Quercetin and quercetin-3-β-D-glucoside improve cognitive and memory function in Alzheimer’s disease mouse

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Abstract We investigated the protective activities of quercetin (Q) and quercetin-3-β-D-glucoside (Q3G) under Aβ25–35-injected Alzheimer’s disease (AD) model mice. To induce an AD-like disease in an in vivo model, mice were injected with Aβ25–35 via the intracerebroventricular route. Q and Q3G were administered orally for 14 days at 30 mg/kg/day. Learning and memory functions were evaluated using behavioral experiments that comprise tests like T-maze, Morris water maze, and novel object recognition. The administration of Q and Q3G improved memory and cognitive function, compared with Aβ25–35-injected control mice in the T-maze and object recognition test. Q and Q3G administration decreased time in order to get to the platform during the Morris water maze test. The group administered Q experienced higher protective effects from long-term spatial memory and learning ability impairments than the group administered Q3G. Furthermore, lipid peroxidation and NO formation in the brain were significantly elevated in Aβ25–35-injected control mice, and this was inhibited by Q and Q3G. These results demonstrated that Q and Q3G improved Aβ25–35-induced memory deficits and cognitive impairment and promoted protection in the brain against oxidative stress.

Keywords Alzheimer’s disease · Amyloid beta · Oxidative stress · Quercetin · Quercetin-3-β-D-glucoside

Introduction

Alzheimer’s disease (AD) is an age-dependent brain degenerative illness, characterized by progressive declines in cognitive and memory abilities, and changes in behavior and personality (Reddy and Beal 2008). It is characterized with aggregation of extracellular amyloid beta peptides (Aβ), intracellular neurofibrillary tangles (NFT), neuronal and synaptic death, and functional loss in the aging brain (Terry et al. 1991; Price and Morris 1999; Reddy and Beal 2008). A spontaneously aggregating peptide comprising 39–43 amino acids, Aβ, is initially from amyloid precursor protein (APP) as a result of cleavage by the sequential enzymatic actions of β- and γ-secretase (Mattson 2004). Excessive accumulation of Aβ is shown in the brain of AD patients and is widely recognized as a histopathological hallmark of AD (Haass and Selkoe 2007).

Although previous studies have reported on intracellular oxidative stress caused by Aβ, the mechanisms of Aβ-induced neurotoxicity have not been clearly elucidated (Butterfield and Lauderback 2002). In neuronal systems, Aβ induces elevated generation of reactive oxygen species (ROS), oxidation of protein, DNA and RNA, and peroxidation of lipid (Keller et al. 1997; Yatin et al. 1999; Butterfield and Lauderback 2002). In particular, the Aβ fragment 25–35 (Aβ25–35) has the most neurotoxicity in the Aβ peptide. In the hippocampus, accumulation of Aβ25–35 leads to oxidative stress, resulting in brain damage that impairs learning and memory processes (Trubetskaya et al. 2003). Thus, Aβ25–35-injection-based AD mouse models have been useful for evaluation of novel therapeutic agents.
for AD (Maurice et al. 1996; Tran et al. 2001). Many studies have reported that natural antioxidants, such as curcumin, catechins, and resveratrol, protect against the development of AD symptoms and pathology in an Aβ-induced AD mouse model (Frautschy et al. 2001; Haque et al. 2008; Kim et al. 2010).

Flavonoids as well-known antioxidants have protection activity against neuronal damage in neurodegenerative diseases (Youdim et al. 2002; Dajas et al. 2003; Youdim et al. 2004). Quercetin (Q) is frequently found in plants, and it has pharmacological effects including anti-cancer, anti-inflammatory, and anti-allergic effects, in addition to anti-oxidative activity (Dajas et al. 2015). Several studies have reported neuroprotective effects of Q in vitro and in vivo systems. Liu et al. (2013) demonstrated that Q regulated receptor for advanced glycation end products-mediated pathway and cholinergic neurons in AD in vivo mouse model. In addition, Q inhibited H2O2-induced apoptotic cell death such as DNA fragmentation, activation of the caspase cascade and Bax gene expression in human neuronal cells (Suematsu et al. 2011). Quercetin-3-β-D-glucoside (Q3G), a glycoside of Q, was reported to exhibit protective effects against H2O2-induced oxidative damage in SH-SY5Y human neuronal cells by reducing lipid peroxidation (Soundararajan et al. 2008). Together, these findings suggest that Q and Q3G display promising protective effects in neurodegenerative disease. However, a study assessing possible protective roles of Q and Q3G in AD has not been carried out. Therefore, we examined the protective activity of Q and Q3G using an Aβ25–35-induced AD mouse model.

