Effect of Supplementation of Vitamin E and Vitamin C on Brain Acetylcholinesterase Activity and Neurotransmitter Levels in Rats Treated with Scopolamine, an Inducer of Dementia

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Summary In the present study, the effects of vitamins E and C on the levels of neurotransmitters and acetylcholinesterase activity in the brains of rats treated with scopolamine, an inducer of dementia, were examined. Fifty male Sprague-Dawley rats at the age of 5 wk were divided into five groups after 1 wk of adaptation and fed five different diets for 6 wk: a no-scopolamine group, which was a scopolamine-untreated group fed only a basal diet; a scopolamine-treated group fed a basal diet; a vitamin E-supplemented scopolamine-treated group; a vitamin C-supplemented scopolamine-treated group; and a vitamins E and C-supplemented scopolamine-treated group. Scopolamine was twice administered by intraperitoneal injection (300 mg/kg, body weight), 3 d and 20 min prior to sacrifice. Brain acetylcholinesterase activity was markedly reduced by scopolamine injection. However, the supplementation of vitamins E and C in the diet significantly increased the reduced brain acetylcholinesterase activity up to the level of the scopolamine-untreated group. Brain serotonin concentration in the vitamin C-supplemented scopolamine-treated group was significantly higher than that in the scopolamine-treated group. However, there were no significant differences in brain dopamine and norepinephrine concentrations among all groups. In conclusion, supplementation with vitamin E and/or vitamin C might be useful in maintaining brain acetylcholinesterase activity at the normal level and serotonin concentration for some extent under the condition to induce dementia by scopolamine administration.

Key Words acetylcholinesterase activity, vitamin E, vitamin C, neurotransmitter, scopolamine

Recently, the elderly population has increased because of increased life expectancy. In turn, dementia, a neurodegenerative disease in older adults, has become a major health concern in modern societies. In America, the prevalence of dementia is approximately 14% in the elderly over 65 years of age (1), and the rates double every 5.1 y as age increases (2). In Korea, there are an estimated 200,000 dementia patients, and this number is expected to reach 500,000 by 2020 (3). Thus the search for measures to prevent dementia has become an urgent one.

Dementia is a progressive and degenerative mental disease resulting in the impairment of memory, thinking, and behavior. Age (4), gender (5), family history (6), and brain damage (7) have been proposed to be risk factors for dementia. Although the mechanism of the development of dementia is unknown, it has been postulated that free radicals may play a role. Free radicals formed by oxidative stress promote the peroxidation reaction in brain, resulting in various biochemical changes: lowered levels of neurotransmitters and enzymes for neurotransmitter synthesis and damage to the brain cell receptors. Brain cells are especially susceptible to oxidative stress because of high concentrations of phospholipids, which contain high polyunsaturated fatty acids (8).

It has been demonstrated that several vitamins and minerals, such as vitamin E, C, β-carotene, and selenium, play roles in removing free radicals. In particular, when vitamin E is insufficient, various biochemical changes occur, such as reduced brain acetylcholinesterase levels (9) and activity (10), decreased brain norepinephrine levels (11), and dopamine release and synthesis (12, 13). Furthermore, it has been reported that vitamin E deficiency leads to oxidative stress on the neuronal tyrosine hydroxylase synthesis (14) and reduction of serotonin uptake in the serotoninergic fiber (15).

Vitamin C is an antioxidant that reduces reactive oxygen radicals such as superoxide and hydroxyl radicals into harmless or nonreactive substances in the body (16). When vitamin C was added to the brain tis-
sue in vitro, the more added, the fewer lipid peroxides produced (17, 18). Deana et al. (19) found that brain acetylcholinesterase and catecholamine levels were reduced in vitamin C deficiency. In another study, damage induced by 3,4-methylene dioxy-methamphetamine (MDMA) (20) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (21) to the serotonergic and dopaminergic nerve systems was prevented by vitamin C supplementation.

Besides these findings, vitamins E and C levels in the plasma (22) and cerebrospinal fluid of Alzheimer’s patients (23) were lower than those in normal subjects. As mentioned above, vitamins E and C could lead to favorable effects on neurotransmitter metabolism in the normal states. In the present study, therefore, we investigated whether supplementation of vitamin E and/or vitamin C can maintain normal brain neurotransmitter status under the condition of induced dementia.

**MATERIALS AND METHODS**

**Animals, diets, and protocols.** Fifty male Sprague-Dawley rats, 5 wk of age, with an average body weight of 133.7 (±13.36) g, were purchased from Hanlim Animal Laboratory (Chuncheon, Korea) and fed a standard rat chow (Samyang Feed Co., Korea) for 1 wk of adaptation. The rats were then divided into five groups: a no-scopolamine group (N-Sc), which was a scopolamine-untreated group fed only a basal diet; a scopolamine-treated group (Sc); a vitamin E-supplemented scopolamine-treated group (VE-Sc); a vitamin C-supplemented scopolamine-treated group (VC-Sc); and a vitamin E and C-supplemented scopolamine-treated group (VCE-Sc) (Table 1). The rats were fed the experimental diet for 6 wk.

