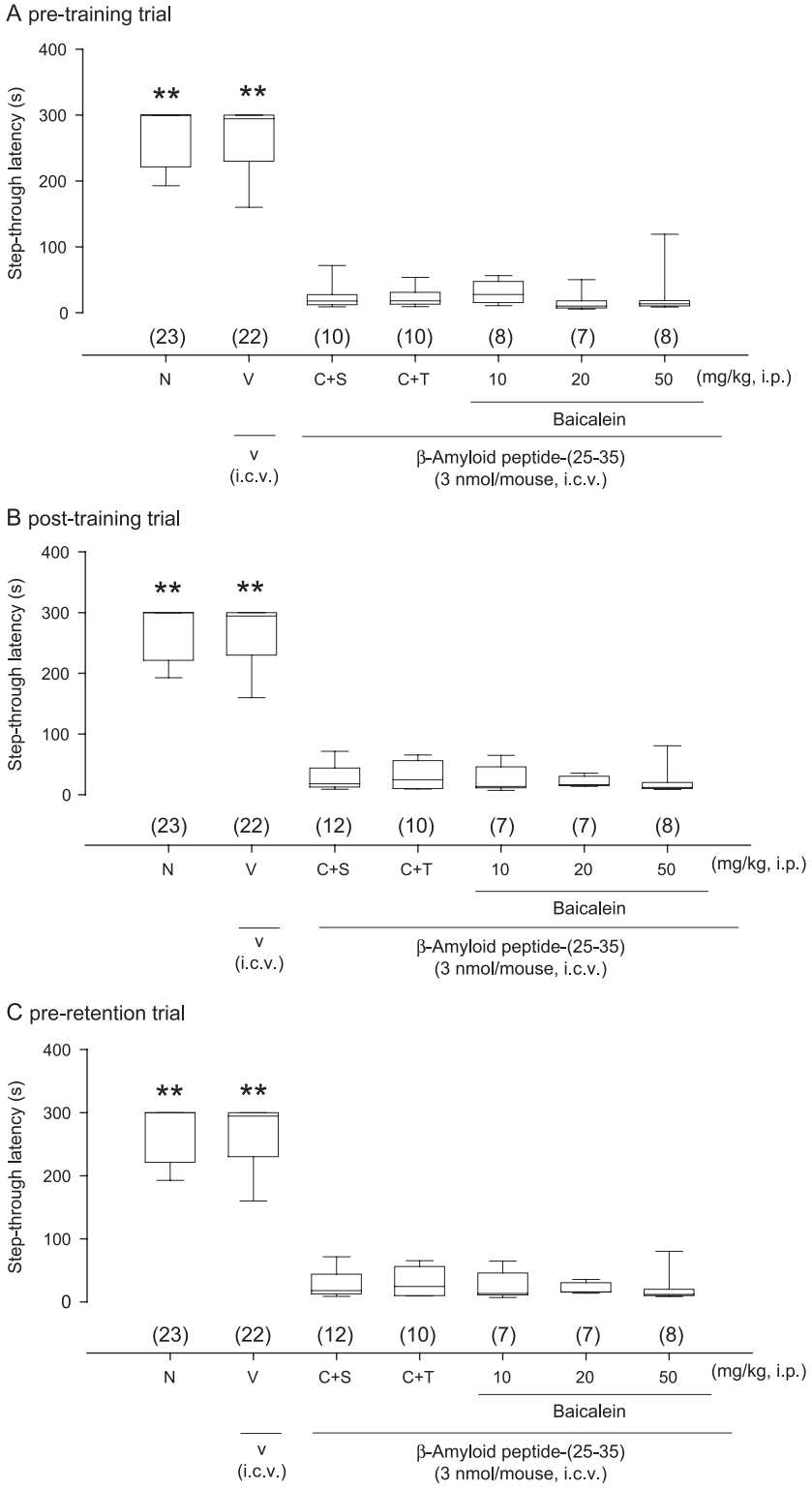

Fig. 3. Effects of baicalein on β-amyloid peptide-(25–35)-induced amnesia in the step-through passive avoidance test in mice. Fourteen days after i.c.v. administration of the vehicle (twice-filtered water, V), or the aggregated form of β-amyloid peptide-(25–35) (3 nmol), mice were subjected to the passive avoidance test. Various doses of baicalein (10–50 mg/kg, i.p.) were administered 30 min before the training trial (A), immediately after the training trial (B), or 30 min before the retention test (C). Data are expressed as medians (horizontal bar within the column), interquartile range (column), and 5th to 95th percentile range. The number of mice in each group is indicated in parentheses. $^{**}P<0.01$, as compared with the β-amyloid peptide-(25–35)-treated control group either treated with saline (C+S) or treated with saline containing 0.6% Tween 80 (C+T) (Mann–Whitney rank sum test).

