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Protective effects of idebenone and α-tocopherol on β-amyloid-(1–42)-induced learning and memory deficits in rats: implication of oxidative stress in β-amyloid-induced neurotoxicity *in vivo*

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Abstract

Amyloid β-peptide (Aβ), the major constituent of the senile plaques in the brains of patients with Alzheimer's disease, is cytotoxic to neurons and has a central role in the pathogenesis of the disease. Previous studies have suggested that oxidative stress is involved in the mechanisms of Aβ-induced neurotoxicity *in vitro*. In the present study, we examined whether oxidative stress contributes to learning and memory deficits caused by continuous intracerebroventricular infusion of Aβ-(1–42). In the Aβ-(1–42)-infused rats, spontaneous alternation behaviour in a Y-maze and spatial memory in a water maze task were significantly impaired, as compared with Aβ-(40–1)-infused control rats. The retention of passive avoidance learning was also significantly impaired by treatment with Aβ-(1–42). Potent antioxidants idebenone and α-tocopherol prevented the behavioural deficits in Y-maze and water maze, but not passive avoidance, tasks in Aβ-(1–42)-infused rats when they were repeatedly administered by mouth once a day from 3 days before the start of Aβ infusion to the end of behavioural experiments. Lipid peroxide levels in the hippocampus and cerebral cortex of Aβ-(1–42)-infused rats did not differ from those in control animals, and neither idebenone nor α-tocopherol affected the lipid peroxide levels. These results suggest that treatment with antioxidants such as idebenone and α-tocopherol prevents learning and memory deficits caused by Aβ.

Introduction

Alzheimer's disease (AD) is the most common cause of progressive decline of cognitive function in aged humans, and is characterized by the presence of numerous senile plaques and neurofibrillary tangles accompanied by neuronal loss. The senile plaques are composed of amyloid β-peptide (Aβ), a 40–42 amino acid peptide fragment of the β-amyloid precursor protein (APP) (Kang *et al*., 1987; Glenner, 1988; Kitaguchi *et al*., 1988; Tanzi *et al*., 1988). Transgenic mice, which overexpress human APP containing the mutations associated with familial AD, develop many of the pathological characterizations associated with AD (Games *et al*., 1995; Johnson-Wood *et al*., 1997; Sturchler-Pierrat *et al*., 1997). Furthermore, Aβ is cytotoxic to neurons (Yankner *et al*., 1990) and renders neurons vulnerable to various insults including excitotoxicity (Koh *et al*., 1990; Mattson *et al*., 1992).

Previous studies, including those from our laboratory, have demonstrated that intracerebral infusion of Aβ causes brain dysfunctions as evidenced by neurodegeneration and an impairment of learning and memory (Kowall *et al*., 1991; Flood *et al*., 1994; Nitta *et al*., 1994a, 1997; Giovannelli *et al*., 1995; Maurice *et al*., 1996; Yamada *et al*., 1998), although neurotoxic effects of Aβ *in vivo* have been somewhat controversial (Clemens & Stephenson, 1992; Podlisny *et al*., 1993; Winkler *et al*., 1994; Fukuchi *et al*., 1996). These previous findings suggest that Aβ has a central role in the pathogenesis of AD while the molecular mechanisms other than those mediated by Aβ could be involved in neuronal death and impaired cognition in AD (Neve & Robakis, 1998).

Accumulating evidence suggests that oxidative stress is involved in the mechanism of Aβ-induced neurotoxicity (Behl *et al*., 1992, 1994; Butterfield *et al*., 1994; Schubert *et al*., 1995), and the pathogenesis of AD (Yankner, 1996; Markesbery, 1997). For instance, Aβ has been shown to increase the levels of hydrogen peroxide and lipid peroxides in cultured cells (Behl *et al*., 1994). Antioxidants such as α-tocopherol protects neurons against Aβ-induced cytotoxicity (Behl *et al*., 1992, 1994; Mattson & Goodman, 1995). In addition, an increased lipid peroxidation (Subbarao *et al*., 1990), increased carbonyl modification of protein (Smith *et al*., 1991), and increased oxidation of mitochondrial DNA (Mecocci *et al*., 1994) have been reported in the brains of AD patients. It has not yet been clarified, however, whether oxidative stress contributes to neurotoxicity caused by Aβ *in vivo*.

We have previously demonstrated that continuous infusion of Aβ-(1–40) into the cerebral ventricle in rats results in learning and memory deficits, suggesting that accumulation of Aβ is related to

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cognitive impairments in AD (Nabeshima & Nitta, 1994; Nitta *et al*., 1994a, 1997; Nabeshima & Itoh, 1997a,b). In rats treated with Aβ-(1–40), dysfunction of cholinergic and dopaminergic neuronal systems are observed as evidenced by the decrease in the nicotine- and KCl-induced stimulation of acetylcholine and dopamine release *in vivo* (Itoh *et al*., 1996). We also observed changes in ciliary neurotrophic factor levels in the brain (Yamada *et al*., 1995), activation of glial cells (Nitta *et al*., 1997) and a deficiency of long-term potentiation in the CA1 field of the hippocampus in this rat model of AD (Nabeshima & Itoh, 1997a).