The composition of the basal diet produced by Purina Feed Company in Korea is presented in Table 2. The supplementation level of vitamin E (dl-α-tocopheryl acetate) was 24,000 mg/kg diet (24,000 IU), which is approximately 320 times the amount (75 mg/kg diet) recommended by the AIN guideline (24). However, this is not regarded as a toxic level according to research results using humans (25) and animals (16, 26). During the experimental period, no sign of toxicity was observed. Vitamin C was supplemented at the same level as vitamin E (24,000 mg/kg diet). The experimental diet was freshly prepared every 3 d to prevent oxidation, according to guidelines of the AIN-76. It was kept at −20°C until used.

The rats were caged individually with free access to food and distilled water (ad libitum) in temperature-controlled rooms maintained at 23±1°C with a 12-h light : dark cycle and at 55±5% of humidity. Food intakes and body weights were recorded weekly.

This animal experiment was conducted in accordance with the ethical guidelines of Chung-Ang University.

**Scopolamine administration.** Scopolamine is a muscarinic receptor antagonist that impairs the memory and verbal ability by blocking the acetylcholine receptor. It is often used intentionally to induce dementia (27, 28). In the previous study in our laboratory, cognitive ability was measured by an active avoidance test by using a shuttle box (Electric shock interface, Muromachi Kikai Co., Japan). There were significant decreases in active avoidance rate and acetylcholinesterase activity in the scopolamine-treated rats (28). Thus the occurrence of dementia in rats treated with scopolamine was confirmed by delayed active avoidance response. In this study, scopolamine was intraperitoneally injected twice at a dose of 300 mg/kg body weight, i.e., 3 d and 20 min prior to sacrifice.

**Sample collections.** The animals that had fasted for 12 h prior to sacrifice were sacrificed by guillotine without anaesthesia at 20 min after intraperitoneal scopolamine injection. The right brain tissue was separated from the left brain, weighed, rinsed with a phosphate buffer solution, and kept at −70°C after blast freezing.

**Biochemical determinations of samples.** Brain tissues were homogenized at a concentration of 20 mg of tissue per mL of 0.1 M phosphate buffer (pH 8.0) after removing the capillaries for assays of acetylcholinesterase,
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Table 3. Body weight gain and brain weight in the experimental groups.

<table>
<thead>
<tr>
<th>Groups*</th>
<th>Initial (g)</th>
<th>Final (g)</th>
<th>Gain (g)</th>
<th>Brain weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-Sc (n=10)</td>
<td>129.50±17.07</td>
<td>455.60±31.33</td>
<td>326.10±24.96</td>
<td>1.53±0.07</td>
</tr>
<tr>
<td>Sc (n=10)</td>
<td>135.00±13.94</td>
<td>417.50±37.44</td>
<td>282.50±29.84</td>
<td>1.47±0.08</td>
</tr>
<tr>
<td>VC-Sc (n=10)</td>
<td>134.50±13.83</td>
<td>397.00±36.45</td>
<td>262.50±33.60</td>
<td>1.42±0.06</td>
</tr>
<tr>
<td>VE-Sc (n=10)</td>
<td>138.50±7.84</td>
<td>397.00±30.66</td>
<td>258.50±33.00</td>
<td>1.46±0.06</td>
</tr>
<tr>
<td>VCE-Sc (n=10)</td>
<td>131.00±14.10</td>
<td>391.00±27.57</td>
<td>260.00±19.58</td>
<td>1.47±0.07</td>
</tr>
</tbody>
</table>

1 Mean±SD.
2 Means with different superscript within the same column are significantly different at p<0.05 by Duncan's multiple range test.
* Group Name: N-Sc: no scopolamine, Sc: scopolamine treatment only, VC-Sc: vitamin C supplementation-scopolamine, VE-Sc: vitamin E supplementation-scopolamine, VCE-Sc: vitamin C plus E supplementation-scopolamine.

Table 4. Acetylcholinesterase activity in the brain for the experimental groups.

<table>
<thead>
<tr>
<th>Groups*</th>
<th>Acetylcholinesterase activity (µmol/min/g wet wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-Sc (n=10)</td>
<td>70.05±15.79</td>
</tr>
<tr>
<td>Sc (n=10)</td>
<td>49.01±20.56</td>
</tr>
<tr>
<td>VC-Sc (n=10)</td>
<td>70.29±15.73</td>
</tr>
<tr>
<td>VE-Sc (n=10)</td>
<td>70.20±17.53</td>
</tr>
<tr>
<td>VCE-Sc (n=10)</td>
<td>88.22±14.44</td>
</tr>
</tbody>
</table>

1 Mean±SD.
2 Means with different superscript within the same column are significantly different at p<0.05 by Duncan's multiple range test.
* Group name: N-Sc: no scopolamine, Sc: scopolamine treatment only, VC-Sc: vitamin C supplementation-scopolamine, VE-Sc: vitamin E supplementation-scopolamine, VCE-Sc: vitamin C plus E supplementation-scopolamine.

