Research Article

Quercetin improved spatial memory dysfunctions in rat model of intracerebroventricular streptozotocin-induced sporadic Alzheimer's disease

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Abstract

Background: Alzheimer's disease (AD) is one of the most common neurodegenerative syndromes characterized by a progressive decline in the spatial memory. There are convincing evidences on the neuroprotective effects of flavonoids against AD. **Aims and Objective:** To determine the effect of quercetin on the acquisition and retention of spatial memory in a rat model of AD. **Materials and Methods:** Twenty-four male Wistar rats were divided into four groups (six in each): group I: control rats receiving intracerebroventricular (ICV) injection of normal saline, group II: rats induced AD by ICV injection of streptozotocin (STZ; 3 mg/kg bilaterally; twice, on days 1 and 3), and groups III and IV: ICV-STZ AD rats treated intraperitoneally (IP) with 40 and 80 mg/kg/day quercetin, respectively, over a period of 12 days. Then, the rats were trained with four trials per day for five consecutive days in the Morris water maze (MWM). On the sixth day, the memory retention was evaluated. **Result:** The ICV-STZ AD groups showed a significant impairment in the acquisition and retrieval of spatial memory when compared with the control group ($P < 0.001$). In the AD groups, the escape latency during the training trials showed a significant decrease ($P < 0.001$). Meanwhile, during the MWM task, these rats spent more time in the target quadrant in probe trials when compared with the controls. **Conclusion:** Quercetin acted as a spatial memory enhancer in ICV-STZ–induced AD rats. Hence, this flavonoid can be considered potentially as a promising agent for developing prophylactic and therapeutic neuroprotection. This neuroprotective effect of quercetin may be attributed to its antioxidant and scavenging properties.

KEY WORDS: Quercetin; Spatial Memory; Streptozotocin; Alzheimer's Disease

Introduction

Alzheimer's disease (AD) is a complex neurodegenerative syndrome characterized by a progressive decline in learning, memory, and other cognitive capabilities.[1] The two relevant major pathological events of AD are: forming the senile plaques, consisting, predominantly, of extracellular amyloid-β (Aβ) peptides, and neurofibrillary tangles, including polymerized hyperphosphorylated tau protein.[2] Sporadic Alzheimer's disease (SAD) constitutes ~99% of all the AD cases, and it is a multifactorial disease caused by genetic, environmental, and metabolic factors.[2,3]

The ICV-STZ–induced AD is a valid experimental model for researchers to evaluate the new treatments for AD.[4,5] To date, it has been reported that various compounds inhibit the formation and extension of Aβ fibrils and destabilizing Aβ fibrils in vitro. Polyphenolic compounds have been extensively

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studied and have shown to exhibit strong anti-Aβ fibrils aggregation effects.[6] Polyphenolic compounds are extensively distributed in fruits and vegetables. Quercetin (3,3′,4′,5,7-pentahydroxyflavone) is one of the most abundant bioflavonoids, which represent more than 60% of the average polyphenol ingestion.[7,8] Quercetin acts as an excellent free radical scavenger and antioxidant. Among its many well-studied health beneficial effects such as neuroprotection,[9,10] anticancer,[11,12] and hepatoprotective effects,[13,14] it has been shown that quercetin also possesses attenuated spatial learning and memory impairment in *d*-galactose–treated mice, suggesting its potential antiaging effect.[15] Moreover, one previous study reported the protective effect of long-term treatment with quercetin on cognition in a mouse model of AD.[16] To our knowledge, there are no reports on the influence of quercetin against ICV-STZ–induced cognitive dysfunction in the rat. The effect of quercetin administration on the spatial memory in ICV-STZ AD rats is not well understood to date. Therefore, this study was performed to evaluate the effect of quercetin on the spatial memory in a rat model of ICV-STZ–induced sporadic AD.

Materials and Methods

Quercetin was purchased from Sigma–Aldrich. Then, it was suspended in 1 mL of 1% ethanol diluted with sterile saline. STZ of analytical grade (Sigma–Aldrich, USA) was dissolved in cold normal saline. Healthy adult male Wistar rats, weighing 250–300 g, were obtained from the Experiment Animal Center of Golestan Medical Sciences University, Gorgan, Iran. All of the animal experiments were performed according to the guidelines of the National Institutes of Health and Animal Ethics Committee of Golestan University of Medical Sciences. The rats were maintained in a 12-h light–dark cycle at a controlled temperature (22 ± 1°C) with food and water ad libitum.