To examine whether oxidative stress contributes to the Aβ-induced behavioural neurotoxicity *in vivo*, we investigated the effects of two potent antioxidants, idebenone [6-(10-hydroxydecyl)-2,3-dimethoxy-5-methyl-1,4-benzoquinone] (Suno & Nagaoka, 1984; Miyamoto & Coyle, 1990) and α-tocopherol, on Aβ-(1–42)-induced learning and memory deficits. As recent evidence suggests that Aβ-(1–42) plays a more important part than Aβ-(1–40) in the pathology of AD (Jarrett & Lansbury, 1993; Iwatsubo *et al*., 1994), rats were continuously infused with Aβ-(1–42) into the cerebral ventricle in the present study.

Materials and methods

Materials

The rats used in the present study were males of the Wistar strain (7 weeks old; Charles River Japan Inc., Yokohama, Japan) weighing 250 ± 20 g at the beginning of the experiments. They were housed in groups of two or three in a temperature- and light-controlled room (23 °C; 12-h light cycle starting at 09:00 h) and had free access to food and water, except during the behavioural experiments. All experiments were performed in accordance with the guidelines for animal experiments of the Nagoya University School of Medicine, the Japanese Pharmacological Society and the National Institutes of Health. The infusion cannula was implanted into the right ventricle (A, −0.3; L, 1.2; V, 4.5) under pentobarbital anaesthesia (50 mg/kg i.p.), according to the atlas of Paxinos & Watson (1986).

Aβ-(1–42) and Aβ-(40–1) were obtained from Bachem (Torrance, CA, USA). Idebenone was kindly provided by Takeda Chemical Industries (Osaka, Japan). α-Tocopherol was purchased from Sigma (St Louis, MO, USA). Aβ-(1–42) and (40–1) were dissolved in 35% acetonitrile containing 0.1% trifluoroacetic acid. Continuous infusion of Aβ-(1–42) (300 pmol/day) was maintained for, at least 2 weeks, by attaching an infusion cannula to a mini-osmotic pump (Alzet 2002; Alza, Palo Alto, CA, USA) (Nitta *et al*., 1994a). The infusion cannula was implanted into the right ventricle (A: – 0.3, L: 1.2, V: 4.5) according to the atlas of Paxinos & Watson (1986). The control rats were infused with Aβ-(40–1). We have confirmed that the vehicle by itself has no effect on learning behaviour at this flow rate (Nitta *et al*., 1994a, 1997).

Drug administration and experimental design

Idebenone (10 and 20 mg/kg) and α-tocopherol (150 mg/kg) were dissolved in soybean oil, and administered p.o., in a volume of 1 mL/kg, to Aβ-(1–42)-infused rats for 23 consecutive days. The vehicle-treated Aβ-(40–1)-infused control and Aβ-(1–42)-infused rats were administered with soybean oil. Each group consisted of seven to nine rats. The experimental schedule is shown in Fig. 1. The drug administration began 3 days before the start of Aβ-(1–42) infusion, and continued throughout the experimental period. The behavioural study began on day 7 after the start of Aβ-(1–42) infusion, and carried out sequentially. In the behavioural study, drug administration was carried out after the behavioural test to avoid a direct effect on performance. On day 19 after the start of Aβ infusion, rats were killed 1 h after the drug administration to measure lipid peroxide levels in the brain.

Measurement of locomotor activity

Locomotor activity was measured on day 7 after the start of Aβ infusion. The experimental apparatus consisted of a locomotor cage (25 × 42 × 20 cm), with photobeams placed 2 cm above the floor at 1-inch intervals along two sides of the cage (Colombus Instruments, USA). Locomotor activity was measured during a 10-min period (Fuji *et al*., 1993).

Y-maze task

The Y-maze task was carried out on day 8 after the start of Aβ infusion, as described previously (Yamada *et al*., 1998). The experimental apparatus consisted of a black-painted Y-maze made of plywood. Each arm of the Y-maze was 35 cm long, 25 cm high and 10 cm wide and positioned at an equal angle. Each rat was placed at the end of one arm and allowed to move freely through the maze during an 8-min session. The sequence of arm entries was recorded manually. A spontaneous alternation behaviour, which is regarded as a measure of spatial memory (Maurice *et al*., 1994; Yamada *et al*., 1996), was defined as the entry into all three arms on consecutive choices in overlapping triplet sets. The percentage spontaneous alternation behaviour was calculated as the ratio of actual to possible alternations (defined as the total number of arm entries – 2) × 100.

Water maze task

The water maze task (Morris, 1984), with some modification (Nitta *et al*., 1994a), was carried out from days 9 to 16 after the start of Aβ infusion. The experimental apparatus consisted of a circular water tank (140 cm in diameter and 45 cm high). A transparent platform (10 cm in diameter and 25 cm high) was set inside the tank, which was filled, to a height of 27 cm, with water of temperature ≈ 23 °C; the surface of the platform was 2 cm below the surface of the water. The pool was located in a large test room, in which there were many cues external to the maze (e.g. pictures, lamps, etc.): these were visible from the pool and could be used by the rats for spatial orientation. The position of the cues remained unchanged throughout the water maze task.

