Ferulic Acid Supplementation Prevents Trimethyltin-Induced Cognitive Deficits in Mice

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Received October 11, 2006; Accepted January 11, 2007; Online Publication, April 7, 2007
[doi:10.1271/bbb.60564]

This study’s objective was to clarify the ameliorative effects ferulic acid (4-hydroxy-3-methoxycinnamic acid) has against cognitive deficits and ChAT activation in trimethyltin (TMT) induced, memory injured mice following a 28-d ferulic acid treatment. After administering TMT for 3 d, each mouse performed Y-maze and passive avoidance tests to check immediate working memory performance and cognitive function. The results showed that ferulic acid administration attenuated TMT-induced memory injury and a decline in ChAT activity in the mice. This suggests that ferulic acid might be useful for preventing cognitive dysfunction as well as for boosting the activation of ChAT in dementia.

Key words: choline acetyltransferase; cognitive function; ferulic acid (4-hydroxy-3-methoxycinnamic acid); trimethyltin

Alzheimer’s disease (AD) is the most common cause of dementia in the elderly. It is a chronic and slowly progressing neurodegenerative disorder with a characteristic deterioration of intellectual capacities in various domains, such as learning and memory, language ability, reading and writing, praxis, and environmental interaction. The most dramatic abnormalities of AD are those of the cholinergic system, which provides the foundation for the so-called cholinergic hypothesis of AD.1) A well-established feature of AD is dysfunction of the basal forebrain cholinergic neurons in which the basal forebrain nuclei lose the cholinergic neurons that innervate the cerebral cortex and hippocampus, resulting in a decrease in choline acetyltransferase (ChAT) and acetylcholinesterase in those terminal fields.2) Choline acetyltransferase (acetyl CoA: choline O-acetyl-transferase, EC 2.3.1.6, ChAT), the enzyme responsible for catalyzing the synthesis of acetylcholine (ACh), a neurotransmitter, is presently the most specific indicator for monitoring the functional state of cholinergic neurons in the central and peripheral nervous systems.3) One strategy for ameliorating the symptoms of AD is to restore a near normal acetylcholine concentration in the synaptic cleft for improved cholinergic neurotransmission. In a previous study, ChAT activators increased ACh synthesis boosting, the endogenous ACh level of the brain and thereby increasing cholinergic neurotransmission, which resulted in improved cognitive function in mild to moderate AD.4)

A. tuberosum Rottl. is an aromatic herb extensively cultivated and consumed in East Asia. It is used not only as a food, but also for medicinal purposes.5) During our screening of traditional herbal plants, ferulic acid was found to be the most potent substance. Therefore, the aim of this study was to determine the cognitive enhancing and ChAT activating properties of ferulic acid in TMT-induced amnesic mice. Ferulic acid was isolated from A. tuberosum Rottl., and its chemical structure was confirmed by 1H/13C-NMR (Avance-600 Brucker, Rheinstetten, Germany) chemical shift and electron impact mass spectrometry (EI-MS; JMS-AX505WA, JELO, Akishima, Japan).

Male ICR mice (Samtaco BioKorea, Seoul, Korea) were used to measure cognitive function after a one-week adaptation period (20–22°C; 12 h light/dark cycle). The mice were divided into five groups, and each group, consisted of eight mice. Water and food in the form of dry pellets were available ad libitum. All experiments were conducted according to Korea University’s Committee on the Care and Use of Laboratory Animals guidelines. Ferulic acid was dissolved every

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Note
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day in tap water at concentrations of 0.002% and 0.005% (w/v). The mice were allowed free access to either normal drinking water or ferulic acid solution (ferulic acid treated groups) for 28 d. Three days before the learning and memory studies, trimethyltin chloride (TMT, Aldrich Chemical, St. Louis, MO) was dissolved in fresh saline and 2.5 mg/kg of TMT was singly injected intraperitoneally into the mice; the normal group was injected only with saline.

