Cherry Tomatoes Ameliorate Scopolamine-induced Amnesia in Mice

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Abstract

Cherry tomatoes are rich in antioxidants, which may protect against neurodegeneration and consequent memory loss. This study was conducted to investigate the effect of cherry tomatoes on scopolamine-induced amnesia in mice. Male ICR mice (4 weeks old) were maintained for 4 weeks on a diet containing 10 or 20% tomato powder (TP), and then administered scopolamine (1 mg/kg body weight, i.p.) 45 min before memory testing. Passive avoidance and Morris water maze testing revealed that scopolamine-induced amnesia was significantly reduced in the TP groups compared to the non TP-received (control) group. Accordingly, acetylcholinesterase activities in the serum and brain of TP groups were lower than those in the control group. These findings suggest that cherry tomatoes may be useful for the prevention of neurodegenerative diseases such as amnesia and Alzheimer’s disease.

Key words: acetylcholinesterase, cherry tomato, Morris water maze task, passive avoidance task, scopolamine

INTRODUCTION

Alzheimer’s disease (AD) is the most common type of dementia in modern societies. This condition is emerging as one of the greatest social problems due to its economic and social impacts. The main symptom of AD is memory loss accompanied by degeneration of basal forebrain cortical and cholinergic neurons. Clinical and (1,2) experimental studies (3,4) have shown that the cholinergic system plays an important role in multiple cognitive functions including learning, memory, arousal, and attention. Accordingly, AD is associated with a decrease in choline acetyltransferase (ChAT) activity in the cortex, hippocampus, and amygdala. AD is also accompanied by a markedly altered distribution of acetylcholinesterase (AchE) in the brain and degeneration of cholinergic neurons in the central nervous system (CNS) (5). According to the cholinergic hypothesis, elevation of acetylcholine (ACh) levels might alleviate symptoms of cognitive deficits in AD patients. This has led to the development of AchE inhibitors for the treatment of AD (6).

Scopolamine is a classical antagonist of muscarinic ACh receptors. This compound can pass through the brain-blood barrier and block muscarinic receptors throughout the brain, including the hippocampus and cerebral cortex (7). Blockade of cholinergic transmission with scopolamine decreases neuronal excitability and impairs learning and memory (8), especially learning acquisition and short-term memory (1,9). Scopolamine-induced learning and memory impairment in experimental animals has been used as a model for screening anti-amnestic drugs.

Many neurodegenerative diseases, including amnesia and AD, are associated with intracellular oxidative stress. Thus, antioxidants might be useful for the prevention of these diseases. Tomatoes contain many kinds of antioxidant compounds, including carotenoids such as lycopene. Numerous studies have shown that, as natural antioxidants, lycopene and other carotenoids are associated with protection against chronic disease such as cardiovascular disease and cancer (10). The antioxidant activity of lycopene is mainly dependent on its ability to scavenge singlet oxygen and peroxyl radicals. Non-oxidative mechanisms are also thought to underlie the ability of lycopene to prevent cancer. These mechanisms include regulation of intercellular gap junction communication, hormonal and immune systems, and xenobiotic metabolism pathways (11-13). Tomato consumption and serum lycopene are associated with a lower risk for several cancers, particularly those of the prostate, gastrointestinal tract, lung, and urinary bladder (12,13). Of the various tomato genotypes, cherry tomatoes have the highest concentration of antioxidants (lycopene, ascorbic acid, and phenols) and highest antioxidant activity (14). However, whether cherry tomatoes have anti-amnestic effects is unclear. In this study, we investigated whether cherry tomatoes could ameliorate scopolamine-induced...
amnesia in mice.

MATERIALS AND METHODS

Preparation of tomato powder (TP)

Goodtrae variety cherry tomatoes were purchased from Buyeo, Chungnam, Korea. The tomatoes were washed and finely diced. They were then dried in a freeze-drier and stored at -20°C until further use.

Animals and diets

Male ICR mice weighing 25 ~ 30 g and 4 weeks of age were obtained from Bio genomics, Inc. (Korea). Mice were housed, three animals per cage, under a 12-h light-dark cycle (lights on at 7:30 a.m.) and at a constant temperature of 23 ~ 25°C and humidity level of 55 ± 5%. After an adaptation period of one week, animals were randomly assigned to one of the following four groups: normal group, control group, TP 10 group, or TP 20 group. Both the normal and control group were maintained on a basal diet (AIN 93G), while the TP 10 and TP 20 group received a basal diet supplemented with 10% and 20% TP, respectively. The normal, TP 10, and TP 20 groups also received an i.p. injection of scopolamine (1 mg/kg body weight). Each group was maintained on the diet for 4 weeks and given free access to water and food during this time. The composition of each diet is detailed in Table 1. Body weight and food intake were measured weekly, with measurements being performed at the same time every week.