Reference memory test

For each training trial, the rat was put into the pool at one of five starting positions, the sequence of the positions being selected randomly. The platform was located in a constant position throughout the test period in the middle of one quadrant, equidistant from the centre and edge of the pool. In each training session, the latency to escape on to the hidden platform was recorded. If the rat found the platform, it was allowed to remain there for 15 s and was then returned to its home cage. If the rat was unable to find the platform within 90 s, the training was terminated and a maximum score of 90 s was assigned. The behavioural changes were recorded automatically using a video image motion analyser (Neuroscience Inc., Tokyo, Japan), and then path length and swim speed were analysed. Training was conducted for 5 consecutive days, twice a day, from days 9 to 13 after the start of Aβ infusion.

Probe test

Immediately after the 10th training trial on day 13 after the start of Aβ infusion, the platform was removed from the pool and animals

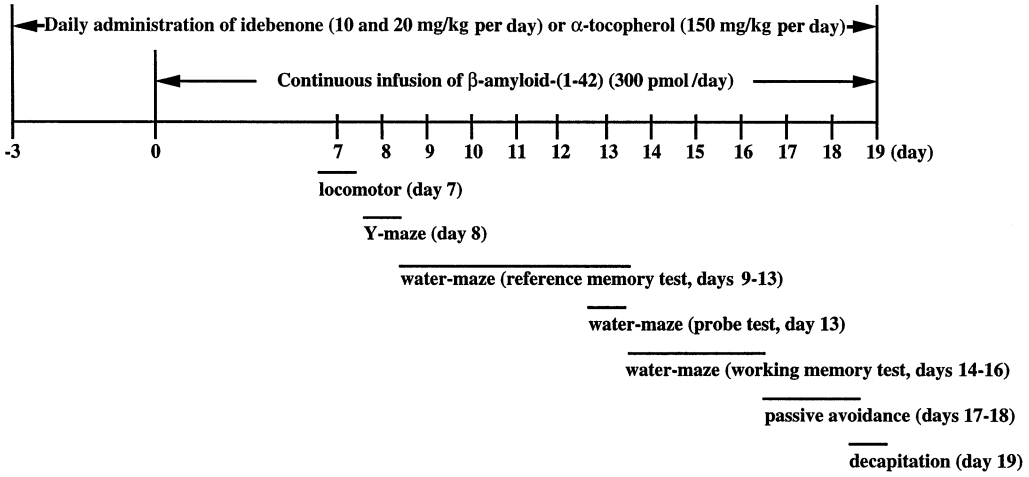

FIG. 1. Experimental schedule.

were tested on a 30 s spatial probe trial. The time spent in the platform quadrant where the platform had been located during training was measured.

Working memory (repeated acquisition) test

Working memory test was conducted for 3 consecutive days from days 14 to 16 after the start of Aβ infusion, and consisted of five trials per day (one session). The working memory test was similar, procedurally, to the standard training of water maze test except that the platform location was changed in each session. As the platform position was changed daily, this task could evaluate the working memory component (Morris *et al*., 1990; Frick *et al*., 1995). For each trial, the rat was put into the pool at one of five starting positions, the sequence of the positions being selected randomly. The first trial of each session is an informative sample trial in which the rat is allowed to swim to the platform in its new location and to remain there for 15 s The rat was then placed in a home cage for an intertrial interval of 1 min. The platform remained in the same location throughout the remaining four trials of the day. Spatial working memory was assessed as the mean performance in the second trial of 3 consecutive days from days 14 to 16 after the start of Aβ infusion.

Multiple-trial passive avoidance task

Multiple-trial passive avoidance task was carried out on days 17 and 18 after the start of Aβ infusion, as described previously (Yamada *et al*., 1996). The experimental apparatus consisted of two compartments (25 × 15 × 15 cm high), one illuminated, and one dark, both equipped with a grid floor. The two compartments were separated by a guillotine door. In the acquisition trial, each rat was placed in the illuminated compartment; when the animal entered the dark compartment, the door was closed and an inescapable footshock (0.3 mA, 5 s) was delivered through the grid floor. The rat was removed after receiving the footshock and was placed back into the light compartment by the experimenter. The door was again opened 30 s later to start the next trial. Training continued in this manner until the rat stayed in the light compartment for 120 s on a single trial. In the retention trial, given 24 h after the acquisition test, the rat was again placed in the illuminated compartment and the time until it entered the dark compartment was measured as step-through latency. When the rat did not enter for at least 300 s, a score of 300 s was assigned. The results were also expressed as the percentage of animals per group that showed a step-through latency of 300 s or more (retention percentage).

Measurement of lipid peroxide levels

The cerebral cortex and hippocampus were homogenized in 0.32 M sucrose, and the homogenate was centrifuged for 10 min at 1000 ***g*** at 4 °C. The resultant supernatant was centrifuged at 20 000 ***g*** for 20 min at 4 °C. The pellet (P2 fraction) was homogenized in 20 mM Tris–HCl buffer (pH 7.4) and used for the assay of lipid peroxide. The sample (1 mL) was incubated at 37 °C for 60 min, and the reaction was stopped by adding 100 μl of 1 M perchloric acid. The mixture was centrifuged at 20 000 ***g*** for 20 min. The lipid peroxide levels of the supernatant were measured with thiobarbituric acid and expressed as malondialdehyde (MDA) per mg protein (Ohkawa *et al*., 1979). Briefly, the supernatant (0.8 mL) was incubated with 0.2 mL of 8.1% of sodium dodecyl sulphate, 1.5 mL of 20% acetic acid (pH 3.5), and 1.5 mL of 0.8% thiobarbituric acid at 100 °C for 60 min. After cooling, the thiobarbituric acid reactive product in the sample was extracted with mixture of n-butanol/pyridine (15 : 1), and was determined spectrophotometrically at 532 nm. Protein content was determined according to the method of Lowry *et al*. (1951) utilizing bovine serum albumin as a standard.

