Effect of Vitamin E on Learning and Memory Deficit in Aged Rats

Hirokatsu Takatsu¹, Kei Owada¹, Kouichi Abe², Masahiro Nakano³ and Shiro Urano⁴,*

¹Division of Biological Chemistry, Shibaura Institute of Technology, 3–7–5 Tatsuso, Koto-ku, Tokyo 135–8548, Japan
²Vitamin E Information and Technology Section, Eisai Co. Ltd., Bunkyo-ku, Tokyo 112–8088, Japan
³Mitsubishi Gas Chemical Co. Inc., Kita-ku, Niigata 950–3112, Japan
⁴Division of Biochemistry, Shibaura Institute of Technology, Minuma-ku, Saitama 337–8570, Japan

(Received April 6, 2009)

Summary In order to verify whether vitamin E improves the cognitive impairment induced through aging, aged rats fed a vitamin E-supplemented diet had their learning and memory functions assessed in comparison with the aged rats fed a normal diet using a Morris water maze test. Although normal aged rats showed very poor learning ability concerning the place of a platform in the water maze apparatus, the aged rats fed the vitamin E-supplemented diet learned the place with a marked speed in only 5 trials. After old animals showed the maximum learning ability, they were kept in a normal atmosphere for 48 h without a trial followed by an assessment of their memory function using the same apparatus. The vitamin E-supplementation to aged rats resulted in marked retention of their maximum memory function, although normal aged rats showed a significant memory loss of about 60%. Pyrroloquinoline quinone (PQQ), which increases in the production of nerve growth factor, and protects neurons, had a similar effect on cognitive function to that of vitamin E in the aged rats. These results suggest that vitamin E may improve cognitive deficit caused through aging by not only its neuro-protecting effect but an antioxidant efficacy.

Key Words vitamin E, cognitive deficit, learning, memory, aged rat

Although it has been recognized that normal aging is accompanied by declines in cognitive performance, the precise mechanisms leading to this deficit during aging are not well understood. It is evident that these declines arise from neurodegeneration through several factors during aging such as stroke, cerebral infarction and oxidative stress. There is substantial notion that oxidative stress is relevant to the aging process. Oxidative stress occurs at the time of an imbalance between reactive oxygen species (ROS) generation and its detoxification by antioxidants in living tissues, so that aging is considered to be accumulation of oxidative damage of living tissues through oxidative stress experienced over a long period of time (1, 2).

Among organs in living tissues, neurons in the brain are considered to be more vulnerable to oxidative stress than other organs, leading to neuronal oxidative damage, and neurodegenerative disorders such as Alzheimer’s disease (AD), Parkinsonism and senile dementia (3). It is well-characterized that there are increased regional levels of oxidative stress in the AD brain, and hence recent studies have demonstrated a decrease in polyunsaturated fatty acids, increased levels of lipid peroxidation markers, protein oxidation, and DNA and RNA oxidation in AD (4). Based on the oxidative stress theory of brain aging, our previous study revealed that the levels of thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides, F2-isoprostane and conjugated dienes increase significantly with oxidative stress in the rat brain, and the activity of antioxidative enzymes and vitamin E content in the brain decrease markedly (5–7). Furthermore, in accordance with these phenomena, young rats subjected to oxidative stress showed marked deficit in learning and memory functions. The delayed-type apoptosis of pyramidal cells and the accumulation of amyloid-β-like substances in the hippocampus of young rats were induced by oxidative stress (7–9). These abnormalities were also observed in both normal old rats and vitamin E-deficient rats not subjected to oxidative stress. It is, therefore, reasonable to suggest that an impairment in cognitive function caused by oxidative stress during aging and in neurodegenerative disease results from oxidative neuronal death.

It has been reported that either vitamin E or pyrroloquinoline quinone (PQQ) prevents such oxidative damage in the brain and cognitive deficit caused by oxidative stress in rats during aging (9, 10). Since both compounds have an antioxidant effect, it is implied that the improvement of such abnormalities by these compounds results from their antioxidant efficacy. However, it is known that vitamin E and PQQ protect neuron by other effects rather than antioxidant. In particular, PQQ increases the production of nerve growth factor (NGF), and protects the N-methyl-d-aspartate (NMDA) receptor, so that it is still unknown from which the effects of both compounds on cognitive deficit result.

*To whom correspondence should be addressed. E-mail: urano@sic.shibaura-it.ac.jp
The brain of aged rats is considered to be more oxidatively damaged already than that of young rats. Consequently, it can be presumed that vitamin E may be ineffective as an antioxidant on the improvement of the cognitive deficit of old rats, even when they fed the vitamin E-supplemented diet. In a positive case, it is reasonable to consider that vitamin E improves the cognitive impairment during brain aging by a different effect from antioxidant. In order to test this notion, here, we examined the effects of vitamin E on the cognitive deficit of old rats in comparison with that of PQQ using a Morris water maze test.