**Materials and methods**

**Flavonoids and reagents**

Q, Q3G, Aβ25–35, and N-(1-naphthyl) ethylenediamine were supplied by Sigma Aldrich (Saint Louis, MO, USA). Phosphoric acid was supplied from Samchun Pure Chemical (Pyeongtaek, Korea), and thiobarbituric acid (TBA) was obtained from Acros Organics (Geel, Belgium).

**Animals and treatment**

Mice from Institute of Cancer Research (5 weeks old, Male, 25–30 g) were purchased from Orient Inc. (Seongnam, Korea). The mice were kept in plastic cages under an environment with a 12-h light–dark cycle. It has standard laboratory humidity (50 ± 10 %) and temperature (20 ± 2 °C). During the period of experiment, the mice were provided free access to a diet (5L79) available in Orient Inc. (Seongnam, Korea) and water. All experimental procedures were permitted (approval No. PNU-2014-0740) using the guidelines established by the Pusan National University Institutional Animal Care and Use Committee (PNU-IACUC). The mice were separated into four groups. Among the groups during the experiment, no significant differences in body weight were observed. The sham-injected and Q/Q3G-untreated Aβ25–35-injected control groups were orally fed 0.5 % carboxymethylcellulose (CMC) solutions; the Q and Q3G-treated groups were orally fed Q and Q3G at concentrations of 30 mg/kg/day in 0.5 % CMC solutions using a zonde for 14 days.

Aβ25–35 was dissolved in 0.9 % NaCl. Before the injection, Aβ25–35 was aggregated and subjected to incubation (37 °C for 3 days). Based on the method of Laursen and Belknap (1986), aggregated Aβ25–35 at a 25 nM/5 μL concentration was administered to the mice via i.c.v. injection. Then, the injection of aggregated Aβ25–35 (5 μL/mouse) was carried out 0.5 mm posterior to the bregma. And it was injected 1.0 mm lateral to the sagittal suture with a Hamilton microsyringe (10 μL, 26-gauge needle, 2.2-mm depth). Sham normal animals (sham-injected controls) were injected with 0.9 % NaCl. Except the sham normal animals, other groups were injected with Aβ25–35. Learning and memory ability were evaluated using the tests of T-maze, Morris water maze, and novel object recognition.

**T-maze test**

A T-shaped maze which has a start box leading to a left route and right route was constructed. During the training period, each mouse was kept in the start box. After they were allowed to search around the maze for 10 min, until the right route was given with a black-colored moveable blocking door. In 24 h after the training, the mouse was permitted to freely move around the left and right route for 10 min, the time with the blocking door was removed. The percentage of space perceptive ability was measured by the ratio of the No. of left (familiar) or right (novel) route entries to the No. of total route entries multiplied by 100 (Montgomery 1952a).

**Novel object recognition test**

During the training period, the mouse was kept in a black-colored cage (40 × 40 × 40 cm) containing two identical objects (A, A’). The mouse was then kept at the middle of the cage, and the No. of contacts with each object was recorded for 10 min. After 24-h test session, the mouse was exposed to one familiar and one novel object (A, B). The mouse was given 10 min to freely search for the original and novel object while the No. of contacts were recorded. In order to calculate the object cognitive ability percentage, the ratio of the No. of contacts with the familiar object or...
the novel object over the total No. of contacts with both objects was determined (Bevins and Besheer 2006).