Statistical analysis

Results were expressed as mean ± SE Statistical significance was determined by one-way analysis of variance (ANOVA) or the Kruskal–Wallis test, followed by Bonferroni's test for multigroup comparison. Two-way ANOVA was also conducted for analysing data of the water maze. Bonferroni's test for multigroup comparison was used for a *post-hoc* analysis. In the passive avoidance task, Fisher's exact probability test was used for the statistical analysis of changes in retention percentage. $P < 0.05$ was regarded as statistically significant.

Results

Effects of idebenone and α-tocopherol on locomotor activity in the Aβ-(1–42)-treated rats

Locomotor activity counts in the Aβ-(40–1)-infused control ($n = 8$) and Aβ-(1–42)-infused group ($n = 9$) were 2150 ± 440 and 2564 ± 343 counts/10 min, respectively. The activity counts in the Aβ-(1–42)-infused rats, which were repeatedly administered with

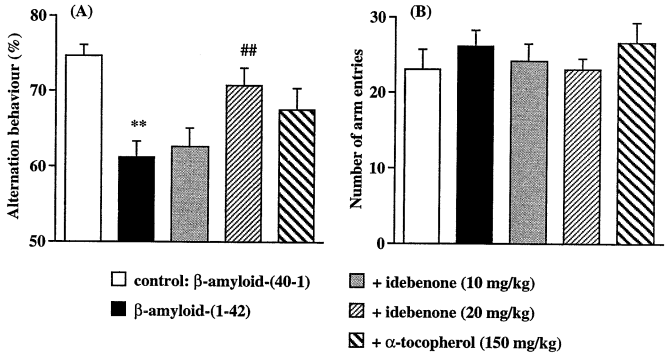

FIG. 2. Effects of idebenone and α-tocopherol on spontaneous alternation behaviour in the Aβ-(1–42)-treated rats. Spontaneous alternation behaviour (A) and the number of arm entries (B) during an 8-min session in the Y-maze task were measured on day 8 after the start of Aβ infusion. Values indicate mean ± SE (n = 7–9). There was a significant group effect on spontaneous alternation behaviour ($F_{4,36}$ = 6.001, $P < 0.001$). **$P < 0.01$ vs. Aβ-(40–1)-treated control rats. ##$P < 0.01$ vs. Aβ-(1–42)-treated rats.

idebenone at doses of 10 and 20 mg/kg, and α-tocopherol at 150 mg/kg, were 2759 ± 377, 2584 ± 362 and 3199 ± 497 counts/10 min, respectively. There was no statistical difference in locomotor activity of the five treatment groups ($F_{4,36}$ = 0.850, $P > 0.05$).

Effects of idebenone and α-tocopherol on spontaneous alternation behaviour in the Aβ-(1–42)-treated rats

There was a significant group effect on spontaneous alternation behaviour ($F_{4,36}$ = 6.001, $P < 0.001$). *Post-hoc* analysis revealed that frequency of spontaneous alternation behaviour in the Aβ-(1–42)-treated group was significantly less than that in the Aβ-(40–1)-infused control group ($P < 0.01$). Idebenone at 20 mg/kg significantly attenuated the impairment of this behaviour induced by Aβ-(1–42) ($P < 0.05$). α-Tocopherol at 150 mg/kg also increased spontaneous alternation behaviour in the Aβ-(1–42)-treated rats, but the effect was not significant. On the other hand, there was no difference in the number of arm entries of the five groups of animals (Fig. 2B) ($F_{4,36}$ = 0.462, $P > 0.05$).

Effects of idebenone and α-tocopherol on performance of the water maze task in the Aβ-(1–42)-treated rats

Changes in escape latency on to the hidden platform produced by training trials in each group of rats are shown in Fig. 3A. Two-way ANOVA with all treatment groups revealed significant main effects of group ($F_{4,360}$ = 4.835, $P < 0.001$) and training ($F_{9,360}$ = 86.354, $P < 0.0001$), but not group by trial interactions ($F_{36,360}$ = 0.898, $P > 0.05$). *Post-hoc* analysis indicated that performance in the Aβ-(1–42)-infused group was significantly impaired, compared with that in the control group ($P < 0.0001$). Repeated daily administration of idebenone at doses of both 10 and 20 mg/kg ($P < 0.01$), as well as α-tocopherol at a dose of 150 mg/kg ($P < 0.05$), significantly ameliorated the impairment of performance caused by continuous infusion of Aβ-(1–42) into the cerebral ventricle (Fig. 3A). Changes in path length produced by training trials in each group of rats showed the similar pattern with the escape latency (data not shown). There were also no significant differences in swim speed among five groups of animals during the course of the 10 training trials.