Body weight gain and brain weight in all the experimental groups are shown in Table 3. Compared with the N-Sc group, the body weight gains of all the scopolamine-treated groups were significantly lower (p<0.05). There was no significant difference, however, in body weight gain between all vitamin-supplemented groups treated with scopolamine and the vitamin-un-supplemented group with scopolamine treatment. No significant difference in brain weight was found between the N-Sc group and the Sc group. Among the scopolamine-treated groups, the brain weights of vitamin-treated groups did not differ from those of the group treated with scopolamine only. But the brain weights of each vitamin-supplemented group treated with scopolamine was significantly lower than of those in the group not treated with it.

Brain acetylcholinesterase activity was markedly reduced by scopolamine injection (Table 4). However, when vitamin E and/or vitamin C were supplemented in the diet, the reduced enzyme activity was significantly increased to the level of the scopolamine-untreated group (Table 4). In the VCE-Sc group, the activity was even higher than in the scopolamine-untreated group (Table 4). The levels of brain neurotransmitters in each experimental group are presented in Table 5. There were no significant differences in brain dopamine and norepinephrine concentrations among all five...
groups. However, brain serotonin concentration was significantly higher in the VC-Sc group than in the Sc group.

**DISCUSSION**

In this study, vitamin E and/or vitamin C were added to the diet to investigate their effects on brain acetylcholinesterase activity and neurotransmitter levels in rats with an administration of scopolamine, which works as a blocking agent of an acetylcholine receptor, leading to dementia. Scopolamine administration reduced body weight but had little effect on brain weight. Moreover, the administration of quite high doses of vitamin E and/or vitamin C did not affect these parameters in scopolamine-treated rats as some other vitamin studies have already indicated (Table 3) (16, 31-33).

In this study, brain acetylcholinesterase activity was markedly reduced by scopolamine injection, and the reduced activity was restored to the original level by supplementation with vitamin C or vitamin E (Table 4). Southam et al. (34) and Goss-Sampson et al. (35) reported that vitamin E deficiency in rats led to the rearrangement of acetylcholinesterase transport in the neurons. Noda et al. (9) also reported that vitamin E deficiency had significantly reduced acetylcholinesterase activity in the cerebral cortex, hippocampus, and hypothalamus, as seen in this study. These findings suggest that vitamin E may prevent oxidative stress in the neurons, as indicated in the study of Vannucchi et al. (36) and Cadenas et al. (37), and that it is helpful in maintaining the function of the cholinergic nervous system. Furthermore, brain acetylcholinesterase activity in the VCE-Sc group was higher than in the N-Sc group. This could suggest that vitamins E and C may have a synergistic effect and could protect the brain from damage induced by scopolamine more strongly than each vitamin supplementation. Thus the supplementation of vitamins E and C may be beneficial in the prevention of dementia.

However, there were no significant differences in dopamine and norepinephrine levels in the brain among all the experimental groups (Table 5). Although supplementation with vitamin E and/or vitamin C did not affect the levels of brain neurotransmitters in this study, several other studies have shown that vitamins E and C affect the levels of neurotransmitters in the brain (38, 39). But brain serotonin level in this study was significantly higher in the VC-Sc group than in the Sc group, though there was no difference in that concentration between the N-Sc group and the Sc group or between the Sc group and the VE-Sc or the VCE-Sc group. We expected that vitamin treatment might improve the brain serotonin level, but a significant difference was observed only in the VC-Sc group. It is very difficult to interpret the result because of the inconsistent outcome, which could be a limitation of the in vivo study. Furthermore, no difference in the level between the VCE-Sc group and the Sc group might be due to the experimental period of our study being not long enough to observe the effect or that there might be some interactions between vitamin C and vitamin E on brain serotonin metabolism.

The result of our study suggests, however, that vitamin C may play a certain role in brain serotonin level. Gudelsky (20) examined the effect of vitamin C on the depletion of serotonin in the 5-HT neuron and found that the brain serotonin level did not decrease in rats when MDMA was administered with vitamin C, though the level was reduced by 30-35% in MDMA-treated rats without vitamin C administration. This may be explained by the study of Todd and Bauer (40) that vitamin C may modulate the serotonergic function by controlling 5-[3H]-hydroxytryptamine binding to bovine frontal cortex membranes. From the findings of our study and others (20, 40), it appeared that vitamin C supplementation might have a beneficial effect on preventing damage to the nervous system. More elaborated studies are needed to investigate the effects of vitamin C or vitamin E supplementation on brain neurotransmitter metabolism.

In conclusion, the results of the present study indicate that supplementation with vitamin E and/or vitamin C might be useful in maintaining brain acetylcholinesterase activity at the normal level and serotonin concentration for some extent after the induction of dementia by scopolamine treatment.

**Acknowledgments**

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REFERENCES