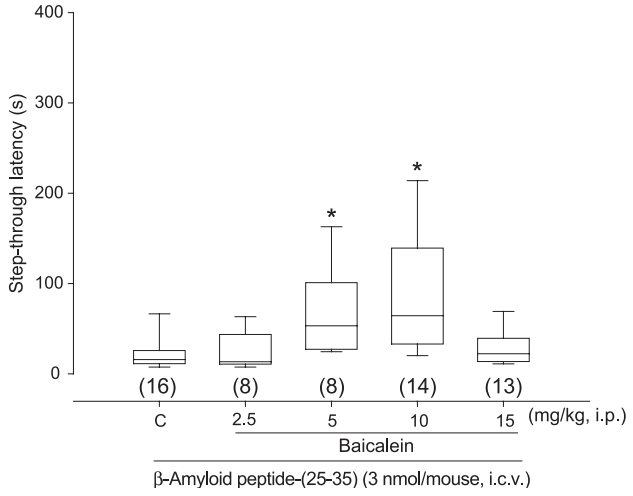

Fig. 4. Effect of baicalein pretreatment on β-amyloid peptide-(25–35)-induced amnesia in the step-through passive avoidance test in mice. Various doses of baicalein (2.5–15 mg/kg, i.p.) were administered 30 min before i.c.v. treatment of β-amyloid peptide-(25–35) (3 nmol/mouse). Mice were subjected to the step-through passive avoidance test 14 days after β-amyloid peptide-(25–35) treatment. Data are expressed as medians (horizontal bar within the column), interquartile range (column), and 5th to 95th percentile range. The number of mice in each group is indicated in parentheses. *P<0.05, as compared with the control group (C) (Mann–Whitney rank sum test).

P<0.001; pre-test group: H(2)=28, P<0.001). Baicalein (10–50 mg/kg, i.p.) had no significant effects on β-amyloid peptide-(25–35)-induced amnesia when administered 30 min before the training trial, immediately after the training trial, or 30 min before the retention test (P>0.05).

As shown in Fig. 4, baicalein attenuated β-amyloid peptide-(25–35)-induced amnesia when administered 30 min before the i.c.v. treatment of β-amyloid peptide-(25–35) (H(4)=17.5, P<0.05). The effective doses for baicalein were 5 and 10 mg/kg. At the dose of 15 mg/kg, baicalein appeared to have no beneficial effect.

3.4. Effects on the progression of β-amyloid peptide-(25–35)-induced amnesia

When mice were trained for the step-through passive avoidance task 14 days after i.c.v. treatment of β-amyloid peptide-(25–35) and the retention test was performed 24 h later, it was found that one dosage of baicalein (5–15 mg/kg, i.p.) on day 3 or day 8 had no significant effect (data not shown). However, as shown in Fig. 5, baicalein attenuated β-amyloid peptide-(25–35)-induced amnesia when administered once per day for 7 days (from day 2 to day 8) (H(3)=13.4, P<0.05) or 13 days (from day 2 to day 14)