MATERIALS AND METHODS

Animals. All animal experiments were performed with the approval of the Animal Protection and Ethics Committee of the Shibaura Institute of Technology. Young male Wistar rats (age, 3 mo; Japan SLC, Inc., Hamamatsu, Japan), aged male Wistar rats (age, 25 mo; obtained from Tokyo Metropolitan Institute of Gerontology), rats fed a vitamin E- or PQQ-supplemented diet (young; age, 3 mo, fed 200 mg of R.R.R-α-tocopherol or 20 mg of PQQ/kg·body weight/d for 8 wk from 4 wk of age; old; age, 25 mo, fed the same diets for 2 wk from 98 wk of age) were used in this study. In order to assess the effect of oxidative stress on cognitive function, young rats were subjected to hyperoxia as oxidative stress in a 100% oxygen chamber at room temperature for 48 h, as described previously (9). Aged rats were kept in a normal atmosphere for 48 h before the behavioral test.

Chemicals. R.R.R-α-tocopherol and PQQ were kindly supplied from Eisai Company Ltd. (Tokyo, Japan) and Mitsubishi Gas Chemical Company, Inc. (Tokyo, Japan), respectively.

Behavioral testing. The learning ability and memory function of the rats were tested using a Morris water maze apparatus (140 cm in diameter and 45 cm in height) (11). The bottom of the pool was divided into quadrants using white lines, and the transparent platform was submerged 2 cm below the surface of the water at the center of one of the quadrants; the water was maintained at 21±1°C. For pre-training, the rats were allowed to swim freely in the pool for 120 s without the platform. Daily training consisted of one trial in which the rats swam from the start point to a fixed goal; this was conducted for 15 consecutive days. Goal time and swimming distance from the start point to the platform were measured, and the rates of decreases in swimming time and distance from their values in the first trial were expressed as learning ability. The swimming distance was measured by tracing the tracks of swimming.

After all the groups had learned the task completely, the control young rats and young rats fed the vitamin E- or PQQ-supplemented diet were kept in 100% oxygen atmosphere as oxidative stress at 21±1°C for 48 h in an oxygen chamber. Aged rats were kept in a normal atmosphere at 21±1°C for 48 h before the behavioral test. The platform was removed, and the rats were placed opposite the quadrant where the platform had been located. The percentage of time spent in the quadrant where the platform had been was used as an assessment of memory retention.

Statistical analysis. Results are presented as means±SE. All data were analyzed with Student’s test, one-way ANOVA followed by a Dunnett’s t test. The experimental data were considered to be statistically different when p-values were less than 0.05.

RESULTS

Learning ability
As shown in Fig. 1a, although the young rats learned the location of the platform after only five trials, the aged rats needed 15 trials to recognize the place, as in our previous report (9). Furthermore, since the rate of decrease in swimming time of the aged rats from the start point to the platform did not change after 15 trials, their maximum learning ability was lower than that of the young rats by about 35%. The learning ability of the young rats fed the vitamin E-supplemented diet was similar to that of the young control, so that
vitamin E supplementation did not improve the learning function of the young control. These results suggest that learning ability using space cognition in a water maze test decreases with age, and that vitamin E does not improve the ability of the young rats.

Interestingly, when aged rats were fed vitamin E for only 2 wk before the start of the trial, their learning ability was enhanced markedly (Fig. 1b). Considering that nerves in the brain of aged rats are damaged oxidatively by oxidative stress experienced for a long period of time, it is difficult to consider that vitamin E improves the cognitive deficit of aged rats through the prevention of oxidative brain damage by its antioxidant effect. In order to verify this notion, the efficacy of vitamin E was compared with that of PQQ, which increases the production of the nerve growth factor, and protects neurons. As shown in Fig. 1b, PQQ had a similar effect to vitamin E on enhancement of learning ability in the aged rats.

Memory retention

After the rats learned the location of the platform in the pool, the effect of vitamin E on memory retention of the rats was assessed in comparison with that of PQQ. On the basis of the oxidative stress theory of brain aging, when young rats were subjected to hyperoxia as oxidative stress for 48 h, their memory suddenly declined 5 d after the stress. Aged rats kept under normal atmosphere for 48 h showed a marked decline of memory retention. In contrast, the memory function of the vitamin E-supplemented young rats was retained significantly even after the oxidative stress, although its efficacy decreased at a point of 9 trials by about 35% (Fig. 2a). As shown in Fig. 2b, aged rats fed the vitamin E-supplemented diet showed a marked improvement over the memory deficit observed in the aged control. It was found that the rate of memory retention of vitamin E-supplemented aged rats was significantly high in comparison with vitamin E-supplemented young rats subjected to oxidative stress. When aged rats were fed the PQQ-supplemented diet, their memory function was also increased markedly. The effect of PQQ on the improvement of memory deficit was similar to that of vitamin E in the aged rats.

