Oligonol improves memory and cognition under an amyloid β25-35–induced Alzheimer’s mouse model

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ABSTRACT

Alzheimer’s disease is an age-dependent progressive neurodegenerative disorder that results in impairments of memory and cognitive function. It is hypothesized that oligonol has ameliorative effects on memory impairment and reduced cognitive functions in mice with Alzheimer’s disease induced by amyloid β25-35 (Aβ25-35) injection. The protective effect of an oligonol against Aβ25-35-induced memory impairment was investigated in an in vivo Alzheimer’s mouse model. The aggregation of Aβ25-35 was induced by incubation at 37°C for 3 days before injection into mice brains (5 nmol/mouse), and then oligonol was orally administered at 100 and 200 mg/kg of body weight for 2 weeks. Memory and cognition were observed in T-maze, object recognition, and Morris water maze tests. The group injected with Aβ25-35 showed impairments in both recognition and memory. However, novel object recognition and new route awareness abilities were dose dependently improved by the oral administration of oligonol. In addition, the results of the Morris water maze test indicated that oligonol exerted protective activity against cognitive impairment induced by Aβ25-35. Furthermore, nitric oxide formation and lipid peroxidation were significantly elevated by Aβ25-35, whereas oligonol treatment significantly decreased nitric oxide formation and lipid peroxidation in the brain, liver, and kidneys. The present results suggest that oligonol improves Aβ25-35-induced memory deficit and cognition impairment.

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1. Introduction

Memory impairment is a key feature of old age and is associated with various types of dementia. Recently, the type of dementia associated with Alzheimer’s disease (AD) has attracted a great deal of attention and concern because AD is the most common cause of progressive cognitive impair-

Abbreviations: AD, Alzheimer’s disease; Aβ, amyloid β peptide; Aβ25-35, amyloid β25-35; BBB, blood-brain barrier; NO, nitric oxide; TBA, Thiobarbituric acid; MDA, Malondialdehyde.

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excessive accumulation of Aβ peptide in the brain is a major characteristic of AD. Therefore, prevention of neurotoxicity caused by Aβ is the most crucial strategy for AD treatment. Oligonol, which is derived from lychee fruit, is a relatively low-molecular-weight polyphenol containing catechin-type monomers and proanthocyanidin oligomers. Oligonol is produced from polyphenol polymers, typically proanthocyanidins, via oligomerization. Therefore, oligonol derived from lychee fruit delivers higher levels of oligomeric proanthocyanidins than other fruit and plant sources [3]. There are many reports that oligonol exhibits some biological effects, including anticancer, antioxidant, and anti-inflammatory as well as beneficial activities, such as increasing nitric oxide (NO) bioavailability and exerting a regulatory effect on lipid metabolism [4]. It is hypothesized that oligonol has ameliorative effects on memory impairment and reduced cognitive functions in mice with AD induced by amyloid β25-35 (Aβ25-35) injection.

Although the safety of oligonol has already been confirmed and it is used for dietary and pharmaceutical supplements [3], studies on the effects of oligonol on cognitive function have not yet been conducted. Therefore, in the present study, the protective role of oligonol against cognitive impairments induced by Aβ25-35 injection in experimental mice was investigated to test the hypothesis that oligonol improves memory and cognition. The study included T-maze, object recognition, and Morris water maze tests. In addition, protective activities resulting from NO production and lipid peroxidation in tissues were also studied.

2. Methods and materials

2.1. Oligonol

Oligonol obtained from lychee fruit (Litchi chinensis Sonn.) using a patented process (international patent WO 2004/103988 Al) [5] was provided by Amino Up Chemical, Co, Ltd (Sapporo, Japan). The composition of the product is shown in the Table. Briefly, the extraction processes performed by Amino Up Chemical, Co, included the combination of lychee fruit extract (750 g) and green tea extract (150 g) (purchased from Guilin Layn Natural Ingredients, Corp, Guilin, China) fruit extract (750 g) and green tea extract (150 g) (purchased from Samchun Pure Chemical, Co (Gyeonggi, Pyeongtaek, Korea)).

2.2. Reagents

Amyloid β25-35 and malondialdehyde bis (dimethyl acetal; 1,1,3,3-tetramethoxypropane) were obtained from Sigma Aldrich (St Louis, MO). Dimethyl sulfoxide and sodium chloride (NaCl) were purchased from Bio Basics, Inc (Ontario, Canada). Thiobarbituric acid (TBA) was provided by Lancaster Synthesis (Ward Hill, NY). Phosphoric acid and 1-butanol were acquired from Samchun Pure Chemical, Co (Gyeonggi, Pyeongtaek, Korea).