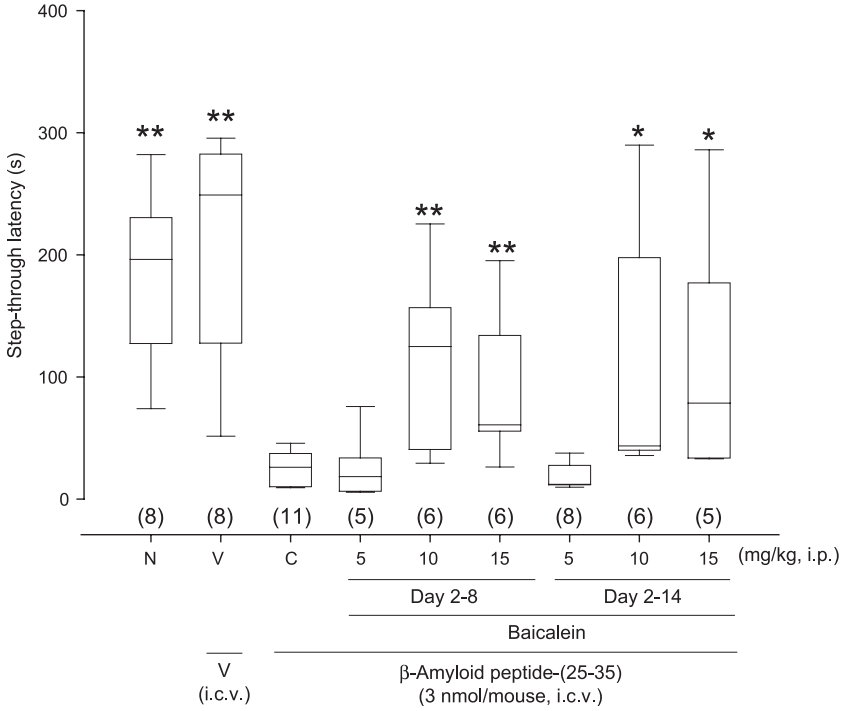

Fig. 5. Effects of multiple dosages post-treatment of baicalein on β-amyloid peptide-(25–35)-induced amnesia in the step-through passive avoidance test in mice. Various doses of baicalein (5–15 mg/kg, i.p.) were administered for 7 days (from day 2 to day 8) or 13 days (from day 2 to day 14) after i.c.v. treatment of β-amyloid peptide (25–35) (3 nmol/mouse) (as day 1). Mice were subjected to the step-through passive avoidance test 14 days after β-amyloid peptide (25–35) treatment. Data are expressed as medians (horizontal bar within the column), interquartile range (column), and 5th to 95th percentile range. The number of mice in each group is indicated in parentheses. N means the group of naïve mice (without any treatment), V means the group of i.c.v. vehicle (twice-filtered water), and C means the i.p. vehicle (saline containing 0.6% Tween 80) control group. * P<0.05, ** P<0.01, as compared with the β-amyloid peptide-(25–35)-treated control group (C) (Mann–Whitney rank sum test).

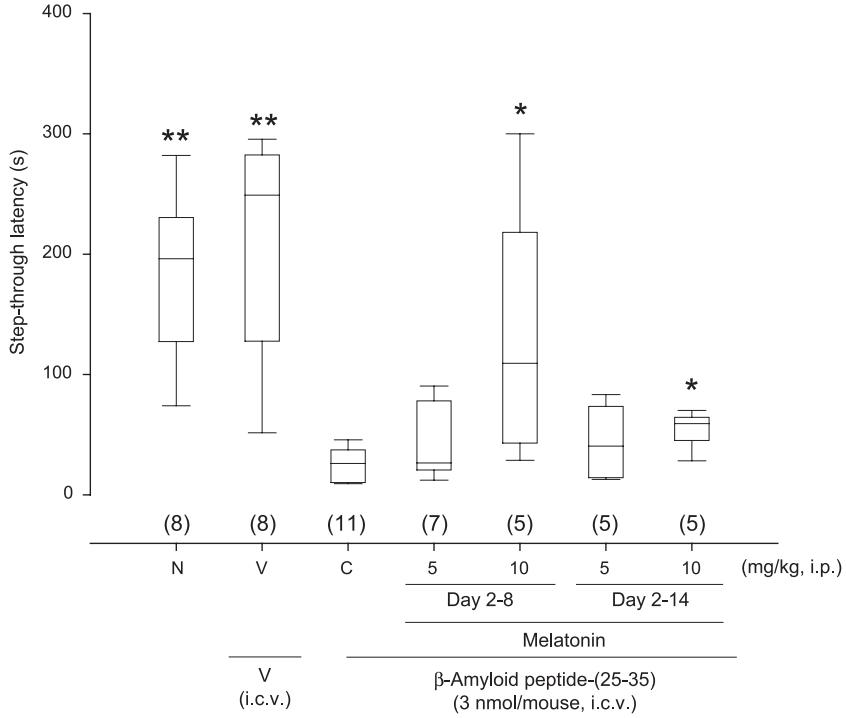

Fig. 6. Effects of multiple dosages post-treatment of melatonin on β-amyloid peptide-(25–35)-induced amnesia in the step-through passive avoidance test in mice. Various doses of melatonin (5 and 10 mg/kg, i.p.) were administered for 7 days (from day 2 to day 8) or 12 days (from day 2 to day 13) after i.c.v. treatment of β-amyloid peptide-(25–35) (3 nmol/mouse) (as day 1). Mice were subjected to the step-through passive avoidance test 14 days after β-amyloid peptide-(25–35) treatment. Data are expressed as medians (horizontal bar within the column), interquartile range (column), and 5th to 95th percentile range. The number of mice in each group is indicated in parentheses. N means the group of naïve mice (with any treatment), V means the group of i.c.v. vehicle (twice-filtered water), and C means the i.p. vehicle (saline containing 0.6% Tween 80) control group. *P<0.05, **P<0.01, as compared with the β-amyloid peptide-(25–35)-treated control group (C) (Mann–Whitney rank sum test).