**Morris water maze test**

The test of Morris water maze was performed in a circular pool (100 cm in diameter, 35 cm in height) filled with water that was made opaque by water-soluble nontoxic white paint at a temperature of 22 ± 1 °C. A hidden platform with 8 cm in diameter located 1 cm below the surface of the water was placed at the center of one “target” quadrant out of four randomly divided quadrants. The hidden platform was placed in the same position. Four posters were placed on the walls to provide visual signals for spatial navigation. On the training days, the mouse was allowed to swim for up to 60 s to look for the escape platform. In the instance that the mouse could not find the platform within 60 s, it was directed to the platform and was given 15 s to stay on the platform. On the day after the training, the same procedure was executed as before. For the second test, the platform was eliminated, and for 60 s, the mice were allowed to swim while their behavior was recorded and in the target quadrant, the time spent (%) was calculated. In the final test, the mouse was provided a visible platform in transparent water, and then the time spent to reach the platform was recorded (Morris 1984).

**Measurement of lipid peroxidation in brain**

The dissected brains were homogenized with 0.9 % NaCl combined with 1 % phosphoric acid and 0.67 % TBA solution. For 20 min, the solution was boiled, and it was mixed with butanol, then the mixture was subjected at 3000 rpm for 10 min by centrifugation. At 540 nm, the absorbance value of the supernatant was determined. The lipid peroxidation yield was calculated with a standard curve derived from malondialdehyde (MDA) at different concentrations (Ohkawa et al. 1979).

**Measurement of nitric oxide (NO) in brain**

Brain homogenates were mixed with distilled water. Then, the mixed solution (20 μL) was added to 20 μL of phosphoric acid and 0.1 % N-(1-naphthyl) ethylenediamine dihydrochloride solution. Then the mixed solution was spectrophotometrically analyzed at 540 nm for its absorbance value. The NO production yield was calculated by a standard curve for NaNO₂ content (Schmidt et al. 1992).

**Statistical analysis**

Data are shown as mean ± SD. The statistical significance of data was tested using one-way analysis of variance (ANOVA), and after by Duncan’s multiple range tests. For two-condition comparisons, Student’s t-test was employed to data of T-maze and novel object recognition. Statistical significance was considered \( P < 0.05 \).

**Results**

**Spatial cognition ability in T-maze test**

The Aβ25-35-injected control group displayed impaired space perceptive ability, as they could not determine between the familiar and novel route in Fig. 1. This suggests that Aβ25-35 injection caused cognitive deficits in these mice. Administration of Q and Q3G led to higher space perceptive ability, as mice preferred the new route rather than the old route. Notably, Q3G-treated mice showed a significant 64.23 % increase directional recognition to the novel route.

**Object recognition ability in novel object recognition test**

The results of the test for novel object recognition are shown in Fig. 2. The Aβ25-35-injected control group showed no significant differences in the ability to recognize.
and explore the novel versus familiar object. However, the sham-injected group spent a significantly higher percentage of time (57.88 %) exploring the novel object compared with the familiar object. Similarly, administration of Q and Q3G led to significantly higher exploration of the novel object of 56.30 and 64.99 %, respectively.

Reference working memory in Morris water maze test

Morris water maze test was carried out to assess if administration of Q and Q3G improved learning ability and memory. As shown in Table 1, the Aβ25–35 sham-injected group reached the hidden platform in less time than the Aβ25–35-injected control group during the training periods. The control group spent a longer time to reach the hidden platform, indicating that Aβ25–35-injection impaired cognition. Administration of Q or Q3G lowered the time to get to the hidden platform, suggesting improved cognitive function. The retention time in the area that has the cue poster after removing the platform for each experimental group is shown in Table 2. The Aβ25–35-injected control group spent a shorter amount of time in the target quadrant than the sham-injected group, and the Q and Q3G groups spent a longer time in the target quadrant than the control group. In the final test, the time to reach the hidden and exposed platforms was recorded (Table 1). The Aβ25–35-injected control group spent a longer time to get to the hidden platform. But in mean latency to the exposed platform, no significant difference was observed in the Q and Q3G groups (data not shown). This indicated that improved cognitive function in the treatment groups was not related with exercise or visual abilities.