2.3. Animals, experimental protocols, and the Aβ25-35-infused mouse model

Twenty male mice (Imprinting Control Region (ICR) strain, 5 weeks old) were purchased from Orient, Inc (Seongnam, Korea). The animals were fed a standard diet and water ad libitum. The animal facility was maintained at 20°C ± 2°C and 50% ± 10% relative humidity under a 12-hour light/dark cycle. The animal protocol used in this study was reviewed and approved (approval no. PNU-2011-0072) by the Pusan National University–Institutional Animal Care and Use Committee. The mice were divided into four groups (5 mice in each group). There were no significant differences in body weight among the groups. The control group (group I) was only provided water by oral administration and 0.9% NaCl solution (5 mL) by injection. The remaining 3 groups were Aβ25-35-infused, as briefly described. Three days after a saline solution of Aβ25-35 (0.9% NaCl) was incubated for aggregation at 37°C, it was injected into mice, according to a previously reported method [7]. Our previous study and one other report focusing on the effectiveness of drugs for AD have demonstrated that the aggregation of Aβ25-35 was well induced by the incubation of Aβ25-35 for 3 days [8,9]. Briefly, after the mice were lightly anesthetized with ether, a 5-μL Aβ25-35 solution (5 nmol/mouse) was injected at each mouse’s bregma (2.2-mm depth) with a 10-μL Hamilton microsyringe that was fitted with a 26-gauge needle. Oligonol was not administered to group II, but it was orally administered to group III (100 mg/kg per day) and group IV (200 mg/kg per day) for 14 days using sonde. After the behavior tests, the mice in each group were anesthetized by perfusion with saline under ether inhalation, according to a previously reported method [7,10].

2.4. Novel object recognition test

The object recognition test was performed in a square open-field apparatus (40 × 30 × 20 cm) that was painted black, as specified in a previously reported method [11]. Two identical objects (plastic bottles) were placed at a fixed distance within the square field. The mice were placed at the center of the

<table>
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<th>Table – Phenol composition of oligonol used in the present study</th>
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<td>Monomers (flavon-3-ols)</td>
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<td>Miscellaneous (water, proteins, fats, etc)</td>
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square field, and the number of touch times for each object was recorded over a 10-minute training session. The mice were placed back into the same field 24 hours after the training session, but one of the original objects used during the training session was replaced with a novel object (another plastic bottle) this time. The mice were allowed to search freely for 10 minutes, and the number of touch times was recorded (test session).

2.5. T-maze test

The T-maze test was conducted according to a previously reported method [12]. The maze apparatus was T shaped; the walls of the maze were made of black painted board (length of start and goal stems, 50 cm; width, 13 cm; height, 20 cm) and were glued to a black square board as the bottom piece. The apparatus consisted of a start box, left arm, and right arm with a block door that could separate the arms from one another. The mice were placed at the start box, and the number of touch and exploration times was recorded over a 10-minute training session. The mice were placed back into the same apparatus 24 hours after the training session. The mice were allowed to search freely for 10 minutes, and the number of touch and exploration times was recorded (test session).

2.6. Morris water maze test

The Morris water maze test was conducted, according to a previously reported procedure [13] with slight modifications. The apparatus used in the Morris water maze test consisted of a water tank pool that was 80 cm in diameter and surrounded by a 40-cm-high wall that was randomly divided into quadrants. White paint was put into the pool to make the water opaque. The water temperature was maintained at 22°C ± 1°C. A platform (8 cm in diameter) was placed 1 cm below the water surface in the middle of 1 quadrant. The position of the platform was unchanged during the training session. Visual cues for navigation were provided by 4 posters on the walls of the apparatus. Each trial consisted of the mouse being placed into the water facing the outer edge of the pool at 1 of 4 starting points. Three training trials per day were conducted for 3 days. In training trials for the Morris water maze test, the mice were randomly placed in the water facing the pool wall and allowed to swim for a maximum of 60 seconds. The latency time required to find the platform was recorded. Upon reaching the platform, the mice were allowed to stay on the platform for 15 seconds. If a mouse failed to reach the platform within 60 seconds, it was gently guided onto the platform for 15 seconds. If a mouse failed to reach the platform, the mice were allowed to remain on the platform. The latency time for the mice to remain at the former location for 60 seconds while looking for the platform, and the latency time for the mice to remain at the former location of the platform in the primary test was recorded. In a tertiary test, the number of times to reach the platform in transparent water was counted.

