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Naringenin ameliorates learning and memory impairment following systemic lipopolysaccharide challenge in the rat

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ABSTRACT

Systemic inflammation following infection is usually associated with long-term complications including cognitive deficit and dementia. Neuroinflammation and cognitive decline are also main hallmarks of several neurological conditions. Naringenin is a citrus flavanone with anti-inflammatory, neuroprotective, and antioxidant potential. In this study, the protective effect of naringenin against lipopolysaccharide (LPS)-induced cognitive decline was evaluated in the rat. LPS was daily injected at a dose of 167 μg/kg for 1 week and naringenin was administered *p.o.* at doses of 25, 50, or 100 mg/kg/day. Treatment of LPS-injected rats with naringenin dose-dependently improved spatial recognition memory in Y maze, discrimination ratio in novel object discrimination task, and retention and recall capability in passive avoidance test. Furthermore, naringenin lowered hippocampal malondialdehyde (MDA) as an index of lipid peroxidation and improved antioxidant defensive system comprising superoxide dismutase (SOD), catalase, and glutathione (GSH) in addition to decreasing acetylcholinesterase (AChE) activity. Additionally, naringenin was able to lower hippocampal nuclear factor-kappaB (NF-κB), toll-like receptor 4 (TLR4), tumor necrosis factor α (TNFα), cyclooxygenase-2 (COX2), inducible nitric oxide synthase (iNOS), glial fibrillary acidic protein (GFAP) level and its immunoreactivity, and to elevate nuclear factor (erythroid-derived 2)-like 2 (Nrf2). Taken together, naringenin could alleviate LPS-induced cognitive deficits and neuroinflammation, as was evident from attenuation of oxidative stress and AChE and modulation of Nrf2/NF-κB/TNFα/COX2/iNOS/TLR4/GFAP.

1. Introduction

Neuroinflammation typified by overactivation of microglia and astrocytes escalates during aging process and is a key hallmark of neurodegenerative disorders including Alzheimer's disease. Glial activation through overproduction of proinflammatory cytokines and reactive oxygen species leads to a chronic neuroinflammatory process (Sawikr et al., 2017). Neuroinflammation is also a crucial risk factor for development of cognitive disturbance and dementia (Bettcher and Kramer, 2014). Lipopolysaccharide (LPS) is an endotoxin derived from Gram-negative bacteria that excites signaling cascades of pro-inflammatory agents with excess generation of pro-inflammatory cytokines (Sun et al., 2015). Systemic injection of LPS is associated with increased beta amyloid deposition and neuroinflammation in those brain areas critical for memory processes like hippocampus (Lee et al., 2013), leading to cognitive debility (Hsing et al., 2015). Increased oxidative stress (Jangra et al., 2018) and weakened antioxidant system (Zarezadeh et al., 2017), nuclear factor (erythroid-derived 2)-like 2 (Nrf2) down-regulation and nuclear factor-kappaB (NF-κB) up-regulation (Zhou et al., 2015), higher activity of acetylcholinesterase (Ming et al., 2015), greater release of proinflammatory mediators like tumor necrosis factor α (TNFα) (Wang et al., 2014), and enhanced expression of glial fibrillary acidic protein (GFAP) (Zarezadeh et al., 2017) are notably observed subsequent to LPS exposure. Nowadays, nutraceuticals targeting neuroinflammation have been explored and proposed as novel therapeutic and preventive agents for neurodegenerative and neuroinflammatory disorders (Qu et al., 2016; Sawikr et al., 2017).

Naringenin, also known as 4′,5,7-thrihydroxyflavanone, is a citrus flavanone and one of the most consumed flavonoids in the society with good bioavailability (Palma-Duran et al., 2015) and possible medical applications in chronic disorders (Fallahi et al., 2012; Sirovina et al., 2016; Yan et al., 2016). Naringenin with its antioxidant (Cavia-Saiz

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et al., 2010; Da Pozzo et al., 2017) and anti-inflammatory (Al-Roujayee, 2017; Manchope et al., 2017; Nyane et al., 2017) actions has the potential to be postulated as a therapeutic agent in inflammation-related conditions. Additionally, naringenin has also shown anti-apoptotic effect (Tang et al., 2017; Wang et al., 2016) and is capable to7 improve learning and memory in various models of cognitive deficits in experimental animals (Baluchnejadmojarad and Roghani, 2006; Ghofrani et al., 2015; Hua et al., 2016; Khan et al., 2012; Yang et al., 2014). Furthermore, this flavanone could reduce LPS-induced inflammatory pain (Pinho-Ribeiro et al., 2016), is able to lower LPS-induced acute lung injury (Fouad et al., 2016) and to suppress LPS-induced inflammatory response in microglial cells (Wu et al., 2016). These beneficial effects of naringenin is also related to its neuroprotective potential (Bai et al., 2014; Chtourou et al., 2014; Muthaiah et al., 2013; Raza et al., 2013). Therefore, naringenin may be considered a potential agent for management and treatment of neuroinflammation-related disorders. Therefore, this study was undertaken to evaluate the efficacy of naringenin against LPS-induced cognitive deficits in the rat and to unravel some of its modes of action.