MDA scavenging effect of Q and Q3G in brain

The effects of Q and Q3G groups on lipid peroxidation in the brain are shown in Table 3. The MDA level of the control group was significantly increased (55.29 ± 3.75 nmol/mg protein) compared with the normal group (28.00 ± 2.64 nmol/mg protein). Q and Q3G administration decreased the level of MDA to 24.59 ± 3.30 nmol/mg protein and 27.37 ± 6.30 nmol/mg protein, respectively. In particular, Q lowered the MDA level to a highly significant degree. These results indicated a protective effect of Q and Q3G against peroxidation of lipid in the Aβ25–35-induced AD brain.

NaNO2 inhibition effect of Q and Q3G in brain

The effects of Q and Q3G treatment on NO formation in the brain are shown in Table 3. Production of NO in the

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**Table 1** Effects of Q and Q3G on spatial learning and memory impairment in Aβ25–35-induced AD mice revealed by the Morris water maze test.

| Training day | Latency to reach hidden platform (second) |
|-------------|------------------------------------------|
|             | Normal | Control | Q                | Q3G              |
| 1 day       | 56.22 ± 13.14 | 50.78 ± 16.66 | 57.78 ± 9.90 | 53.22 ± 15.89  |
| 2 day       | 54.72 ± 14.65 | 50.72 ± 15.50 | 37.44 ± 21.67 | 43.33 ± 19.05  |
| 3 day       | 30.89 ± 17.01 | 54.94 ± 9.42  | 26.44 ± 20.14 | 33.44 ± 20.35  |
| Final test  | 22.33 ± 20.49b | 59.17 ± 2.04a | 17.83 ± 11.79b | 29.33 ± 8.33b |

Values are mean ± SD

**Normal** 0.9 % NaCl injection + oral administration of 0.5 % CMC; **Control** Aβ25–35 injection + oral administration of 0.5 % CMC; **Q** Aβ25–35 injection + oral administration of quercetin (30 mg/kg/day); **Q3G** Aβ25–35 injection + oral administration of quercetin-3-β-D-glucoside (30 mg/kg/day).

Means with different letters indicate significant differences (P < 0.05) by Duncan’s multiple range test.
Table 2 Effects of Q and Q3G on memory impairment induced by injection of Aβ25-35 as assessed by the Morris water maze test

| Group               | Occupancy in target quadrant (%) |
|---------------------|----------------------------------|
| Normal              | 27.83 ± 6.62*                    |
| Control             | 13.67 ± 1.97b                    |
| Q                   | 23.33 ± 4.37a                    |
| Q3G                 | 20.20 ± 7.91a                    |

Values are mean ± SD

Normal 0.9 % NaCl injection + oral administration of 0.5 % CMC; Control Aβ25-35 injection + oral administration of 0.5 % CMC; Q Aβ25-35 injection + oral administration of quercetin (30 mg/kg/day); Q3G Aβ25-35 injection + oral administration of quercetin-3-β-D-glucoside (30 mg/kg/day)

ab Means with different letters indicate significant differences (P < 0.05) by Duncan's multiple range test

Table 3 Effect of administration of Q and Q3G on lipid peroxidation and NO formation in the brain

| Group               | MDA (nmol/mg protein) | NaNO2 (µmol/L/mg protein) |
|---------------------|------------------------|---------------------------|
| Normal              | 28.00 ± 2.64c          | 9.05 ± 2.14c              |
| Control             | 55.29 ± 3.75a          | 13.66 ± 1.34a             |
| Q                   | 24.59 ± 3.30f          | 10.22 ± 2.28b             |
| Q3G                 | 37.37 ± 6.30b          | 11.89 ± 0.98ab            |

Values are mean ± SD

Normal 0.9 % NaCl injection + oral administration of 0.5 % CMC; Control Aβ25-35 injection + oral administration of 0.5 % CMC; Q Aβ25-35 injection + oral administration of quercetin (30 mg/kg/day); Q3G Aβ25-35 injection + oral administration of quercetin-3-β-D-glucoside (30 mg/kg/day)

ab Means with different letters indicate significant differences (P < 0.05) by Duncan's multiple range test

