Effect of *Camellia sinensis* on Spatial Memory in a Rat Model of Alzheimer’s Disease

Tahereh Mahmoodzadeh,1 Maryam Haji Khasem Kashani,2 Hassan Ramshini,3 Alireza Moslem,4 and Mohammad Mohammad-Zadeh4,*

1Department of Anatomy, School of Biology, Damghan University, Damghan, IR Iran
2Damghan University, Damghan, IR Iran
3Department of Biology, Payam Noor University, Tehran, IR Iran
4Cellular and Molecular Research Center, Sabzevar University of Medical Sciences, Sabzevar, IR Iran

*Corresponding author: Mohammad Mohammad-Zadeh, Cellular and Molecular Research Center, Sabzevar University of Medical Sciences, P. O. Box: 96159635, Sabzevar, IR Iran. Tel: +98-9126048374, E-mail: mohamadzadehm@medsab.ac.ir

Received 2016 January 09; Accepted 2016 January 26.

Abstract

**Background:** Alzheimer’s disease (AD) is one of the most common types of neurodegenerative disorders. The accumulation of Aβ plaques in the hippocampus contributes primarily to memory impairment. Green tea polyphenols prevent brain aging.

**Objectives:** In this study, green tea extract was used to prevent the generation of Aβ plaques in a rat model of AD induced by hen egg white lysozyme (HEWL).

**Materials and Methods:** Rats (n = 36) weighing 250 - 280 g were divided into six groups: control and positive control groups received normal saline and scopolamine, respectively; the lesion group received HEWL; and the treated groups received a mixture of HEWL and green tea extract at three doses into the hippocampus. Twenty days after injection, spatial memory was assessed by Morris water maze.

**Results:** Treated rats showed a significant decrease in escape latency, compared with lesion and positive control groups, indicating improvement in spatial memory. The AD groups showed significant decrease in escape latency than the control group, indicating impairment of spatial memory. Histological analysis revealed more number of Aβ plaques in the hippocampus of the injured group than that in the treated animals.

**Conclusions:** Our results suggest that the green tea extract is effective in preventing amyloid fibril formation and lysozyme fibrillization that in turn results in the improvement of memory deficits in the rat model of AD.

**Keywords:** Alzheimer’s Disease, Spatial Memory, Lysozyme

1. Background

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder, accounting for more than 35 million cases worldwide (1). It is characterized by progressive loss of memory and other cognitive and executive functions, with two types of pathological deposits found in the brain, the extracellular amyloid (Aβ) plaques and the intracellular tau neurofibrillary tangles (2).

Studies have shown that Aβ plaques in AD transgenic mice are associated with activated microglia and astrocytes and triggering of inflammatory responses. However, astrocytes and microglia proliferate in the vicinity of Aβ and clear Aβ deposits (3). Yet, other studies have reported that interactions among Aβ, glia, and astrocytes cause inflammation in the AD brain, which can lead to altered neuronal homeostasis and oxidative injury (4).

Recently, the potential role of polyphenols in aging and neurodegeneration has widened with discoveries reporting that they can modulate various important pathways in the pathogenesis of AD by reducing amyloid aggregation and inflammation and modulating a class of proteins known as sirtuins that are involved in cell survival. Polyphenols are well known as antioxidants and direct scavengers of free radicals (5, 6).

Polyphenols can induce antioxidant enzymes such as glutathione peroxidase, catalase, superoxide dismutase, and hydrogen peroxide and superoxide anions and inhibit the expression of enzymes such as xanthine oxidase, which is involved in the generation of free radicals (7-9). Green tea is a source of polyphenols.

2. Objectives

Therefore, in this study, we used green tea extract to prevent the generation of Aβ plaques in a rat model of AD induced by hen egg white lysozyme (HEWL).
3. Materials and Methods

3.1. Animals

A total of 36 male Wistar rats weighing 250 - 280 g were individually housed and maintained under a 12-hours light-dark cycle and standard temperature (20 - 22°C) with free access to food and a liquid source. Procedures involving animals and their care were conducted in accordance with the “Guide to the care and use of experimental animals” (10). All the experiments were performed during the same time of the day (8:00 a.m. to 2:00 p.m.) in the morning to avoid the bias of circadian rhythms (11, 12).