Passive avoidance task

Passive avoidance tests were carried out in a shuttle chamber (256000 series, TSE Systems, Germany) that was equipped with a grid floor and shock generator (8). For the acquisition trials, each mouse was placed in the lighted compartment, and the door between compartments was opened 10 s later. When the mice entered the dark compartment, the door was immediately closed and an electrical foot shock (0.3 mA) of 3 s duration was delivered through the stainless steel rods. The latency period before entering the dark compartment was measured. The next day, for induction of amnesia, mice were injected with scopolamine 45 min before the avoidance trial to induce amnesia. The mouse was again placed in the lighted compartment, and the time until it returned to the dark compartment was recorded as the step-through latency (with a maximum of 300 s).

Morris water maze task

The water maze was a slightly modified version of the Morris water maze (15). The experimental apparatus consisted of circular water tank (diameter, 100 cm; height, 35 cm) containing water at 23°C to a depth of 15 cm and rendered opaque by the addition of powdered milk. A platform was positioned inside the tank with its top submerged 2 cm below the water surface in the target quadrant of the maze. Each mouse was given three trials per day for two consecutive days to find the hidden platform. Once the mouse located the platform, it was placed on the platform for 10 s. If the mouse could not locate the platform within 180 s, then it was lead to the platform by experimenter. On the third day, amnesia was induced with scopolamine, which was injected 45 min prior to the water maze test. In each training trial, the time required to escape onto the hidden platform was recorded.

Measurement of AchE activity

The mice were decapitated 120 min after injection with scopolamine, and the serum and the brains were collected. AchE activity was measured using the method of Ellman et al. (16) with slight modification. The whole brain was rapidly homogenized in sodium phosphate buffer (0.1 mM, pH 7.4). For assay of AchE activity, the reaction mixture contained sodium phosphate buffer (0.1 mM, pH 8.0) 2.9 mL, 0.01 M DTNB 100 mL, 0.075 M acetylcholine iodide 20 μL, and 100 μL of homogenate. Changes in absorbance at 412 nm were recorded. Protein concentration was determined by the method of Lowry et al. (17).

Statistics

Results are expressed as mean ± standard error. Group comparisons were performed using a one-way analysis of variance with Duncan’s multiple tests. p < 0.05 was
The effect of TP on scopolamine-induced impairment in passive avoidance. One day after passive avoidance training, mice were tested for avoidance of the dark compartment by measuring step-through latency. A subset of animals received scopolamine (1 mg/kg body weight, i.p) 45 min prior to avoidance testing. *p < 0.01 vs. normal and **p < 0.05 vs. control (n=12 per group).

Fig. 1. Effect of TP on scopolamine-induced impairment in passive avoidance. One day after passive avoidance training, mice were tested for avoidance of the dark compartment by measuring step-through latency. A subset of animals received scopolamine (1 mg/kg body weight, i.p) 45 min prior to avoidance testing. *p < 0.001 vs. normal and **p < 0.05 vs. control (n=12 per group).

Effect of TP on AchE activity
AchE, which is found at neuromuscular junctions and cholinergic synapses in the CNS, catalyzes the degradation of ACh into choline and acetate. AchE inhibition is known to antagonize scopolamine-induced amnesia in experimental animals. Analysis of the AchE activity in scopolamine-treated animals revealed that both serum and brain AchE activity were significantly higher in this group than that in the normal (vehicle-treated) group.
Fig. 2. Effect of TP on scopolamine-induced impairment in Morris water maze task performance. Following two consecutive days training, mice were tested for spatial memory of the platform by determining escape latency. A subset of mice received scopolamine (1 mg/kg body weight, i.p) 45 min prior to testing. \( p < 0.01 \) vs. normal and \( p < 0.05 \) vs. control \((n = 12 \text{ per group})\).

This result is in agreement with other reports that scopolamine blocks cholinergic neurotransmission and increases AchE activity. In accord with its ability to block scopolamine-induced impairments in learning and memory, TP blocked scopolamine-induced increases in brain and serum AchE activity. AchE activity did not significantly differ between the TP groups. These results are encouraging, as inactivation of AchE is considered to be the most successful approach for AD treatment. Many researchers have tried to increase ACh levels in synaptic regions using AchE inhibitors such as tacrine, though some of these drugs are associated with adverse effects (22,23).

Tomatoes, including cherry tomatoes, contain many kinds of carotenoids, such as lycopene and beta carotene, that have potent antioxidant activity and lower the formation of reactive oxygen species (24-27). Intracellular oxidative stress has been implicated in many neurodegenerative diseases such as senile dementia and AD, and antioxidants might be useful for the prevention of these diseases. Accordingly, we have previously shown that, in mice, scopolamine-induced memory dysfunction is ameliorated by seaminol glucosides, a type of antioxidant compounds found in sesame seed (28). Taken together, the current findings suggest that cherry tomatoes are useful for the prevention of amnesia and other neurodegenerative diseases. The protective effect of cherry tomatoes may be mediated by antioxidant activity and/or AchE inhibitory activity. Further studies will be necessary to clarify the mechanism of action.

Fig. 3. Effect of TP on AchE activity in the serum and brain. \( p < 0.05 \) and \( *** p < 0.001 \) vs. normal. \( p < 0.05 \) and \( ### p < 0.001 \) vs. control \((n=12 \text{ per group})\).

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