Aβ25-35-injected control group in the brain was significantly increased (13.66 ± 1.34 µmol/L/mg protein), compared to the sham-injected group (9.05 ± 2.14 µmol/L/mg protein). However, Q and Q3G administration decreased the production of NO to 10.22 ± 2.28 µmol/L/mg protein and 11.89 ± 0.98 µmol/L/mg protein, respectively. These results demonstrated that Q and Q3G attenuated NO formation induced by Aβ25-35 in the AD brain.

Discussion

This research demonstrated that the administration of Q and Q3G attenuated learning and memory impairments in an Aβ25-35-injection AD mouse model, and likewise protected against Aβ25-35-induced oxidative stress in the brain. Aβ peptide accumulation is a neuropathological hallmark of AD that leads to oxidative stress by inducing overproduction of ROS, which is neurotoxic (Varadarajan et al. 2000). Aβ25-35-injection mouse and cultured models of neuronal cell have been used to evaluate the role of Aβ-induced toxicity owing to oxidative stress in vitro (Loo et al. 1993; Yamaguchi and Kawashima 2001). Therefore, scavenging the Aβ-induced excess ROS with antioxidants might be an effective protective strategy in treating AD.

We indicated the safe and established AD mouse model with related references. The Aβ-injection to intracerebroventricular route (i.c.v.) to mice is well established model to study AD pathology (Ji et al. 2008; Kim et al. 2009; Choi et al. 2015). According to Glascock et al. (2011), i.c.v. injection is safe and can be performed freely, with mice recovering shortly after injection without any detrimental side effects. Therefore, the i.c.v. injection of Aβ into mice was well used to study AD pathological conditions and the agents for treatment of AD.

Flavonoids are plant polyphenolic compounds abundant in vegetables and fruits that have been suggested to improve cognitive function (Rendeiro et al. 2015). According to Shimmyo et al. (2008), flavonoids can cross the blood–brain barrier and protect neuronal cells. In addition, previous reports have demonstrated that flavonoids directly reduce Aβ plaque pathology by exerting antiamyloidogenic and fibril-destabilizing activity in in vitro systems (Ono et al. 2003; Jiménez-Aliaga et al. 2011). Consumption of flavonoid-supplemented foods has been related with cognitive benefits in both rodents and humans. Previous researches indicated the beneficial effects of flavonoid-rich diet by protecting neurons, declining neuroinflammation, enhancing the neurocognitive ability, and changing in synaptic plasticity in humans and animals (Spencer 2007; Vauzour et al. 2008; Rendeiro et al. 2009; Janssen et al. 2015). In addition, a dietary intake with flavonoid-rich foods showed the improvement of cognitive impairment with aging in cohort studies over a 10-period and also increasing in cerebral blood flow velocity (Letenneur et al. 2007; Sorond et al. 2008). Q, the most common of the flavonoids, has been reported to show protection against Aβ-induced oxidative stress in neuronal cells (Heo and Lee 2004; Zhu et al. 2007; Ansari et al. 2009). In an AD transgenic mouse model, Q administration led to a reduction of senile plaques, elevation in the activity of activated protein kinase, improvement of mitochondrial dysfunction by decreasing mitochondrial membrane potential, and reduced ROS levels in the brain (Wang et al. 2014; Sabogal-GuáQueta et al. 2015). Furthermore, Q improved cognition by affecting cholinergic neuronal regulation in an Aβ25-35-injection mouse AD model, and Q3G was reported to defend against oxidative damage caused by H2O2 in in vitro neuroblastoma SH-SY5Y cells (Soundararajan et al. 2008; Liu et al. 2013). However, a study of potential protective role of Q and Q3G from...
Aβ25-35-induced oxidative stress in AD had not here-tofore been carried out.