A 30-s spatial probe trial was carried out on day 13 after the start of Aβ-(1–42) infusion, following the 10th training trial, to examine whether the rats had learned the position of the platform (Fig. 3B). One-way ANOVA indicated that there was a significant group effect on the time spent in the platform quadrant where the platform had

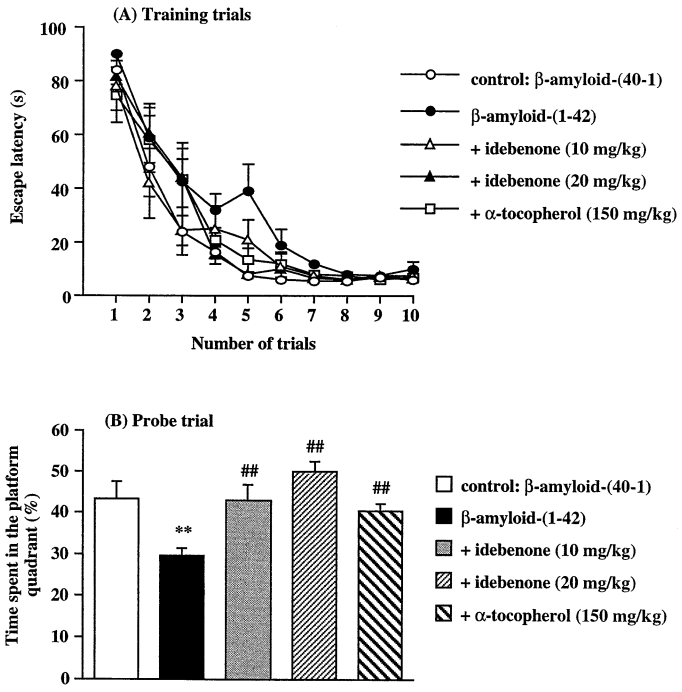

FIG. 3. Effects of idebenone and α-tocopherol on performance in the training trials (A) and in the probe trial (B) of the water maze task in the Aβ-(1–42)-treated rats. The training trials were carried out on days 9–13 after the start of Aβ infusion. The probe trial was carried out on day 13 after the start of Aβ infusion, immediately after the 10th training trial. Values indicate mean ± SE (n = 7–9). Two-way ANOVA with all treatment groups revealed that there were significant main effects of group ($F_{4,360}$ = 4.835, $P < 0.001$) and training (F_{9360} = 86.354, $P < 0.0001$), but not group by trial interactions ($F_{36,360}$ = 0.898, $P > 0.05$) in the training trials (A). **$P < 0.01$ vs. Aβ-(40–1)-treated control rats. ##$P < 0.01$ vs. Aβ-(1–42)-treated rats.

been located during training trials ($F_{4,36}$ = 6.403, $P < 0.001$). The Aβ-(1–42)-treated rats searched the platform quadrant for a significantly less amount of time than the Aβ-(40–1)-treated control rats ($P < 0.01$). Idebenone, at both 10 and 20 mg/kg, significantly reversed the decrease in time spent in the platform quadrant in the Aβ-(1–42)-infused group. α-Tocopherol also increased the time spent in the platform quadrant ($P < 0.01$).

Performance in the working memory (repeated acquisition) test is shown in Fig. 4A. There were no significant differences at all in the escape latencies of the sample trials (first trial) of 3 consecutive days among five treatment groups (KW = 2.171, P = 0.7043). Spatial working memory was assessed as the mean performance in the second trial for 3 days. As shown in Fig. 4B, a significant difference in performance among five treatment groups was observed (KW = 22.101, P = 0.0002). *Post-hoc* analysis revealed that performance in the Aβ-(1–42)-treated rats was significantly impaired as compared with the control rats ($P < 0.001$), suggesting that spatial working memory is impaired in the Aβ-(1–42)-treated rats. Idebenone at 20 mg/kg ($P < 0.05$) and α-tocopherol at 150 mg/kg ($P < 0.05$) significantly ameliorated the Aβ-(1–42)-induced impairment of working memory. Impairment of performance in the Aβ-(1–42)-treated rats was also evident in the third to fifth trials, which was significantly improved by treatment with idebenone at 20 mg/kg (fourth trial) and α-tocopherol at 150 mg/kg (third and fourth trials).

Effects of idebenone and α-tocopherol on performance of the multi-trial passive avoidance task in the Aβ-(1–42)-treated rats

In the acquisition trial, all rats of five treatment groups stayed in the light compartment for 120 s following one trial. The step-through

