The effect of tea polyphenol (TP) on cognitive and anti-cholinesterase activity was examined in scopolamine-treated mice. Chronic administration of TP significantly reversed scopolamine-induced retention deficits in both step-through passive avoidance and spontaneous alternation behavior tasks. Furthermore, TP exhibited a dramatic inhibitory effect on acetylcholinesterase activity. This finding suggests that TP might be useful in the treatment of Alzheimer’s disease.

Key words: tea polyphenol; cognitive function; scopolamine; acetylcholinesterase; Alzheimer’s disease

Alzheimer’s Disease (AD) is the most common type of dementia in modern societies and had a profound economic and social impact as the aging of populations continues. AD is characterized by alterations at the level of various neurotransmitters and related markers and receptors. Of all these, the most severely affected by far is the cholinergic system.1) The cholinergic system is responsible for the storage and retrieval of items in memory and its degradation correlates well with the severity of cognitive and memory impairment. Hence it has been suggested that elevation of the Acetylcholine (ACh) level might be helpful in attempts to improve the symptoms of cognitive deficit in AD.2) Loss of cholinergic innervation, demonstrated by reduced choline acetyltransferase (ChAT) and elevated acetylcholinesterase (AChE) activity, is correlated with the degree of dementia and the severity of the neuropathological hallmarks of AD.3,4)

Tea is the most widely consumed beverage in the world, aside from water. While tea contains a number of bioactive chemicals, it is particularly rich in flavonoids, including catechins and their derivatives. These polyphenolic compounds have been found to be efficient scavengers of reactive oxygen species (ROS).5) and to possess neuroprotective properties under conditions like hypoxia, ischemia, and Parkinson’s disease.6) In spite of the great potential of tea polyphenol (TP) in the treatment of neurodegenerative disease, the effects of TP on AD and its learning and memory enhancing properties have not been investigated. In that the modulation of AChE is presently the most accepted and recognized therapeutic marker for development of cognitive enhancers, it is pertinent to study this enzyme. The aim of the present study, then, was to determine the cognitive enhancing and anti-cholinesterase properties of TP in scopolamine-induced amnesic mice. Learning and memory parameters were evaluated by single trial step-through passive avoidance test and spontaneous alternation behavior. AChE activity in the whole brain was analyzed following behavioral paradigms. Male ICR mice 4–5 weeks old were housed under standard housing conditions (24–27°C, 60–65% humidity) with a 12-h light/dark cycle. Food in the form of dry pellets and water were available ad libitum. The animal experiments were performed according to internationally recognized ethical standards and the guidelines of the Animal Care and Use Committee of Kyunghee University. 0.2% TP (w/w) was added to the commercial chow diet which was fed for 7 weeks. At the end of the experimental period, scopolamine HBr (1 mg/kg, i.p., Sigma, U.S.A.) was injected 30 min prior to the passive avoidance test, as described previously.7) The apparatus consisted of two compartments, one illuminated and one dark, with a grid floor. During the training trial, each mouse was placed in the lighted compartment and when it entered the dark compartment the door was closed and it received an inescapable shock (0.5 mA, 1 sec). In the test trial, given 1 day after the training trial, the mouse was again placed in the lighted compartment and when it entered the dark compartment the door was closed and it received an inescapable shock (0.5 mA, 1 sec). In the test trial, given 1 day after the training trial, the mouse was again placed in the lighted compartment and the time until it re-entered the dark compartment was measured (the step-through latency maximum testing limit was 300 sec). Immediate working memory...
performance was assessed by recording spontaneous alternation behavior in a single session in a Y-maze. The procedure was basically the same as that described previously. Each mouse, naive to the maze, was placed at the end of one arm and allowed to move freely through the maze during an 8-min session. The series of arm entries was recorded visually, and alternation was defined as successive entries into the three arms non-overlapping triplet sets. Animals were sacrificed by decapitation 2 weeks after the behavioral test and the whole brain was removed and homogenated with sodium phosphate buffer (30 mM, pH 7.2). The homogenates were centrifuged at 10,000 × g at 4°C for 30 min and the resulting supernatant was used as an enzyme source for the AChE assay, which was measured by the method of Ellman et al. The rates of hydrolysis by AChE were monitored spectrophotometrically. Protein concentration was determined using the BCA kit (bicinchoninic acid; Sigma Co., U.S.A.). The inhibitory dose of TP required for 50% AChE inhibition (IC50) was examined using control mice brains as enzyme source and calculated with the SPSS program (version 8.0). Results were expressed as mean ± SEM, and the data were analyzed by one-way ANOVA followed by Dunnett’s test. The criterion for statistical significance was p < 0.05.

Intraperitoneal injection of scopolamine hydrobromide, a muscarinic cholinergic receptor blocker, causes memory deficits and decreases cholinergic activity in behavioral performance. Hence this method of scopolamine exposure is a useful in vivo model for AD. Scopolamine-treated mice significantly shortened the latency time (80% decrease in step-through latency) as compared to vehicle-treated mice (Fig. 1). Lower latency time indicates impairment of memory retention in the passive avoidance task. Chronic administration of TP significantly increased latency time.

Spontaneous alternation behavior, which is regarded as a measure of spatial memory, was investigated using the Y-maze test. Mice injected with scopolamine exhibited significantly impaired spatial working memory (28% decrease in alternation behavior) (Fig. 2), and administration of TP blunted the scopolamine-induced decrease in alternation behavior. In contrast, the numbers of arm entries were not changed among the various experimental groups (data not shown), indicating that general locomotive activity was not affected by scopolamine. TP administration dramatically inhibited AChE activity (71% inhibition) as compared to the control (Fig. 3). TP inhibited AChE activity in a dose-dependent manner (Fig. 4). The concentration required for 50% enzyme inhibition (IC50) was 248 μg/ml. Rizvi and
Zaid,11) however, have reported that epicatechin caused an elevation in AChE activity in diabetic erythrocytes, in which a result opposite to our results.

The central cholinergic system is considered to be the most important neurotransmitter involved in the regulation of cognitive functions. Cholinergic neuronal loss in the hippocampal area is the major feature of AD, and enhancement of central cholinergic activity through the use of anti-cholinesterase is presently the mainstay of pharmacotherapy for senile dementia of the Alzheimer type.12,13) Several groups have tried to supplement the use of anti-cholinesterase is presently the mainstay of pharmacotherapy for senile dementia of the Alzheimer type.12,13) Several groups have tried to supplement the use of anti-cholinesterase inhibitors, such as tacrine and physostigmine, which prevent ACh hydrolysis. But most of these drugs failed to effectively ameliorate the symptoms of AD and had many side effects.12–15) Therefore, seeking a new active constituent from a natural source that has a potent inhibitory effect on AChE and anti-dementia activity is very promising. The results of the present study clearly indicate that chronic administration of TP improved cognitive performance and inhibited AChE activity in scopolamine-induced amnesic mice. This is an important addition to the neuroprotective properties of TP, and to our knowledge this is the first report suggesting that TP may be useful in the treatment of AD.

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References