Animals were anesthetized with an intraperitoneally (IP) injection of ketamine (80 mg/kg) and xylazine (2.5 mg/kg) and, then, surgery was performed according to the previously described protocol using a stereotaxic (Stoelting, USA) apparatus. The coordinates of the ICV cannula implantation were 0.8 mm anterior to posterior (AP) bregma, 1.8 mm midline to lateral (ML), and 4 mm dorsal to ventral (DV) dura.[16] STZ dissolved in normal saline was infused bilaterally into the cerebroventricles (5 μL on each site and the final concentration of 3 mg/kg body weight) through microinjection pump, which was connected to a Hamilton microsyringe.[4,17] One minute after infusion, the needle was removed slowly from the site. The same administration of STZ was repeated 48 h after the first infusion. The solutions were freshly prepared just before the injection to avoid their decomposition. The same volume of sterile saline was infused as the vehicle control.

The rats were divided randomly into the following four groups (n = 6 each):

group I: control normal saline-injected group, included normal healthy rats that received intracerebroventricular (ICV) injection of normal saline (5 μL/site/rat).

group II: STZ group, included rats with induced AD by ICV injection of STZ as mentioned earlier.

group III: STZ + Qu40, rats in this group received quercetin in a daily dose of 80 mg/kg IP[18] for 12 consecutive days, followed by 1 week after induction of AD by ICV injection of STZ.

group IV: STZ + Qu 80, rats in this group received quercetin in a daily dose of 100 mg/kg IP[18] for 12 days, followed by 1 week after induction of AD, as previously mentioned.

Morris Water Maze

The Morris water maze (MWM) task was done according to a protocol described elsewhere.[18] The water maze consisted of a swimming pool that was adapted for rats. It consisted of a circular tank (150 cm diameter, 60 cm high), filled to a depth of 50 cm with water maintained at 26°C. Located inside the pool was a removable, circular platform made of Plexiglas, positioned such that its top surface was 0.5 cm below the water. Data were collected using a video camera fixed to the ceiling of the room and connected to a video recorder and to a video-tracking system (Maze router, Urmia, Iran), both located in an adjacent room, which housed the home cages of the rats undergoing testing. The swimming pathway and initial latency in locating the hidden escape platform were recorded for each trial.

For 5 consecutive days, on each day, the rats were subjected to one session of four trials. A trial terminated when the animal reached the platform, where it was allowed to remain for 15 s. On the sixth day, following 24 h after the last training session, the retention of the spatial memory was assessed with a 60-s probe trial; each rat was randomly released at any one of the starting points (N–S–E–W), facing the wall of the pool with the removed platform. The parameters measured on the probe trial were the time spent in the target quadrant and the initial latency to find the escape platform. All the behavioral tests were conducted at the same time of the day (8:00 a.m. to 4:00 p.m.). The timeline of experiments is shown in Figure 1.

Statistical Analysis

The results are presented as mean ± SEM and analyzed using SPSS software, version 17.0. All the data were analyzed by the one-way analysis of variance and Student's t-test. $P < 0.05$ was considered statistically significant.

Result

Figure 2 shows the time taken to reach the hidden platform (A) and swim path (B) on each training day for ICV-STZ–induced AD and control groups. The mean escape latency for the trained rats was decreased over the course of the 20 learning trials in both the groups. The control group rats (vehicle treated) exhibited significantly lower escape latency in all days during the training trials when compared with the AD group ($P < 0.01$). The two-way repeated measurement of ANOVA test revealed that ICV-STZ model significantly increased escape latency when compared with the control group rats on days 1–5 ($P < 0.001$). As shown in Figure 2B, there was a significant difference in swimming path

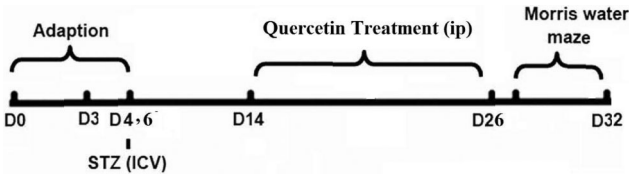

Figure 1: Timelines of experiments.

lengths to reach the platform among the control and SAD groups ($P < 0.01$ and $P < 0.001$).

The mean latencies and swim path lengths in both the groups suggest that their motor performance (ability to swim) was unaffected by the stereotaxic surgery, whereas the ICV-STZ group tended to use more time than the controls in the following trials. ICV-STZ–induced AD animals showed a lower ability to find the platform and learn its location in all the 5 days of training. Furthermore, statistical analysis revealed that ICV-STZ–induced AD rats showed longer escape latencies and swim path length than controls during the all 5 days of training trials.