2. Materials and methods

2.1. Experimental design

Male albino Wistar rats (Pasteur's institute, Tehran, Iran, 190–240 g, 10–12 weeks old) were housed under standard laboratory conditions (a temperature of 21–23 °C, a humidity of 40–60% and with 12:12 h lighting cycle) and freely provided with food and water. Procedures involving animals and their care were conducted in conformity with the NIH guidelines for the care and use of laboratory animals. The timeline of experimental procedures has been outlined in Fig. 1.

The rats (n = 72) were randomly allocated and divided into 6 groups, i.e. control, LPS, naringenin25-, naringenin50- and naringenin100-treated LPS (receiving naringenin at doses of 25, 50, or 100 mg/kg), and dexamethasone-treated LPS (as the positive control group). To induce systemic inflammatory response and consequent neuroinflammation, LPS from *Escherichia coli* (SigmaAldrich, St Louis, MO, USA; 0111:B4) dissolved in cold normal saline was *i.p.* injected at a dose of 167 μg/kg for seven days. Systemic LPS administration is a widely-accepted model for neuroinflammation induction in rodents with elevation of brain cytokines and microglial activation (Czerniawski et al., 2015; Henry et al., 2008; Zarezadeh et al., 2017). This dose of LPS was selected from earlier studies (Czerniawski et al., 2015; Zarezadeh et al., 2017) and is in a range that could impair memory processes without inappropriately affecting exploratory behavior (Bassi et al., 2012; Hennigan et al., 2007). Rats in control group received normal saline. Naringenin (SigmaAldrich, St Louis, MO, USA) was administered *p.o.* (dissolved in Kolliphor) at doses of 25, 50, or 100 mg/kg/day, 1 h after LPS, for seven days. Dexamethasone was administered *p.o.* at a dose of 0.2 mg/kg with a timetable similar to naringenin. This dose of dexamethasone has been shown to be effective for sepsis treatment and its associated depressive-like parameters and memory impairment (Cassol-Jr et al., 2010). Behavioral tests were performed 3 h after LPS administration on testing days. All behavioral experiments were carried out from 10:00–16:00 by a trained experimenter blind to interventions. Behavioral experiments including novel object recognition (discrimination), Y-maze, and passive avoidance paradigms were done at week 1 post-LPS, as depicted in Fig. 1. All animals were euthanized on day 7 following the last behavioral test.

2.2. Y-maze task

Y-maze task is a valid tool to judge spatial recognition memory of short-term nature in rodents through evaluation of spontaneous alternation behavior (Nasri et al., 2012; Roghani et al., 2006). The maze included three arms and a central interconnecting arena. All animals were tested once in a randomized manner. After allowance of a 15 min period for adaptation to testing room, animals were individually placed at the end of one arm and allowed to move freely through the arms for a period of 8 min. An arm entry was counted when the hind paws were totally within the arm zone. Alternation was defined as successive entries into the three arms on overlapping triplet sets (i.e. A, B, C, B, C, A, etc.). The number of maximum spontaneous alternation was the total number of arms entered-2 and the percentage was calculated as the ratio of actual to possible alternations (defined as the total number of arm entries-2). In addition, total number of arm entries was used as an index of general locomotive activity. The maze was wiped with alcohol to diminish odor cues. Y-maze task was conducted on day-2.

2.3. Novel object discrimination (NOD) task

The used protocol of this test has been described before (Stuart et al., 2013). In this experiment, each rat received two consecutive 5 min object exploration trials separated by a 4 h inter-trial interval (ITI). Rats were exposed to two objects during the first (familiarization) trial, and one of the objects was randomly selected and replaced with a third, novel object in the second (choice) trial. During the two trials exploration of each object, defined as sniffing, licking, chewing, or having moving vibrissae while directing the nose toward and ≤1 cm from the object, was separately recorded. Sitting on an object in the absence of any directed interest was not regarded as exploratory activity. The objects and test areas were wiped with 70% (v/v) ethanol between trials to reduce odor cues. The discrimination (D) ratio was calculated as time spent exploring the novel object compared with the familiar object relative to the total time spent exploring all objects, according to the formula: (t [novel]-t [familiar]) / (t [novel] +t [familiar]) *100. NOD task was conducted on day-3.

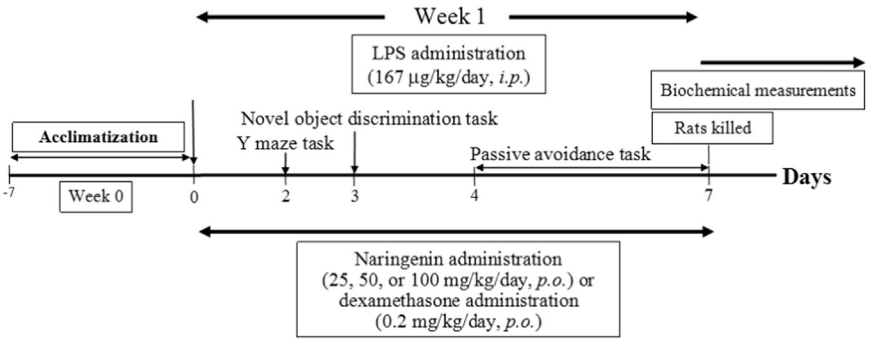

Fig. 1. Schematic experimental design for treatments and behavioral tests. Animals received daily treatment of naringenin (25, 50, or 100 mg/kg) or dexamethasone (0.2 mg/kg) and lipopolysaccharide (167 μg/kg) for 7 days. Behavioral tasks including Y-maze, novel discrimination and passive avoidance tests were conducted on week 1.