A step-through passive avoidance test was used to evaluate the effects of ferulic acid on learning and memory. A shuttle box was divided into two chambers, one illuminated and one dark, and separated by a guillotine door. During the training trial, each mouse was placed in the lighted compartment, then when the mouse entered the dark compartment the door was closed and the mouse received an inescapable electric shock (0.5 mA, 1 s). The test trial was given 1 d after the training trial, and again the mouse was placed in the lighted compartment and the latency time to enter the dark compartment was measured. If the mouse did not enter the dark chamber within the cut-off time (300 s), it was assigned a latency value of 300 s. A Y-maze was used to assess the immediate working memory performance of the mice by recording their spontaneous alternation behaviors in a single session. Each mouse, unfamiliar with 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 were recorded visually, and alternation was defined as successive entries into the three arms in non-overlapping triplet sets. The percentage alternation was calculated as the successive entries divided by total arm entries minus two, multiplied by 100. The animals were sacrificed by decapitation after the end of the Y-maze and passive avoidance test. The whole brain tissues were immediately removed and homogenized with 12.5 mM sodium phosphate (pH 7.0), and the mixture was centrifuged for 30 min at 34,000 × g. The supernatant obtained was used to determine ChAT activity. The activity of ChAT was measured by the formation of [14C] acetylcholine from [acetyl-1-14C]-coenzyme A and choline based on the method of Fonnum. Brain protein concentration was measured using the Bradford assay with bovine serum albumin as the protein standard. All data are presented as mean ± SE. A one-way ANOVA test was used to determine the effects of the treatment. The differences among the means were inspected using Duncan’s multiple range tests, and were considered to be significant at a P value of <0.05.

In this experiment, the mice were treated with ferulic acid, which was present in their drinking water at various concentrations, for up to 28 d prior to TMT administration. The intraperitoneal injection of TMT caused memory deficits and decreased cholinergic activity during behavioral performance, hence this method of TMT exposure is a useful in vivo model for AD. Spontaneous alternation behavior was evaluated via Y-maze tests, and was regarded as a measure of immediate spatial working memory. No significant difference in number of arm entries was detected in the ferulic acid treated groups (Fig. 1A). In contrast, the mice treated only with TMT showed significantly reduced alternation behavior (approximately 20%) as compared with that of the normal control group. However, pretreatments of 0.002% and 0.005% ferulic acid blunted the TMT-induced decrease in alternation behavior by approximately 14% and 28% respectively, compared with that of the TMT-only group (Fig. 1B). These results indicate that TMT injection did not affect the general locomotor activity of the mice.

In the passive avoidance performance tests, the TMT-treated mice showed significantly shorter latency times during the retention trials, with a 52% decrease in step-through latency compared to the normal controls. Mice treated with ferulic acid for 28 d prior to testing had attenuated TMT-induced memory impairment. At ferulic acid concentrations of 0.002% and 0.005% (w/v), step-through latency decreased by approximately 33% and 43% respectively (Fig. 2). This result indicates that 4 weeks of pretreatment effectively prevented TMT-induced decrement in the passive performance test.

ChAT activity was significantly reduced (approximately 20%) in the TMT-treated mice as compared to the normal controls. However, administration of ferulic acid ameliorated ChAT activity to a level that was similar to the normal controls, and treatments of 0.002% and 0.005% (w/v) ferulic acid resulted in ChAT activation levels that were approximately 21% and 30% higher respectively as compared to the TMT control group (Fig. 3). These results reflect disposal of TMT-injured neuronal cells and degeneration of neurotransmitter synthesis system. However, administering ferulic acid improved the memory deficit and behavioral disabilities caused by neuronal damage, including those caused by toxic compounds or amyloid peptides. The pretreatment with ferulic acid for 28 d blunted the Aβ-induced decrease in alternation behavior and effectively prevented an Aβ-induced decrement in the passive avoidance test. Aβ is a neurotoxicant, and it induced neuronal cell death by oxidative stress. The acetylcholine level in the cortex did not decrease upon administration of ferulic acid in the Aβ-treated mice. Also, in another study, administration of ferulic acid after injection of Aβ tended to ameliorate Aβ-induced toxicity in vivo, although less effectively. These results were similar to those of this study. Thus treatment with ferulic acid in vitro inhibited lipid peroxidation and protein oxidant against Aβ-induced oxidative stress. Administration of ferulic acid protected the mice against TMT-induced toxicity. It is suggested that ferulic acid might be a potential chemical agent against AD.