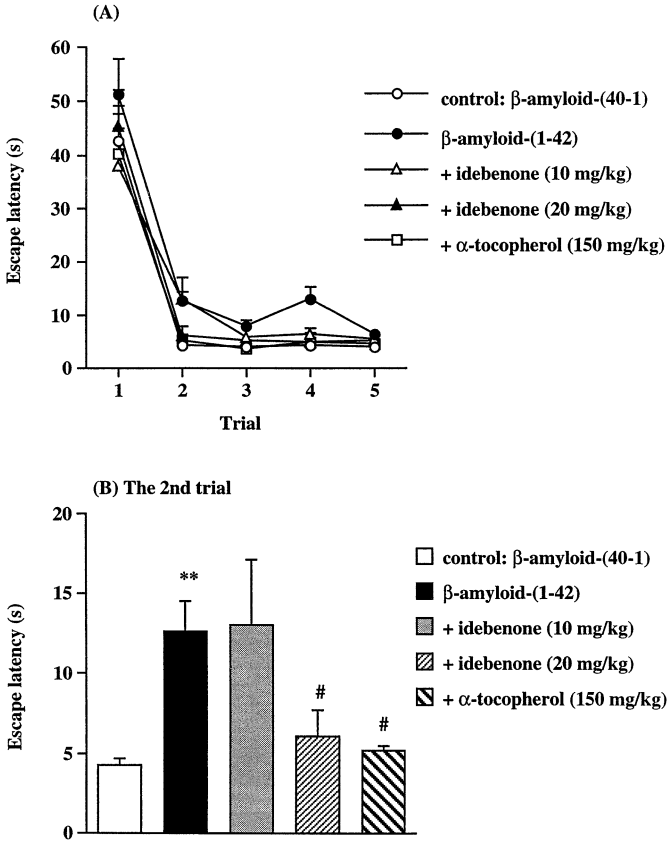

FIG. 4. Effects of idebenone and α-tocopherol on performance in the working memory test of the water maze task in the Aβ-(1–42)-treated rats. The working memory test (five trials per day) was carried out on day 14–16 after the start of Aβ infusion. Values indicate mean ± SE (n = 7–9). There were no significant differences among the five groups in the first trial. Significant differences between Aβ-(1–42) and Aβ-(40–1)-treated rats were observed in the second to fifth trials. Idebenone at 20 mg/kg significantly ameliorated performance of Aβ-(1–42)-treated rats in the second and fourth trials whereas the effects of α-tocopherol were significant in the second to fourth trials. $^{**}P < 0.01$ vs. Aβ-(40–1)-treated control rats. $^{\#}P < 0.05$ vs. Aβ-(1–42)-treated rats.

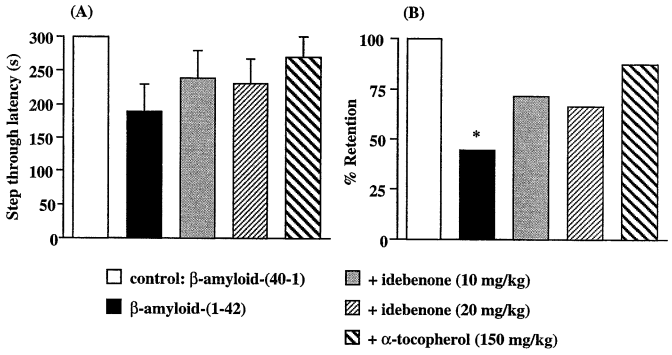

FIG. 5. Effects of idebenone and α-tocopherol on step-through latency (A) and retention percentage (B) in the retention trial of the multi-trial passive avoidance task in the Aβ-(1–42)-treated rats. The passive avoidance task was carried out on days 17 and 18 after the start of Aβ infusion. Values indicate mean ± SE (n = 7–9). Retention (%) represents the percentage of animals per group that showed a step-through latency of 300 s or more. $^{*}P < 0.05$ vs. Aβ-(40–1)-treated control rats.

latencies in the acquisition trial were not different between five groups ($F_{4,36}$ = 0423, $P > 0.05$). There was no apparent difference in vocalization of rats in each treatment group when they received a footshock. In the retention trial, all rats in the control group stayed in the light compartment for 300 s or more, thus the retention percentage was 100. The step-through latency in the Aβ-(1–42)-treated groups was 189 ± 41 s, and the retention percentage was significantly reduced to 44.4. The step-through latencies in rats which were treated with idebenone at 10 and 20 mg/kg, and α-tocopherol at 150 mg/kg were 238 ± 41, 231 ± 36 and 270 ± 30 s, respectively. The retention percentage in these treatment groups was 71.4, 66.7 and 87.5, respectively. Although there was a clear trend of improvement, these values were not statistically different [$F_{4,36}$ = 1.625, $P > 0.05$).

TABLE 1. Effects of idebenone and α-tocopherol on lipid peroxide levels in the brains of rats continuously infused with Aβ-(1–42) into the cerebral ventricle

Treatment	n	Lipid peroxide levels (as MDA nmol/mg protein) Hippocampus	Cerebral cortex
Control: β-amyloid-(40–1)	8	0.647 ± 0.019	0.525 ± 0.027
β-amyloid-(1–42)	9	0.689 ± 0.029	0.523 ± 0.014
+ idebenone (10 mg/kg)	7	0.698 ± 0.036	0.509 ± 0.011
+ idebenone (20 mg/kg)	9	0.673 ± 0.019	0.524 ± 0.027
+ α-tocopherol (150 mg/kg)	8	0.605 ± 0.030	0.469 ± 0.031

Rats were killed 1 h after the last administration of test drugs on day 19 after the start of the β-amyloid-(1–42) infusion. Control rats were infused with β-amyloid-(40–1).
Each value represents the mean ± SE.

