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Naringenin improves learning and memory in an Alzheimer's disease rat model: Insights into the underlying mechanisms

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ABSTRACT

Alzheimer's disease (AD) is one of the prevalent neurological disorders of the central nervous system hallmarked by increased beta-amyloid (Aβ) deposition and ensuing learning and memory deficit. In the present study, the beneficial effect of naringenin on improvement of learning and memory was evaluated in an Alzheimer's disease rat model. The Aβ-injected rats showed a lower alternation score in Y-maze task, impairment of retention and recall capability in passive avoidance test, and lower correct choices and higher errors in radial arm maze (RAM) task as compared to sham group in addition to enhanced oxidative stress and apoptosis. Naringenin, but not a combination of naringenin and fulvestrant (an estrogenic receptor antagonist) significantly improved the performance of Aβ-injected rats in passive avoidance and RAM tasks. Naringenin pretreatment of Aβ-injected rats also lowered hippocampal malondialdehyde (MDA) with no significant effect on nitrite and superoxide dismutase (SOD) activity in addition to lowering apoptosis. These results suggest naringenin pretreatment attenuates Aβ-induced impairment of learning and memory through mitigation of lipid peroxidation and apoptosis and its beneficial effect is somewhat mediated via estrogenic pathway.

1. Introduction

Alzheimer's disease (AD) is known as the most causative factor for dementia and well characterized by the aggregated β-amyloid (Aβ) (Bao et al., 2013). AD is a progressive neurodegenerative disorder that with time impairs cognitive skills and learning and memory abilities (Mimura, 2008). The main pathogenic mechanisms responsible for AD include cholinergic dysfunction, enhanced oxidative stress burden and disturbed antioxidant defense system, augmented inflammatory response, and an excitotoxic insult (Grothe et al., 2014; Obulesu and Jhansilakshmi, 2014; Subash et al., 2014). Currently, there is no effective cure for AD, so the focus of treatments is on stopping or slowing the progressive decline in cognitive functions (Zhu et al., 2013).

Naringenin is a natural flavanone, richly found in citrus and grape fruits, exhibits antioxidant potential, improves brain insulin signaling and cognitive functions and ameliorates AD-type neurodegeneration due to intracerebroventricular-streptozotocin (Khan et al., 2012; Yang et al., 2014). In addition, naringenin exhibits anti-inflammatory effect (Esmaeili and Alilou, 2014), exerts neuroprotective effect in 6-hydroxydopamine (6-OHDA)-induced model of Parkinson's disease and also against 6-OHDA neurotoxicity (Lou et al., 2014; Zbarsky et al., 2005). Of interest, cholinergic function is improved by naringenin due to its antioxidant property and through inhibition of cholinesterase activity in the hippocampal region, in this way could improve type-2 diabetes-induced memory dysfunction (Rahigude et al., 2012). Based on these findings, we tried to evaluate the protective potential of naringenin and to assess the involvement of estrogenic pathway, oxidative stress, and apoptosis in relation to learning and memory deficits in an intrahippocampal Aβ-injected rat model of AD.

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2. Material and methods

2.1. Animals

Adult male Wistar rats (Pasteur's Institute, Tehran), weighing 240–300 g at the start of the experiment were housed three to four per cage in a temperature-controlled colony room (room temperature was 21–23 °C) under 12:12 light/dark cycle (lights on: 06-18). Animals were allowed to acclimate to their environment for 10 days prior to being tested and handled daily. The animals were given free access to water and kept at 80–85% of their free feeding body weight throughout the experiment. All behavioral experiments were carried out between 10 a.m. and 4 p.m. This study was conducted in accordance with the policies stipulated in the Guide for the Care and Use of Laboratory Animals (NIH) and approved by the Research Council of Iran University of Medical Sciences (Tehran, Iran).