A consistent finding for AD patients is the loss of cholinergic markers, including reduced levels of ACh and ChAT in the brain. The cholinergic approach to treat
AD involves counteracting this loss of cholinergic activity by increasing cholinergic transmission through pharmacological intervention. A relevant animal model for AD is an important tool in the ongoing work of understanding its pathology and finding better treatments. Although no current model can develop the full pathologic spectrum of the disease, injection of an excitotoxin has been found to impair memory and elicit a degree of Alzheimer-type neurodegeneration. TMT has been employed as a means of studying pathological induction of the limbic system and damage to neuronal cells. TMT-induced lesions have received increased attention due to the relative lack of specificity of TMT in producing brain damage. The damage is not dependent on changes in any one neurotransmitter system. TMT-induced neurodegeneration is characterized by massive neuronal death, mainly localized in the limbic system, especially in the hippocampus, accompanied by reactive gliosis, epilepsy, and marked neurobehavioral alterations, and is considered a useful model of neurodegeneration and selective neuronal death. The mechanisms of TMT-induced degeneration are not yet understood. The key role of the elements of the acetylcholine system (ChAT, ACh, acetylcholinesterase, AChE) in normal brain functions and in the memory disturbances of AD has been well documented. For induction of neuronal cell death and neuron injury by oxidative stress and apoptosis or necrosis, the activity and contents of ChAT are reduced, and the synthesis and release of ACh neurotransmitter in cholinergic neuron is decreased. Also, AChE activity declines. Although the mechanisms by which TMT induces degeneration are not

![Fig. 1. The Protective Effects of Ferulic Acid against TMT-Induced Learning and Memory Impairments in Mice.](image)

Each behavioral test was conducted 3 d after TMT injection. The number of arm entries (A) and spontaneous alternation behavior (B) were measured during an 8-min session. Control mice were injected only with the vehicle (100 μl). The data represent the mean ± SE (n = 8). A is non-significant. *P < 0.05 vs. normal control. # P < 0.05 vs. TMT-treated control. A, TMT-treated mice fed on ferulic acid (0.002%); B, TMT-treated mice fed on ferulic acid (0.005%).
yet well understood, perhaps the cholinergic system of synapses in the hippocampus was damaged or injured by oxidative stress and apoptotic or necrotic cell death with TMT. Therefore, the enzyme or protein which relates to neurotransmission in cholinergic neurons in the brain is lowered. Also, TMT-treated animals have shown hyperactivity and abnormal responses on passive avoidance tasks and radial-maze tests. Finally, treatment with TMT has inhibited ChAT activity in the cerebral cortex of mice. Therefore, studies suggest that TMT can be used to induce irreversible changes within the rodent brain that may be similar to those that are observed in AD. In this study, TMT-treated mice showed a reduction in memory and behavioral abilities; specifically, they had remarkably shorter step-through latency times and reduced ChAT activity as compared to the normal control mice.

In conclusion, ferulic acid was used to protect against...
Ferulic acid is contained at relatively high concentrations in the cell walls of several plants. In addition, A. tuberosum can be used not only as a culinary herb, but also in treating infection, coronary thrombosis, and atherosclerosis. In A. tuberosum infection, coronary thrombosis, and atherosclerosis, TMT-induced memory impairment. A. tuberosum is rich in flavonols and organosulfur compounds. It can be used internally as an anti-emetic herb to treat urinary incontinence and kidney and bladder weakness, and as a carminative and stomachic and a treatment for spermatorrhoea. Generally, the compound is known to manifest profound antioxidant activity. It has also been utilized as an anti-hepatotoxic agent and offers a variety of cardiovascular system benefits. Recently, studies have shown that ferulic acid improved or protected against neuronal cell damage due to toxic compounds and pesticides. Such studies are part of an attempt to find an agent that exerts protection or mitigating effects against disease, or has anti-inflammatory effects.

To our knowledge this study is the first to show that the administration of ferulic acid protects against TMT-induced memory injury in mice, suggesting that it might be a potential chemo-preventive agent against AD and the memory injuries that are caused by neurotoxic compounds.

Acknowledgment

This study was supported by a grant (code #20050401034615) from the BioGreen 21 Program, Rural Development Administration, Republic of Korea.

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