2.7. Measurement of lipid peroxidation by MDA assay

The degree of lipid peroxidation in the mice used for the behavioral experiments was determined by measuring MDA levels, according to a previously reported method [14]. Briefly, the mice in each group were euthanized with ether, and then the brain, liver, and kidney were immediately removed and placed on ice. The dissected tissue was homogenized with saline solution. This homogenate was mixed with 1% phosphoric acid and 0.67% TBA solution, and then it was boiled for 45 minutes. After 2-ml 1-butanol was added to the reaction mixture, it was centrifuged at 3000 rpm for 10 minutes. Then, the absorbance values of the supernatant were measured at 535 nm for MDA-TBA adduct.

2.8. Measurement of NO scavenging activity

The NO concentrations in tissues were determined by a previously reported method [15]. The supernatant (150 μL) prepared for the MDA assay was mixed with 130 μL of distilled water. After this solution (20 μL) was mixed with a 20 μL of a phosphoric acid solution containing 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, the absorbance was measured at 540 nm for determination of NO levels. A standard curve for the quantitative analysis of NO was prepared with a sodium nitrite (NaNO2) solution.

2.9. Statistical analysis

The experimental results were expressed as means ± SD (n = 5). A 1-way analysis of variance followed by Dunnett post hoc test was performed. Differences with P < .05 or P < .01 were considered statistically significant.

3. Results

3.1. Novel object recognition task

Fig. 1 shows the effect of oligonol on object recognition ability. After 24 hours of training, the cognition ability toward the old object increased after the Aβ25-35 injection from 37.1% ± 2.5% (group I) to 48.7% ± 5.3% (group II). However, oligonol administrations (groups III and IV) brought the cognition ability back to the same level of the control group.

In the case of the ability to recognize the new object, the Aβ25-35 treatment decreased recognition from 62.9% ± 2.9% (group I) to 51.27% ± 5.7% (group II). The administration of oligonol also brought that ability back to the same level as that of the control group. The groups that were administered oligonol at doses of 100 and 200 mg/kg per day for 14 days revealed significantly greater numbers of touches and longer times orienting toward the novel object than the old object during the test session. This indicates that oligonol improved recognition ability in mice impaired by Aβ25-35 infusion.

3.2. T-maze test

Fig. 2 shows the results of the T-maze test. Group II showed lower spatial perception ability toward the novel route than
Fig. 1 – Effects of oligonol on object recognition tests. After the mice were trained with 2 identical objects, they were allowed to explore 1 old object from the training and 1 novel object. The time that the mice spent with the novel object was recorded. The groups (5 mice per group) are defined as follows: group I, 0.9% NaCl injection + oral administration of water; group II, Aβ25-35 injection + oral administration of water; group III, Aβ25-35 injection + oral administration of oligonol (100 mg/kg per day); group IV, Aβ25-35 injection + oral administration of oligonol (200 mg/kg per day). Values are means ± S.D. The object recognition ability for the old object was not significantly different among the experimental groups. Data represent means ± SD. * P < .05 compared with the control group (group I).

3.3. Morris water maze task

This test was used to investigate the effect of oligonol on spatial learning and memory function in Aβ25-35-injected ICR mice. Fig. 3 shows the results of spatial learning in the Morris water maze test. Overall results indicate that Aβ25-35 treatment increased the time of latency to reach the platform, but oligonol administration recovered it. For example, group II (59.0 ± 2.1 seconds in the final test) took a longer time to reach the platform than group I (15.0 ± 14.5 seconds in the final test). However, latency decreased with the administration of oligonol. Moreover, these results were dose dependent. In the final test, the administration of 100 mg (group III) and 200 mg (group IV) of oligonol decreased latency from 59.0 ± 2.1 seconds to 36.6 ± 16.4 seconds and to 17.8 ± 24.4 seconds, respectively. In the final test, the 200 mg oligonol dose helped the mice recover the time required for the task by 93%. Even the latency of the control group (group I) was improved by a
200 mg oligonol administration (group IV). This was up to the tertiary test on the third day, except in the case of the tertiary test on the second day.