(H(3)=13.7, P<0.05). The effective doses for baicalein were 10 and 15 mg/kg on each case, and the effect of baicalein for 13 days was comparable to that for 7 days. Similarly, one dosage of melatonin (5 and 10 mg/kg, i.p.) administered on day 3 or day 8 had no significant effect (data not shown) but multiple dosages of melatonin administered for 7 days (H(2)=7.77, P<0.05) or 13 days (H(2)=7.23, P<0.05) attenuated β-amyloid peptide-(25–35)-induced amnesia (Fig. 6). The effective dose for melatonin was 10 mg/kg, and the effect of melatonin for 13 days was comparable to that for 7 days.

4. Discussion

Although baicalein (10 mg/kg, i.p.) may act on the benzodiazepine binding site of gamma-aminobutyric acid $GABA_A$ receptor to exert an anxiolytic-like effect in the Vogel conflict test in mice (Liao et al., 2003), the present study demonstrated that baicalein (10–100 mg/kg, i.p.), unlike the benzodiazepine drug chlordiazepoxide (10 mg/kg, i.p.), had no amnesic effect in the step-through passive avoidance test. This finding indicated that baicalein is similar to other naturally occurring flavonoids such as chrysin and apigenin (Salgueiro et al., 1997; Viola et al., 1995; Wolfman et al., 1994), which may act on the benzodiazepine binding site of $GABA_A$ receptor to exert an anxiolytic effect with no significant amnesia effect.

In both scopolamine and β-amyloid peptide-(25–35)-induced amnesia models, the present study also demonstrated that baicalein (10 mg/kg, i.p.) had no significant effect on the impairment of learning and memory when administered 30 min before or immediately after training trial, or 30 min before the retention test. These results indicated that baicalein has no facilitating effect on the cognitive functions in the acquisition, consolidation and retrieval stages of learning and memory. However, one dosage of baicalein (5 or 10 mg/kg, i.p.) attenuated β-amyloid peptide-(25–35)-induced amnesia when administered 30 min before i.c.v. treatment of β-amyloid-(25–35). This result is consistent with its protective effect on β-amyloid peptide-(25–35)-induced neurotoxicity in vitro (Lebeau et al., 2001). However, it should be noted that higher dosage of baicalein (15 mg/kg, i.p.) may have no such protective effect.

It is well known that baicalein has antioxidant (Hamada et al., 1993; Shieh et al., 2000) and anti-inflammatory activity (Lin and Shieh, 1996; You et al., 1999). Since reactive oxidative stress and inflammatory process are involved in the progression of β-amyloid-induced amnesia (Butterfield et al., 2001; McGeer and McGeer, 2003), the present study examined whether baicalein has an effect on the progression of β-amyloid peptide-induced amnesia, in comparison with

melatonin, an effective free radical scavenger and antioxidant (Reiter, 1997; Tan et al., 1993). The results showed that one dosage of baicalein (5, 10, or 15 mg/kg, i.p.) or melatonin (5 or 10 mg/kg, i.p.) had no significant effect when i.p. administered on day 3 or day 8, but multiple dosages of baicalein (10 or 15 mg/kg) or melatonin (10 mg/kg) had attenuating effect when i.p. administered once per day for 7 days (from day 2 to day 8) or 13 days (from day 2 to day 14) after i.c.v. treatment of β-amyloid peptide-(25–35) (as day 1). Melatonin has been reported to effectively reduce lipid peroxidation and enhance the antioxidative enzyme activities in the brain tissue in β-amyloid peptide-(25–35)-treated rats, which may contribute to its attenuating effect on β-amyloid peptide-(25–35)-induced impairment of learning and memory in Morris water maze test (Shen et al., 2002). These results together suggest that baicalein may exert a similar action mechanism as that of melatonin on its protective effect on β-amyloid peptide-(25–35)-induced amnesia in mice. However, further study is needed to elucidate the detailed action mechanism for baicalein.

In summary, the present study demonstrated for the first time that baicalein has no amnesic and cognition facilitating effects, but has protective effects on β-amyloid peptide-(25–35)-induced amnesia in mice. The beneficial effect of baicalein on the progression of Alzheimer's disease-type dementia is worthy of further study.

Acknowledgements

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