Figure 3 shows the time taken to reach the hidden platform on each training day among the quercetin-treated and control groups. The MWM performance task data indicated that quercetin in both doses (40 and180 mg/kg) significantly attenuate escape latency ($P < 0.001$) in SAD model of rats when compared with control group on days 1–5. No significant differences were observed among the quercetin-treated groups. For all the days, the quercetin-treated animals showed a significant difference in the escape latency, suggesting that quercetin groups performed significantly better than the controls over time.

The swimming speed during the acquisition test for representative animals in each group are shown in Figure 4. There were no significant difference changes among the four groups from the mean swimming speed between the days during the study, indicating that all of the groups revealed similar motor capabilities. From this, we assumed that the rats were able to swim acceptably.

The probe trial data of the MWM study on the last day (sixth) showed that the average time spent in target quadrant, where the hidden platform was previously placed, significantly

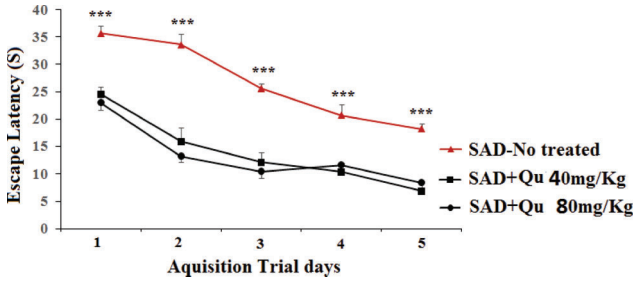

Figure 3: Comparison of escape latency during the training days. The figure shows the escape latency for control, SAD-nontreated, and SAD + Qu–treated (80 mg/kg and 100 mg/kg) groups. Values are mean ± SEM. $n = 6$ in each groups. ***$p < 0.001$ in two-way repeat-measured ANOVA.

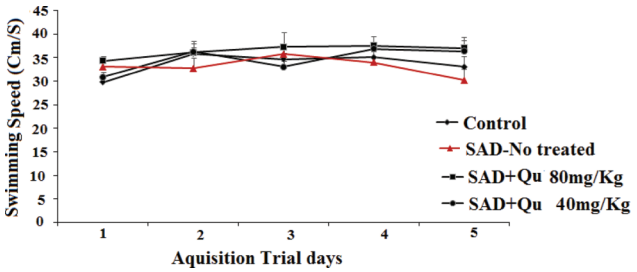

Figure 4: Comparison of swimming speed during the training days. The figures show the escape for control, SAD-nontreated, and SAD + Qu–treated (80 mg/kg and 100 mg/kg) groups. Values are mean ± SEM. $n = 6$ in each group. There were no significant differences in swimming speed between the different groups.

increased with quercetin treatment when compared with SAD nontreated group [Figure 5]. It was further observed that the ICV-STZ group significantly impaired the memory retention, and the target quadrant preference diminished significantly in SAD animals model ($P < 0.05$). The treatment with quercetin in both the doses significantly prevented the memory impairment as indicated by the increase in the time spent in the target quadrant ($P < 0.01$). The data from the probe trial test

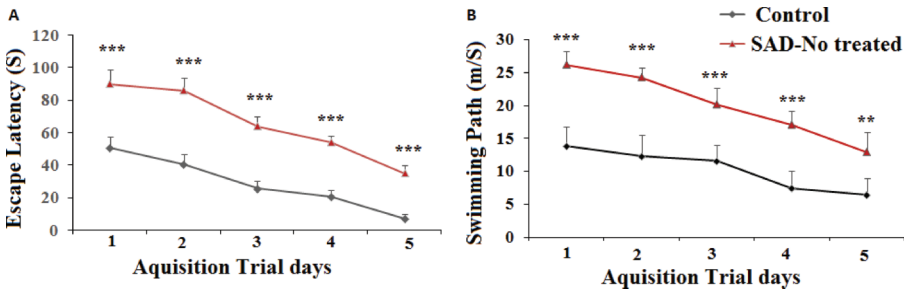

Figure 2: Comparison of escape latency (A) and traveled swimming distance path (B) during training trial days. The figures show that the escape latency and traveled distance significantly increased in SAD model group rats at all the 5 days of training compared with the control group (**$p < 0.01$, ***$p < 0.001$, respectively). Data expressed as mean ± SEM of six rats in each groups.