2.2. Experimental procedure

Rats ($n=45$) were randomly allocated to the following equal-sized groups: sham, naringenin-pretreated sham, beta amyloid (A-beta), naringenin-pretreated Aβ, and naringenin-pretreated Aβ receiving fulvestrant as an estrogen receptor antagonist. For stereotaxic surgery, rats were anesthetized with a combination of ketamine (80 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) and then placed in a Stoelting stereotaxic apparatus (incisor bar −3.3 mm, ear bars positioned symmetrically). The scalp was cleaned with iodine solution and incised on the midline, and a burr hole was drilled through the skull and $A\beta_{1-40}$ (Sigma-Aldrich, USA) was injected at coordinates of −3.6 mm posterior to bregma, 2 mm lateral to sagittal suture, and 2.6–2.8 mm below dura, according to the stereotaxic atlas (Paxinos and Watson, 1986). Naringenin (Sigma-Aldrich, USA) was dissolved in 10% Cremophor and administered orally by gavage at a dose of 100 mg/kg one hour before surgery. The dosage was chosen according to the results of our pilot study and its efficacy in an earlier study (Yang et al., 2014). Animals in the Aβ group were bilaterally injected in the dorsal hippocampus with 4 μl of a solution containing $A\beta_{1-40}$ (2 nmol/4 μl). The amount of Aβ (0.5 nM/μl dissolved in 0.9% normal saline; pH=8.0) was chosen based on our earlier experiment, and the solution was prepared according to previous studies (Bagheri et al., 2011; Miguel-Hidalgo et al., 2002) and then immediately stored at −70 °C until used. Sham group received 4 μl of 0.9% normal saline instead of Aβ solution. The ER antagonist fulvestrant (Sigma-Aldrich, USA) was injected i.c.v. at a dose of 10 μg/rat (5 μl) at coordinates of −0.8 mm posterior to bregma, 1.4 mm lateral to bregma, and 4 mm below dura 30 min before Aβ injection. Fulvestrant was dissolved in dimethyl sulfoxide (DMSO) and diluted to the required volume with artificial CSF (ACSF) containing the following: 120 mM NaCl, 3 mM KCl, 1.15 mM $CaCl_2$, 0.8 mM $MgCl_2$, 27 mM $NaHCO_3$, and 0.33 mM NaH_2PO_4; pH adjusted to 7.2. Postoperatively, the rats were given special care until spontaneous feeding was restored. Behavioral tests were conducted after two weeks post-surgery as depicted in Fig. 1 and were evaluated blind to the treatments by the observer.

2.3. Y maze task

Spatial recognition memory was assessed by recording spontaneous alternation behavior in a single-session Y-maze on the 14th day post-surgery, as described before (Baluchnejadmojarad and Roghani, 2011). The maze was made of black Plexiglas. Each arm was 40 cm long, 30 cm high and 15 cm wide. The arms converged in an equilateral triangular central area that was 15 cm at its longest axis. The procedure was as follows: each rat, naive to the maze, was placed at the end of one arm and was allowed to move freely through the maze during an 8-min session. The series of arm entries were recorded visually. Entry was considered to be complete when the base of the animal's tail was entirely within the arm. Alternation was defined as successive entries into the three arms on overlapping triplet sets. The maximum number of possible spontaneous alternations was determined as the total number of arms entered minus 2, and the percentage was calculated as the ratio of actual to possible alternations × 100.

2.4. RAM task

Spatial memory were tested using a radial arm maze (RAM) according to the paradigm described previously (Bagheri et al., 2011; Baluchnejadmojarad and Roghani, 2006). The apparatus consisted of a 50-cm-elevated (above the floor) eight-armed radial maze (RAM) made of black Plexiglas. The maze was placed in a sound-attenuated and dimly lit room. The 60-cm-long, 10-cm-wide, and 15-cm-high arms extended radially from a central octagonal starting platform (35 cm in diameter), and there was a recessed food cup at the end of each arm. In some of the arms, the cup contained a single small food pellet as a reinforcer. A plastic cylinder (30 cm in diameter, 20 cm high) was placed on the central platform, and a rat was placed inside this cylinder 15 s before the test. Following this interval, the rats were allowed to move freely and timing began. The RAM was surrounded by various extra-maze cues; their orientation relative to the maze was kept constant throughout the experiment. The maze was cleaned with diluted ethanol between trials.

Prior to acquisition (i.e., before surgery), the rats were maintained on a restricted feeding schedule designed to keep their body weight at about 85% of the free-feeding level. The rats learned to visit each arm, eat the pellet, and not re-enter the arm that had been visited during the same test. Each entry into each arm with all four paws was scored during a period of 10 min. Behavioral observation was discontinued after 10 min, even if the animal did not finish the task. The number of correct choices or errors was used to assess the performance of the animal in each session. An error was defined as a re-entry into an already visited arm. Rats that made at least seven correct choices in each of three consecutive sessions were used in the subsequent behavioral experiments. Training was performed at 24-h intervals, and rats that

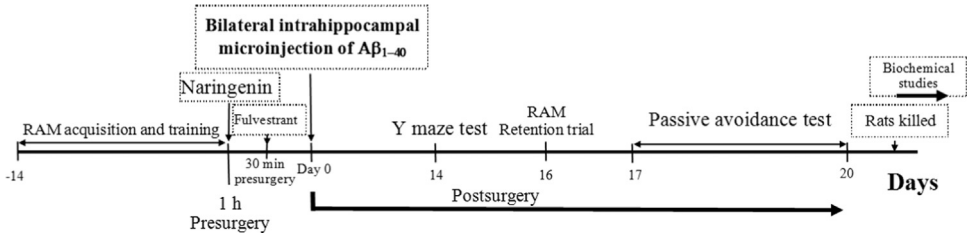

Fig. 1. Experimental scheme for treatments and behavioral tests. RAM stand for radial arm maze.