3.2. Groups

Animals were divided into six groups: control (group 1) and positive control groups (group 2), respectively, received 1 µL normal saline and 3 µg/rat scopolamine (inhibitor of acetylcholine muscarinic receptor causing AD (13)) 30 minutes before Morris water maze (MWM) trials; lesion group (group 3) received HEWL; treated groups received a mixture of HEWL and green tea extract (inhibitor of amyloid fibrillization) at three doses into the hippocampus. For treatment groups (groups 4 - 6) green tea extract is used at the base concentration and diluted with normal saline at a rate 50% and 75% respectively. In groups 3 - 6, MWM trial was performed 20 days after the injections (14).

3.3. Surgery and Microinjection

The rats were first anesthetized with intraperitoneal injections of ketamine (100 mg/kg) and xylazine (10 mg/kg) (15) and, after scissoring the head hair and restraining in a stereotaxic apparatus, microinjections were given at the CA1 region of the hippocampus (V = 3/3 mm; L = -2 mm, and AP = -3/3 mm from the bregma). All injections were performed (1 µL/rat) at a rate of 1 µL/5 minutes. The position of the injection site was examined in Nissl hippocampal sections.

3.4. Amyloidic Lysozyme Preparation

In this experimental study, acidic pH and high temperatures were used to drive the protein toward amyloid formation. 2 mg/ml HEWL protein (Sigma) was dissolved in 50 mM glycine buffer (pH = 2.5) and incubated at 57°C for 48 hours with gentle stirring by teflon magnetic bars. Then, the mixture was centrifuged at 13,000 rpm for 25 minutes. The supernatant was removed and a similar volume of normal saline was added. This solution (amyloidic lysozyme) was used to inject group 3 for AD induction (16).

3.5. Preparation of Amyloidic Lysozyme Under Induction With Green Tea Leaf Extract (GTLE)

GTLE (Sigma) at a concentration of 1 mg/mL and a ratio of 2% and HEWL protein at a concentration of 2 mg/mL and a ratio of 98% were mixed in glycine solution at pH = 2.5 and 57°C for 48 hours. The resulting mixture was used for treatment groups 4 (at the base concentration), and diluted with normal saline 50% for group 5 and 75% for group 6.

3.6. Histological Staining

To study the effect of amyloidic lysozyme in the formation of amyloidic plaques in the hippocampus, we performed spatial amyloidic plaque staining. The samples of hippocampal sections were stained in 0.5% Congo red solution at 50% alcohol for 50 minutes then in 0.2% HOK solution at 80% alcohol (17).

3.7. Morris Water Maze (MWM)

MWM was selected as a method of evaluation of spatial learning and memory (18). A circular blank water tank (152 cm in diameter and 60 cm in height) was filled with water (23°C) to a depth of 30 cm. An escape platform (10 cm in diameter) was placed inside the tank, with the top sinking 2 cm below the water surface. The platform was in the middle of the target quadrant and its position remained fixed during the experiment. Above the tank, a white floor-to-ceiling cloth curtain was drawn around the pool and four types of black cardboard (circular, triangular, rhombus, and square) were hung equidistantly on the interior of the curtain to serve as spatial cues. Each rat had daily sessions of four trials for four consecutive days. For each trial, a rat was placed into the water facing the wall at one of the four standard start locations selected at random (N, S, W, and E) and then released. When the rat succeeded, it was allowed to stay on the platform for 20 seconds. When the rat failed to find the platform within 60 seconds, it was assisted by the experimenter and allowed to stay there for the same time. All trials were videotaped by a camera located 2 m above the water surface. The swim speed, the time required to find the hidden platform, and the swimming distances were recorded. A probe trial was performed 24 hours after the last training session. In this trial, the platform was removed from the tank and the rats were allowed to swim freely for 60 seconds. The percentage of distance traveled in the target quadrant and the time spent in the target quadrant were taken to indicate the degree of memory consolidation after learning.
3.8. Statistical Analysis