Effects of idebenone and α-tocopherol on lipid peroxide levels in the brains of Aβ-(1–42)-treated rats

Lipid peroxide levels in the synaptosomal fraction are shown in Table 1. Continuous infusion of Aβ-(1–42) into the cerebral ventricle failed to increase lipid peroxide levels in either hippocampus or cerebral cortex when rats were killed on day 19 after the start of Aβ-(1–42) infusion. Idebenone at doses of 10 and 20 mg/kg had no effect on lipid peroxide levels in the Aβ-(1–42)-treated rats. α-Tocopherol at 150 mg/kg reduced the lipid peroxide levels in the hippocampus and cerebral cortex of Aβ-(1–42)-treated rats, although the effect was not significant.

Discussion

In the present study, we observed that continuous infusion of Aβ-(1–42) into the cerebral ventricle caused an impairment of spontaneous alternation behaviour in a Y-maze and performance in a water maze task. Retention of long-term memory in a passive avoidance task was also significantly impaired by the continuous infusion of Aβ-(1–42), although the deficits were relatively small. These results are, in general, consistent with our previous reports (Nitta *et al*., 1994a, 1997), in which Aβ-(1–40) was continuously infused into the cerebral ventricle. As in these three behavioural tasks, different motivations are involved and different skills are required for better performance, it is unlikely that the impairment of performance of the Aβ-(1–42)-treated rats in these tasks is due to changes in motivation or sensorimotor function. Actually, the locomotor activity and the number of total arm entries in the Y-maze task in the Aβ-(1–42)-treated rats did not differ from those in the Aβ-(40–1)-treated control rats. Furthermore, we have previously observed that there are no changes in ambulation, rearing, grooming and the latency to start exploration of a novel field in an open-field test, and the head-dipping time in a hole-board test (K. Nishimura, K. Yamada and T. Nabeshima;

unpublished observation). These results suggest that continuous intracerebroventricular (i.c.v.) infusion of Aβ-(1–42) has no effect on motor function and exploratory activity. There were also no apparent differences between the Aβ-(1–42)-treated and control rats in vocalization when the animals received an electric footshock in the acquisition trial of the passive avoidance task. Neither the escape latency on to the submerged platform in the first training trial nor the swim speed during the training trials of water maze task were affected by continuous i.c.v. infusion of Aβ-(1–42). The results suggest that there are no major changes in shock sensitivity and swimming ability. Taken together, it is likely that impairment of performance in the Aβ-(1–42)-treated rats is due to learning and memory deficits.

We studied the effect of Aβ-(1–42) on spatial memory in detail, by dividing it into two memory categories, spatial reference and working memory. The standard water maze task, in which a rat is required to locate a submerged platform, measures predominantly spatial reference memory. Reference memory refers to memory for information that remains constant over repeated trials and is therefore, trial independent (Olton *et al*., 1979). Reference memory is required to learn the general rules of the task (e.g. swim to a platform). The present and our previous studies (Nitta *et al*., 1994a, 1997) suggest that continuous infusion of Aβ-(1–42) or Aβ-(1–40) impairs spatial reference memory formation in the standard water maze training. The probe test, in which performance is largely independent of swimming ability and speed, requires memory for the precise location of the platform, so it more reliably assessed the accuracy of spatial reference memory than the standard training. In the probe test, we observed a clear deficit in the Aβ-(1–42)-treated rats compared with Aβ-(40–1)-treated animals, providing an additional evidence that continuous i.c.v. infusion of Aβ-(1–42) results in an impairment of spatial reference memory. Acute i.c.v. injection of Aβ-(25–35), the biologically active neurotoxic fragment of Aβ (Yankner *et al*., 1990), in mice is also reported to impair spatial reference memory in a water maze task (Maurice *et al*., 1996). These results suggest that accumulation of neurotoxic Aβ fragments such as Aβ-(1–42), Aβ-(1–40) and Aβ-(25–35) causes an impairment of spatial reference memory, although non-toxic Aβ fragment, Aβ-(40–1), has no effect.

Working memory refers to memory in which the information to be remembered changes in repeated trials (Olton *et al*., 1979). Thus, working memory is trial dependent, and can be assessed in versions of water maze task which require learning rapidly changing information. In the present study, by repeated acquisition test of the water maze task (Frick *et al*., 1995), we demonstrated for the first time that accumulation of neurotoxic Aβ-(1–42), but not Aβ-(40–1), caused an impairment of spatial working memory, as evidenced by an increase in the escape latency in the second trial. It is unlikely that the poor performance of the Aβ-(1–42)-treated rats in the working memory test is due to deficit of procedural (reference) memory, as the escape latency in the sample trial (first trial) in each session did not differ at all between the Aβ-(1–42)-treated and the Aβ-(40–1)-treated control groups. To confirm the disrupting effects of Aβ-(1–42) on spatial reference and working memory, further experiments, using a radial arm maze task by which these two memory categories can be measured separately, should be carried out (Olton *et al*., 1979; Jarrard *et al*., 1984; Diamond *et al*., 1996; Zou *et al*., 1998).

Stress can impair water maze learning (Selden *et al*., 1990; Brucato *et al*., 1996). In this regard, it is important to note that change in the passive avoidance learning in Aβ-(1–42)-treated rats was relatively small, although marked deficits of performance in the Y-maze and water maze tasks were observed. Accordingly, as there are some differences in the effects of Aβ-(1–42) between two stressful tasks (water maze and passive avoidance tasks), it is unlikely that spatial memory deficits in the Aβ-(1–42)-treated rats are due to a difference in fear conditioning and/or stress levels.