In this study, AD model mice were generated by i.c.v. injection of Aβ25-35. Q or Q3G were then orally administered to experimental mice for 14 days. The tests of T-maze and novel object recognition were carried out to confirm the effects of Q and Q3G on short-term spatial learning and memory. Both tests are considered appropriate for evaluating learning and memory ability, because the curiosity levels of mice are known to affect exploratory behaviors toward a novel route or object (Montgomery 1952b). The groups administered Q and Q3G showed higher exploration of the novel route or object than in the Aβ25-35-injected control group. Consequently, the results indicated that the Aβ25-35-injected mice had greatly impaired spatial working memory indicative of impaired cognition, which was reversed by Q and Q3G treatment.

The Morris water maze test is a behavioral task method; therefore, it has been widely employed to evaluate the long-term memory of mice, whereas the tests of T-maze and novel object recognition evaluate working memory. Over the training periods, administration of Q and Q3G led to significant decreases in the time find the hidden platform. In contrast, treated Aβ25-35-injected control mice did not show decreases in escape latencies over training. Similar to these results, several reports have demonstrated that the i.c.v. injection of Aβ25-35 induced learning and memory impairment observed through the Morris water maze test (Choi et al. 2014). In addition, the protective effect of Q and Q3G did not involve in effects on in swimming or visual abilities, as confirmed by evaluating the recorded time for each group to reach the exposed platform (data not shown). The present results therefore indicated that Q and Q3G treatment improved cognitive function in an AD mouse model.

Aβ25-35-induced toxicity has been shown to increase the abundance of inflammatory mediators, such as higher inducible nitric oxide synthase and cyclooxygenase-2 levels, which results in increased lipid peroxidation or protein nitration in the AD brain (Avdulov et al. 1997; Stepanichev et al. 2008). Furthermore, in brain cells and neurons, the peroxidation of membrane lipids and proteins was caused by Aβ, eventually resulting in cell death (Butterfield and Lauderback 2002). Previous reports have described significantly increased MDA levels in the brains of AD patients and in an AD mouse model (Markesbery and Lovell 1998; Butterfield and Lauderback 2002). Therefore, an increased level of MDA due to lipid peroxidation is a remarkable oxidative marker for AD. We confirmed that the increase of MDA levels was detected in the Aβ-injected control group, while the administration of Q and Q3G significantly showed a decrease of MDA levels in the brain, especially in the Q group. This suggests that administration of Q and Q3G attenuated oxidative stress induced by Aβ-injection.

Aβ25-35 administration significantly increases reactive oxygen/nitric species (ROS/RNS) in the AD brain (Smith et al. 1997). NO is an important molecule as a physiological messenger in neuronal tissues, but overproduction of NO may result to the accumulation of peroxynitrite in the brain (Bredt and Snyder 1994; Smith et al. 1997). The production of peroxynitrite by Aβ can lead to substantial neuronal damage through processes such as tyrosine nitration of the synaptic protein synaptophysin and peroxidation of lipids in neuronal cells, thus resulting in memory deficits (Tran et al. 2003). In the present study, Aβ25-35-injected control group was increased brain NO levels, and this effect was significantly decreased by administration of Q and Q3G.

According to previous researches, several studies demonstrated neuroprotective effects at dose of 30 mg/kg/day of Q in mice (Liu et al. 2013; Wang et al. 2014). On the basis of these evidences, we were determined the dose used in this study with consideration of safety. Furthermore, dietary supplement of Q was available daily doses of 200–1200 mg (Manach et al. 2005; Egert et al. 2008). It suggests that the dietary supplement dose may play protective role from AD.

In conclusion, this research demonstrates that oral administration of Q and Q3G at a dose of 30 mg/kg/day provided protection against Aβ and oxidative stress in an AD mouse model. We confirmed neuroprotective effects of Q and Q3G by conducting behavioral tests and determined that in the brain, these compounds protect against oxidative stress by inhibiting lipid peroxidation and NO production. Therefore, Q and Q3G might be involved in a protective role in AD that improves memory and learning by conferring protection against oxidative stress. In summary, the results suggest that Q and Q3G improve memory and cognitive dysfunctions in AD by attenuating oxidative stress.

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