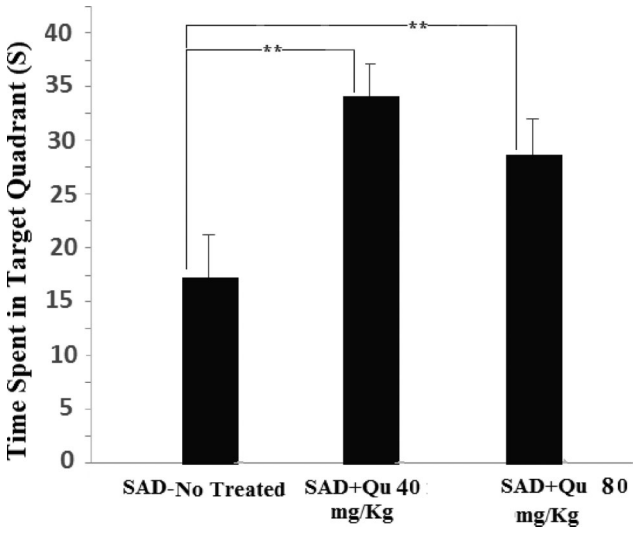

Figure 5: Time spent in the target quadrant against the average time spent in three other quadrants in probe trial on the sixth day for sporadic AD-nontreated, sporadic AD treated with quercetin 80 mg/kg, and sporadic AD treated with quercetin 100 mg/kg groups. Values are mean ± SEM. $^{**}p < 0.01$.

indicated that quercetin treatment significantly improved the memory deficits seen in SAD model rats, and both the quercetin-treated groups showed a clear preference for the former platform quadrant (ANOVA, $P < 0.01$). There were no significant differences in the time spent in the platform quadrant between the quercetin groups.

Discussion

Our results showed that ICV injection of STZ deteriorates spatial learning and memory. We found that the escape latency and swim path to find a hidden platform in MWM significantly increased in all 5 days of trials in ICV-STZ treated rats, and the time spent in the platform quadrant significantly decreased at the sixth day in ICV-STZ–treated rats, which confirmed the STZ-induced memory deficits. Quercetin treatment with two doses caused a significant decrease in escape latency during the training days when compared with the control group. Moreover, quercetin-treated animals found the hidden platform sooner than the ICV-STZ AD group in the probe trial. These data provide evidences that quercetin improved spatial learning and memory capabilities both in the acquisition and retention of spatial memory in the MWM performance task. Previous report has shown that quercetin treatment (5–20 mg/kg, po, twice daily, 30 days) in STZ-induced diabetic rats prevented the changes in MWM performance.[19] Furthermore, Mohammadi et al.[20] also showed that quercetin (50 mg/kg) treatment during restraint stress (21 days) significantly decreased escape latency and increased the time spent in target quadrant during water maze task.

It has been shown that vitamin D[21,22] and flavonoids[20] are involved in brain function and some of behavioral aspects of neurocognition. On the basis of literature, the positive effects of quercetin on cognition may be attributed to a variety of its biological effects.

Quercetin is considered as an excellent free radical scavenger and possesses strong antioxidant properties.[23,24] The antioxidant and anti-inflammatory properties of quercetin have been associated with its ameliorating effect against cellular oxidative stress[25] and related diseases. Thus, it is possible that quercetin creates a good environment in CNS because of its antioxidant capacity. On the other hand, similar to the beneficial effects of traditional antioxidant nutrients, quercetin inhibits the production of NO as dose-dependent manner[26] that serves to protect the brain from free radicals-induced damage. The other possibility is that, although it is generally agreed that quercetin is a proapoptotic agent,[26] but owing to its antioxidant and scavenging effects, which might suggest a different function for it as antiapoptotic activity, the issue will need to be investigated further. In this study, quercetin might have protected ICV-STZ–induced AD-associated cognitive dysfunction by reducing oxidative stress. It might be concluded that quercetin may be able to stimulate hippocampal cells regeneration in a rat model of ICV-STZ–induced AD.

One potential mechanism for the memory-enhancing effect of quercetin may be related with its interaction to membrane receptors. It is plausible that quercetin may interact with transmembrane protein-bound receptors such as adrenergic and glutamatergic receptors. Accordingly, it has been shown that the quercetin may be involved in negative modulation of NMDA receptors and can inhibit these receptors.[27] Moreover, the chemical structure of quercetin is similar to the mammalian estrogen. As an estrogen receptor regulator, quercetin has a high affinity to estrogen receptor binding points.[28,29] However, this mechanism needs to be elucidated, and it would be of interest to further investigate to clarify the quercetin mechanisms of memory enhancing.

Conclusion

In summary, our study demonstrates that quercetin, the widespread consumed dietary flavonoid, can improve brain cognitive function and, thus, may potentially exert a useful effect for memory and learning disturbance caused by ICV-STZ–induced rat model of sporadic AD, which could be related to its free radical-scavenging effects, antioxidant activity, and possibly interaction to membrane-bound receptors. However, the exact mechanism of these effects remains to be elucidated.

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