Fig. 4 shows the time each experimental group remained in the zone containing the cue poster after the platform was removed. Amyloid \( \beta_{25-35} \) treatment reduced the time spent in the target quadrant by mice from 29.4% ± 5.0% (control, group I) to 6.4% ± 2.3% (group II). However, the groups administered oligonol (groups III and IV) spent significantly more time in the target quadrant than the group not administered oligonol (group II) in the probe test. Therefore, the administration of oligonol was seen to improve the memory of the mice regarding the original platform position by showing a longer time spent on the platform.

Fig. 5 shows the time it took the various groups to reach the hidden and the exposed platforms in the Morris water maze test. The times to find the hidden and the exposed platforms were recorded on the final test day of the water maze test. In the probe trial, there were no significant differences in mean latency between any of the groups of mice, in the case of the exposed platform (Fig. 5).

In the case of the hidden platform, \( \beta_{25-35} \) treatment increased latency significantly, from 15.0 ± 14.5 seconds (group I) to 59.0 ± 2.2 seconds (group II). As with the other

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**Fig. 3** – Effects of oligonol on spatial learning in the Morris water maze test. The mice were trained to swim and to find the platform for 3 days. The latency time to reach the platform during training and on the final test day was calculated. The groups (5 mice per group) are the same as those outlined in Fig. 1. Values are expressed as means ± SD. **\( P < .01 \)** compared with the group not administered oligonol (group II).

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**Fig. 4** – Effects of oligonol on memory impairment induced by \( \beta_{25-35} \) in the Morris water maze test. The percentage of time spent in the target quadrant was calculated in the water maze test on the final test day. The groups (5 mice per group) are the same as those outlined in Fig. 1. Values are expressed as means ± SD. **\( P < .01 \)** compared with the control group (group I), **\( P < .01 \)** compared with the group not administered oligonol (group II).
tests, administration of oligonol significantly restored times to completion of the test to 36.6 ± 16.4 seconds (group III) and 17.8 ± 24.4 seconds (group IV).

3.4. Effect on NaNO₂ level of oligonol in brain, liver, and kidney tissues

Fig. 6 shows the effects of oligonol toward NaNO₂ levels in the brain, liver, and kidney tissues. In the case of tissues from the control group (group I), the level of NO in the liver was the highest (56.8 ± 1.2 μmol/L per milligram of protein), followed by kidney (32.3 ± 4.8 μmol/L per milligram of protein), and then brain (15.9 ± 3.9 μmol/L per milligram of protein) tissues. The same trends in respective levels were observed in the other groups (groups II, III, and IV). It is obvious that Aβ₂₅₋₃₅ treatment increased the levels of NaNO₂, whereas oligonol administration reduced them in all three kinds of tissues. This suggests that oligonol plays an important role in recovery from NO-induced oxidative stress.

3.5. Inhibitory effects of oligonol against lipid peroxidation induced by Aβ₂₅₋₃₅

Fig. 7 shows the inhibitory effects of oligonol against lipid peroxidation induced by Aβ₂₅₋₃₅. Amyloid β₂₅₋₃₅ treatment increased MDA levels by 156% in brain tissue, 102% in liver...
tissue, and 25% in kidney tissue, thus suggesting that lipid peroxidation was induced by Aβ25-35. Oligonol administration reduced the levels of MDA in all tissues from Aβ25-35-treated mice, thereby suggesting that oligonol possesses antioxidant properties.

4. Discussion

Amyloid β peptide is the main constituent of senile plaques found in the aging brain. An increase in the number of senile plaques in the brain is known to be associated with disturbances in learning and memory processing, such as AD [16]. The extent of Aβ deposition correlates with the degree of neuronal damage, cognitive impairment, and memory loss. The deposition of Aβ-treated mice could be used as an animal model for the study of AD. A number of studies have demonstrated that injections of Aβ into the brains of mice causes various biological effects, including impairments of learning and memory [17-19] and neuronal degeneration [20,21]. Therefore, the AD mouse model, in which AD is induced by Aβ25-35, has been widely used for studies associated with AD. The injection of Aβ25-35 into the brain is known to induce pathological and biochemical damage in the hippocampus. Although the hippocampus is a region the brain regions with the highest concentration of Aβ-containing senile plaques in AD, the senile plaques of Aβ also affect other areas of the brain and, consequently, cause impairments of memory and cognition. Therefore, Aβ25-35-treated animals are useful models for understanding various problems of AD, such as the pathogenesis and progression of changes [18]. The results of the present study are consistent with previously reported results from a study conducted using the same animal model [22]. In the present study, the protective effect of oligonol against the cognitive dysfunction caused by Aβ25-35 treatment was investigated using an in vivo AD model.