DISCUSSION

There is a theory based on the notion that most changes during aging arise from free radical reactions and the formation of lipid peroxides in tissues, leading to age-related damage and eventually to various aging processes and phenomena (1, 2, 12). In case of the brain damage caused by ROS induced through oxidative stress during aging, it is evident that nervous systems in the brain are injured oxidatively, and hence cognitive deficit may be induced by dysfunction in neurotransmission. Thus, Harman proposed that one of the pathogenesis of dementia including Alzheimer’s disease is ROS generated by oxidative stress (13). In fact, up to the present day, it has been recognized that patients with Alzheimer’s disease are typically subjected to oxidative stress. On the basis of this consideration, with accumulating research on the development of substances which may improve cognitive function in aging, pharmacological and nutritional interest has been focused on an antioxidant treatment. The effect of antioxidants and antioxidant-rich extracts from natural products such as vitamin E (9), coenzyme Q10 (CoQ10) (14), vitamin C and β-carotene (15), lipoic acid (16), melatonin (17), ginkgo biloba (18), apple juice (19), cocoa (20), and green tea (21), on cognitive deficit has been widely investigated. Although there is still no clear explanation...
of the range and nature of their potential effect, antioxidants have been used in the treatment of neurodegenerative disease. In fact, it has been revealed that long-term high-dose vitamin E supplementation in the elderly significantly enhances cognitive function (22, 23). Furthermore, a clinical trial on vitamin E supplementation in patients with moderately severe Alzheimer’s disease showed delays in institutionalization and the onset of severe dementia (24). Thus, it has been believed that antioxidants prevent or improve cognitive impairment through protection of neurons against ROS, although there was no obvious evidence. Previously, it has been suggested that although the significance of vitamin E has been subsequently proven as a radical chain-breaking antioxidant that can protect the integrity of tissues, vitamin E has been found to possess functions that are independent of its antioxidant/radical scavenging ability (25). For example, Sen et al. discovered using a cell culture system that tocotrienol protects against neuronal cell death by an antioxidant-independent mechanism through inhibition of c-Src kinase activation and phosphorylation of ERK (26), although in vivo study has not been reported yet.

In this study, vitamin E and PQQ improved deficit of learning ability and memory retention in aged rats (Figs. 1 and 2). It is evident that neuronal cells in the aged rat brain are more oxidatively damaged than those of young rats, because aged rats were subjected to oxidative stress over a long period of time. Consequently, it is difficult to consider that since aged rats fed vitamin E and PQQ for only 2 wk, the improvement in impaired cognition arose from only their antioxidant properties. This notion is supported by the findings in this study that although young rats fed the vitamin E-supplemented diet retained their memory even when they were subjected to oxidative stress, the efficacy decreased gradually after five trials (Fig. 2a), and that aged rats fed the vitamin E-supplemented diet showed significant memory retention in comparison with the young rats fed the vitamin E-supplemented diet for 8 wk (Fig. 2b). Furthermore, it is also supported by the fact that the learning ability of aged rats was increased markedly by vitamin E and PQQ, although young rats fed the vitamin E-supplemented diet did not show an improvement in their ability (Fig. 1a and b). Since neuronal cells of young control rats were not damaged oxidatively compared to those of the aged rats, the learning ability of young rats maintained normal function (5, 9), and hence, it seems likely that vitamin E was not necessarily needed to improve the ability of the young rats. This notion is also supported by the previous findings that the content of TBARS increased, and docosahexaenoic acid and vitamin E content decreased with age in the rat brain synapse, and that the cholesterol/phospholipids ratio and the fluidity of synaptic plasma membranes in the brain increased markedly with age (27). Thus, it is obvious that oxidative neuronal damage in the brain increased with age, resulting in the dysfunction of cognition in the aged rats. In the face of such brain damage in the aged rats, it is a wonder that vitamin E improved their cognitive deficit (Figs. 1b and 2b). This phenomenon implies that vitamin E has a different effect from the antioxidant effect.

Although it has been recognized that PQQ acts as an antioxidant, this compound increases the production of NGF, and protects the NMDA receptor. Consequently, since PQQ revealed a similar effect to vitamin E on the improvement of cognitive deficit in aged rats in this study, it is reasonable to consider that the beneficial effect of vitamin E may also be caused by such a non-antioxidant effect.

In conclusion, these results suggest that not only neuronal cells in aged rats were protected against further oxidative damage by vitamin E, but also this efficacy may arise from beyond antioxidant effect of vitamin E. Consequently, these phenomena observed in this study imply that the inhibitory effect of vitamin E on cognitive deficit during aging is caused by its antioxidant property as well as non-antioxidative neuro protection. In order to understand the veritable mechanism of an improvement of cognitive deficit in aged rats by vitamin E, further investigations such as the effect of a concurrent diet of vitamin E and PQQ, changes in ROS induction in the brain of aged rats before and after the test of cognition, and inhibition of c-Src kinase activation and phosphorylation of ERK by vitamin E are now in progress.

Acknowledgments
This study has been supported, in part, by MEXT HAITEKU (2004), and a Grant-in-Aid from Eisai Company Ltd. We would like to gratefully acknowledge Messrs. Hironobu Takeda, Hiroshi Isogai, and Ms. Makiko Yamazaki for their excellent technical assistance.

REFERENCES
Improvement of Cognitive Impairment of Rat by Vitamin E