Data were analyzed using Statistica software. All results are expressed as mean (SEM). Data were analyzed by one-way analysis of variance (ANOVA) (in the probe trial) and two-way ANOVA (in the training days). Least significant difference (LSD test) was used to compare groups, and the difference was considered statistically significant when P value was < 0.05.

4. Results

4.1. Effect of Green Tea Leaf Extract (GTLE) on Escape Latency

Escape latency was used for the evaluation of spatial learning and memory of the rat. Two-way ANOVA of escape latency showed significant differences between training days [day: F(5,120) = 43, P = 0.001] (Figure 1). Furthermore, the rats showed significant differences between groups [groups: F(5,120) = 39, P = 0.000] (Figure 1), but there was no interaction between the factors of day and group [day × group: F(15,120) = 1.1, P = 0.32]. In addition, the LSD test showed that all groups exhibited a decreasing trend in escape latency at day 4 compared with that at day 1 (P = 0.000). Moreover, treatment groups also showed a decreasing trend in escape latency at day 3 compared with that at day 1. Lysozyme and scopolamine groups did not show significant difference between each other in none of the days. These two groups showed an increase in escape latency compared with the control group (P = 0.000). All the treatment groups showed a decrease in escape latency compared with the lesion groups (Figure 1).

4.2. Effect of Green Tea Leaf Extract (GTLE) on Velocity

Two-way ANOVA showed no significant difference among the six groups [F(5,120) = 69, P = 0.6] and also among the days [F(5,120) = 58, P = 0.6]. However, there was an interaction between the factors day and group [day × group: F(15,120) = 1.2, P = 0.02].

4.3. Effect of Green Tea Leaf Extract (GTLE) on the Percentage of Distance Traveled in the Target Quadrant in the Probe Trial

One-way ANOVA showed a significant difference between the groups [F(5,30); P = 0.000]. The LSD test showed that the lysozyme group had a significant decrease in the percentage of distance traveled compared with both control (P = 0.000) and scopolamine (P = 0.009) groups. Group 5 (tea2) and group 6 (tea3) of the treatment groups showed a significant increase in the percentage of distance traveled compared with the lysozyme group (Pteal2 = 0.04; Pteal3 = 0.000), which indicated improvement of spatial memory (Figure 2).

4.4. Effect of Green Tea Leaf Extract (GTLE) on the Time Spent in the Target Quadrant in the Probe Trial

One-way ANOVA revealed a significant difference between the groups [F(5,30); P = 0.000]. The LSD test showed that time spent in the target quadrant for the scopolamine and lysozyme groups was significantly less compared with that by the control group (P = 0.000). All the three treatment groups showed an increase compared with the lysozyme group (P = 0.000) (Figure 3).
4.5. Histological Staining

Micrographs taken from the hippocampus by spatial amyloid plaque staining indicated amyloid plaque formation only in the lysozyme group (Figure 4). These results suggest that GTLE can effectively inhibit amyloid plaque formation and prevent AD.