Several recent studies have demonstrated the antioxidant and anti-inflammatory potential of oligonol [23]. There is some evidence that oligonol has the capacity to modulate the pathologic conditions of chronic diseases, such as cancer, diabetes, cardiovascular disease, hypertension, immune deficiency, and neurodegenerative disorders as well as to slow the aging process [4,23]. However, studies regarding the protective effect of oligonol against Aβ-induced cognitive and memory impairment had not been done before the present study. The present study showed improvement in the cognition function of Aβ25-35-treated mice upon oligonol administration, suggesting that oligonol protects against impairments in learning and memory.

The T-maze test is considered a model suited to short-term episodic memory study. The Morris water maze test is a swimming-based model in which animals learn to escape a pool of water by locating and climbing onto a hidden platform. The probe trials are often run following acquisition trials that are the indicators of memory function. The Morris water maze test involves a complex performance drawing upon many factors, including spatial memory. The Morris water maze test used in the present study is one of the most widely accepted model tests used to assess learning and memory in mice. In the case of the Aβ25-35-treated groups in this study, the groups administered oligonol at oral doses of 100 mg (group III) and 200 mg (group IV) took less time to reach the hidden platform than the group not administered oligonol. However, the latency to reach the exposed platform was not significantly different among experimental groups, thus indicating that oligonol protects against impairments in learning and memory.

Nitric oxide is associated with the mechanisms of neurodegenerative diseases, including AD, and the overproduction of NO in the brain plays a role in the pathologies of AD [24]. In
addition, NO has several roles, such as a neurotransmitter, a producer of free radicals, and a mediator of inflammation in the brain [25]. In particular, the production of free radicals is strongly associated with lipid peroxidation, which has been known to cause various diseases that include AD [26]. In the present study, when the Aβ25-35-treated group was administered oligonol, the levels of MDA and NO in liver, kidney, and brain tissues decreased significantly. MDA is a well-known lipid peroxidation product and has been widely used as an indicator of the oxidative damage associated with various diseases, including AD [26]. The present study suggests that Aβ induces lipid peroxidation, which can be inhibited by oligonol in the brain.

There is some evidence that the consumption of polyphenols attenuates the accumulation of the Aβ peptides, contributing to the process of neurodegeneration in AD. For example, epigallocatechin-3-gallate, the main polyphenolic component of green tea, inhibits Aβ aggregation and protects against the neurodegeneration and dementia associated with the progression of AD [27]. Tannic acid and ferulic acid exhibited resistance to Aβ 1-42 toxicity in the brain and displayed neuroprotective action [28,29]. In addition, in our previous study, oligonol attenuated oxidative stress significantly [23]. These reports suggest that the main active components in oligonol are oligomerized polyphenols (monomer, dimer, and trimer).

The blood-brain barrier (BBB) is known to regulate and to protect the microenvironment of the brain. The protection afforded by the BBB is essential for neuronal survival and proper functioning of the central nervous system. Blood-brain barrier dysfunction is one of the earliest pathologic events in AD [30,31]. Blood-brain barrier disruption under pathologic conditions prevents normal drug penetration into the brain [32]. Oligonol protected glial cells from Aβ-induced inflammation and proanthocyanidin while passing through the BBB, affording a level of neuroprotection [33]. Studies reporting these findings also suggest that oligonol possesses a protective effect against the BBB dysfunction associated with memory impairment in AD patients.

It is reported that oligonol is safe, at repeated intakes, and in doses less than 200 mg/d for 6 months [34,35]. This amount is equal to 12 g/d for an adult at 60-kg body weight. In addition, studies conducted on 30 healthy volunteers that consumed oligonol at doses of 100 mg/d and 200 mg/d for 92 days showed good bioavailability [34].

In conclusion, the present study demonstrates that injection of Aβ25-35 causes impairment of memory and learning ability as well as oxidative stress, in mice. Based on these results, we contend that our hypothesis, which states that oligonol improves memory impairments and cognition function in the Aβ25-35-injected mice, is supported. The results of the present study suggest that oligonol would be a potentially beneficial supplement for the improvement of memory and cognitive function in humans.

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REFERENCES