Idebenone is a quinone derivative that has antioxidant properties *in vitro* (Suno & Nagaoka, 1984), and protects against glutamate receptor agonist-induced excitotoxicity *in vitro* (Miyamoto *et al*., 1989) and *in vivo* (Miyamoto & Coyle, 1990), in which oxidative stress is involved. α-Tocopherol is the most prevalent and efficacious lipid-soluble antioxidant in biological systems, and inhibits the chain reaction of lipid peroxidation by trapping the chain carrying peroxyl radicals (Niki *et al*., 1991). Previous studies *in vitro* have shown that α-tocopherol inhibits Aβ-induced neuronal cell death and lipid peroxidation (Behl *et al*., 1992, 1994; Schubert *et al*., 1995; Mark *et al*., 1997), suggesting that oxidative stress is one of the mechanisms of the neurotoxicity of Aβ. In the present study, we found that repeated administration of idebenone and α-tocopherol ameliorated learning and memory deficits in the Aβ-(1–42)-infused rats.

There are several mechanisms that might contribute to the apparent neuroprotective effects of idebenone and α-tocopherol against the Aβ-(1–42)-induced memory deficits. First, it is plausible that oxidative stress contributes to learning and memory deficits caused by Aβ-(1–42). However, we failed to demonstrate an increase in lipid peroxide levels in the brains of the Aβ-(1–42)-infused rats on day 19 after the start of Aβ-(1–42) infusion. Therefore, one might be opposed to the hypothesis that oxidative stress is involved in learning and memory deficits caused by Aβ-(1–42). One possible explanation for the discrepancy between behavioural and neurochemical examinations is that lipid peroxidation caused by Aβ-(1–42) may be transient, and/or occur locally in the brain. Therefore, we could not detect the increase on day 19 after the start of Aβ-(1–42) infusion. Other markers of oxidative damage not evaluated in the present study, such as protein carbonyl content, could be affected to a greater extent than lipid peroxidation (Carney *et al*., 1991).

Other mechanisms than antioxidant effects could be related to the neuroprotective effects of idebenone and α-tocopherol as no changes in lipid peroxide levels were observed in the brains of Aβ-(1–42)-infused rats. For instance, idebenone has been reported to stimulate nerve growth factor (NGF) synthesis and secretion in quiescent astroglial cells (Takeuchi *et al*., 1990). Our laboratory has previously demonstrated that idebenone increases NGF content in the brains of aged rats (Nitta *et al*., 1993), and its mRNA and protein levels in basal forebrain-lesioned rats (Nitta *et al*., 1994b). We consider that idebenone at doses of 10 and 20 mg/kg acts as an orally active NGF synthesis stimulator (see for review, Yamada *et al*., 1997). Accordingly, the protective effects of idebenone against Aβ-(1–42)-induced learning and memory deficits may be not only due to its antioxidant effect, but also the increasing effect of NGF levels in the brain, as another orally active NGF synthesis stimulator, propentofylline [3,7-dihydro-3-methyl-1-(5-oxohexyl)-7-propyl-1H-purine-2,6-dione] (Yamada *et al*., 1997), also prevents Aβ-induced learning and memory deficits (Yamada *et al*., 1998). In our knowledge, α-tocopherol does not have such neurotrophic effects, and thus we believe that neuroprotective effects of α-tocopherol in the Aβ-(1–42)-infused rats is mediated through its antioxidant effect.

Aside from the mechanisms of the ameliorating effects of idebenone and α-tocopherol on Aβ-(1–42)-induced learning and memory impairment, it is not clear in the present study whether the effects of antioxidants depends on the continuous infusion of Aβ-(1–42). It has been previously reported that idebenone ameliorates learning impairment in rats with cerebral hypoperfusion induced by permanent internal carotid ligation (Ohta *et al*., 1997) and in aged rats (Pelleymounter & Cullen, 1993). α-Tocopherol is also reported to prevent spatial learning deficit induced by i.c.v. injection of the cholinotoxin

ethylcholine mustard aziridinium ion (AF64A) without affecting the memory in control animals (Wörtwein *et al*., 1994). Although the doses of drugs, and the duration and route of administration, as well as age and species of the experimental animals are different among the studies, it appears that idebenone and α-tocopherol ameliorate learning and memory deficits under the conditions where oxidative stress is involved in the pathophysiology.

In conclusion, we have demonstrated that the repeated administration of antioxidants, idebenone and α-tocopherol, prevents learning and memory deficits in rats continuously infused with Aβ-(1–42) into the cerebral ventricle. However, there were no changes in lipid peroxide levels in the brains of the Aβ-(1–42)-infused rats when assayed after behavioural experiments. Therefore, although our behavioural results are consistent with the reported protective effects by α-tocopherol and other antioxidants on Aβ-(1–42)-induced neurotoxicity *in vitro*, further studies should be carried out to clarify whether oxidative stress contributes to learning and memory deficits caused by Aβ-(1–42). We consider that clinical trials of antioxidants such as idebenone for the treatment of Alzheimer's disease are warranted.

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Abbreviations

AD, Alzheimer's disease; APP, β-amyloid precursor protein; Aβ, αmyloid β-peptide; MDA, malondialdehyde; NGF, nerve growth factor.

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