5. Discussion

It has been well established that the hippocampus is necessary for spatial learning and memory involved in MWM (19). Changes in the hippocampal functions seem to be critical for cognitive impairment in AD. Although there is eventual plaque formation and neuron degeneration, deficits in spatial memory and inhibition of synaptic plasticity precede morphological alterations, suggesting earlier biochemical changes in the disease (20). Multiple cellular targets and signaling pathways are susceptible to Aβ oligomers, including the protein kinase A/CREB pathway (21, 22), which plays a crucial role in the late LTP and consolidation of long-term memory, such as the eventual consolidation of spatial information in MWM (18). The AD brain pathology is characterized by deposits of amyloid plaques and neurofibrillary tangles. The primary constituents of the plaques are aggregates of Aβ peptides. Aβ monomers in the specific situation form oligomers that assemble into protofilaments and then into fibrils. There is a great deal of interest in developing inhibitors of the amyloidogenic processes. In traditional herbal medicine, numerous plants have been used to treat age-related cognitive disorders. A previous study showed that polyphenols of the extracts (such as GTLE) directly insert into the amyloidogenic core of early aggregates and inhibit amyloid fibril formation (16). In this study, we used GTLE at a concentration of 1 mg/mL (the best range for inhibition of lysozyme fibrillization (16)). MWM was used for the evaluation of spatial memory. The swimming velocity was not different between the groups, indicating that AD was not due to different motor activity. In the MWM, escape latency is one of the important factors that increase in the AD brain. Our results showed that escape latency was longer in the scopolamine and lysozyme groups (AD groups). This factor was much shorter in the treatment groups. Add-over escape latency deduction at day 4 compared with that at day 1 in all the groups indicated that the animals could learn the maze, and training trials resulted in increased learning. Previous studies have conducted training trials for 7 days (23). Furthermore, the percentage of distance spent in the target quadrant and the time spent in the target quadrant increased in the treatment groups and showed a deduction in groups 2 and 3 (AD groups). Our findings are consistent with previous reports that indicated that long-term green tea catechin administration prevents spatial learning and memory impairment in senescence-accelerated mouse prone-8 mice by decreasing Aβ1-42 oligomers and upregulating synaptic plasticity-related proteins in the hippocampus [18]. Moreover, rats pretreated with green tea polyphenols exhibited reduced memory and cognitive impairment due to okadaic acid (OA) microinjection, particularly in the G625 OA group (24). Lee et al. demonstrated that green tea (-)-epigallocatechin-3-gallate inhibits beta-amyloid-induced cognitive dysfunction (25). Assunciao et al. showed that chronic green tea consumption prevents age-related changes in rat hippocampal formation (26). The results of amyloid plaque staining are consistent with previous reports that indicated that green tea polyphenol (-)-epigallocatechin gallate reduces neuronal cell damage and upregulation of MMP-9 activity in the hippocampal CA1 and CA2 areas following transient global cerebral ischemia (27). Lim et al. showed that green tea catechin leads to global improvement among AD-related phenotypes in NSE/hAPP-C105 Tg mice (28).

The oriental medicine Jangwonhwan reduces Abeta (1-42) level and beta-amyloid deposition in the brain of Tg-APPswe/Ps1dE9 mouse model of AD (29).

5.1. Conclusions

The primary finding of this study is that GTLE treatment may prevent spatial memory deficits in a rat model of AD by inhibition of amyloid plaque formation. Thus, drinking tea every day might be an effective habit in preventing the onset of AD and in ameliorating the memory defects in the early phases of the disease.
Figure 4. Coronal 6-µm Section of the Hippocampus Stained With Hematoxylin-Eosin in the Experimental Groups

Histological analysis revealed more number of Aβ plaques in the hippocampus of C, the injured group than that in the treated rats. In A, control; B, scopolamine; E, tea2; and F, tea3 groups, Aβ plaques were never observed and in D, tea1 group; Aβ plaques formation was very low. Magnification × 500. Scale bar 100 µm.

Footnotes

Authors' Contribution: Mohammad Mohammad-Zadeh, designing the project, editing the proposal, and preparing the paper; Tahereh Mahmoodzadeh, performing all experiments; Maryam Haji Khasem Kashani, preparing proposal and help performing experiments; Hassan Ramshini, troubleshooting of experiments; Alireza Moslem, preparing the draft of manuscript.

Financial Disclosure: This study was supported by the Sabzevar University of Medical Sciences.

Funding/Support: This work was supported in part by grants-in-aid for scientific research from the deputy of research and technology of ministry of health and medical education, Iran.

References
