The effect of quercetin on learning and memory deficit, lipid peroxidation, and cholinesterase activity following lipopolysaccharide in the rat

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Abstract

Background and Objective: Lipopolysaccharide (LPS) is a large molecule isolated from bacteria such as the enterobacteriaceae family with a negative effect on memory and learning through disturbing the balance of free radicals and creating oxidative stress conditions. In this study, we evaluated the effect of quercetin on oxidative stress and LPS-induced memory impairment in the rat.

Materials and Methods: Male rats (n=40) were randomly divided into 5 groups: control, control under treatment with quercetin at a dose of 50 mg/kg, LPS, and LPS groups treated with quercetin at doses of 10 or 50 mg/kg. For induction of inflammation, LPS dissolved in normal saline (500 μg/kg) was injected intraperitoneally. After one week, the passive avoidance behavior was tested in the shuttle box and hippocampal homogenate was prepared. Acetylcholinesterase (AChE) activity and lipid peroxidation (malondialdehyde, MDA) were measured using specific kits. Data were analyzed by SPSS software (version 16).

Results: Step-through latency (STL) in quercetin50-treated LPS group was significantly greater than control group (p<0.05). In addition, AChE activity and level of MDA was significantly lower in quercetin50-treated LPS group versus LPS group (p<0.05). Meanwhile, quercetin at a dose of 10 mg/kg did not have such a significant effect.

Conclusion: Quercetin at a dose of 50 mg/kg has a protective effect on learning and memory impairment due to LPS and part of its beneficial effect is mediated via attenuation of lipid peroxidation and AChE.

Key words: Lipopolysaccharide, Quercetin, Neuroinflammation, Lipid peroxidation, Cholinesterase.

1. Introduction

Development of systemic inflammation is as an important risk factor for occurrence of cognitive decline (1). The elderly people are highly vulnerable to the adverse effects of infections on cognition (2). Greater generation of pro-inflammatory cytokines contributes to development of neuroinflammation (3, 4). Inflammation is also seen in the brains of patients with Alzheimer’s disease (5). Lipopolysaccharide (LPS) is an endotoxin separated from Gram-negative bacteria that could stimulate pro-inflammatory cascades with generation of pro-inflammatory cytokines (6). LPS challenge is associated with cognitive deficit (7). Enhanced oxidative stress (8) and increased acetylcholinesterase activity (9) are usually seen after LPS exposure.
Quercetin is a naturally occurring bioflavonoid that is found in many fruits and vegetables (10). Quercetin have multiple beneficial effects such as anti-cancer, anti-inflammatory and protective effects against neurodegeneration (11-14). In addition, quercetin could inhibit neuronal apoptosis due to cytotoxic treatments (14, 15). It has shown that this flavonoid could attenuate neuroinflammation and may be beneficial for treatment of neural disorders (16-18). Furthermore, quercetin could reduce oxidative damage due to chemotherapeutic agents (19). This study was undertaken to assess the possible efficacy of quercetin against LPS-induced cognitive decline in the rat and to find out some related modes of action.

2. Materials and Methods

2.1. Experimental design

Male albino Wistar rats (Pasteur’s institute, Tehran, Iran, 180-210 g) were kept under standard housing conditions (temperature: 21-23°C; humidity: 40-60%; 12:12 h lighting cycle) with food and water available ad libitum. Procedures involving animals and their care were conducted in conformity with the NIH guidelines for the care and use of laboratory animals.

The rats (n=40) were randomly divided into 5 groups: control, quercetin50-treated control, LPS, and LPS groups receiving quercetin at doses of 10 or 50 mg/kg. To induce systemic inflammation, LPS from Escherichia coli (SigmaAldrich, St Louis, MO, USA; 0111:B4) was injected intraperitoneally at a dose of 500 μg/kg for seven days. Systemic LPS administration is a widely-accepted model for neuroinflammation induction in rodents with elevation of brain cytokines (20, 21). All behavioral experiments were carried out from 11:00 to 16:00 by an experimenter blind to groups and treatments. All animals were sacrificed on day 7 after retention and recall trial of passive avoidance test.

2.2. Passive avoidance test

The used devise was composed of two compartments, i.e. one illuminated and one dark chamber with grid floor connected by a guillotine door. Electric shock was delivered by an isolated stimulator. On the first and second days, each rat was placed into the apparatus and left for 5 min to explore the chambers and to habituate. During the acquisition trial (third day), rats were placed in the illuminated chamber and after a 5 min habituation period, the guillotine door was opened and the latency to enter the dark chamber was recorded. After the rat entering the dark chamber, the door was closed and an electric foot shock (1 mA, 1 s) was delivered to floor grid. On the acquisition day, the initial latency (IL) of entrance into the dark chamber was recorded and rats with ILs greater than 60 s were excluded from the study.

Twenty-four hours later, a retention test was done and the interval between the placement in the illuminated chamber and the entrance into the dark chamber was measured as step-through latency (STL up to a maximum of 240 s as cut-off).

2.3. Determination of hippocampal MDA and AChE

At the end of week 1, hippocampal tissue (n = 5-6 from each group) was separately dissected out and 10% homogenate was prepared in lysate buffer and in the presence of protease inhibitor cocktail and the supernatant was aliquoted and stored at -70°C for the following experiments.

Malondialdehyde (MDA) level in the supernatant was measured as described before (22). For determination of MDA concentration (thiobarbituric acid reactive substances, TBARS), trichloroacetic acid and TBARS reagent were added to supernatant, then mixed and incubated at boiling water for 90 min. After cooling on ice, samples were centrifuged at 5000×g for 10 min and the absorbance of the supernatant was read at 532 nm.

AChE activity was determined on the basis of degradation of acetylthiocholine iodide into a product that binds to 5,5-dithiobis-2-nitrobenzoic acid and turns yellow (23). The kinetics of the reaction was followed spectrophotometrically over 5 min at 412 nm. AChE activity was expressed as mM of substrate hydrolyzed/min/g protein.

2.4. Statistical analysis

All results were expressed as mean ± S.E.M. The parametric test one-way ANOVA was used for data analysis and if a significant difference was found out, pair-wise comparison was done using the Tukey post-hoc test. In all calculations, a difference at p<0.05 was regarded as significant.

3. Results

Figure 1 shows the performance of rats in passive avoidance paradigm as shown by initial (IL) and step-through (STL) latencies. With respect to IL, no significant differences were obtained amongst the groups. Meanwhile, LPS and LPS+Quercetin5050 groups exhibited a significant disturbance of retention and recall in this test (p<0.05-0.01), as it was evident by a lower STL and quercetin50-treated LPS group had a significantly higher STL versus LPS group (p<0.05).
Figure 1. Initial (IL) and step-through (STL) latencies of treated-control and LPS-challenged rats in single-trial passive avoidance task (n = 8 for each group) * P<0.05, ** p<0.01 (vs. control) # p<0.05 (vs. LPS).

LPS-injected rats had a significantly elevated hippocampal level of MDA (Fig. 2) (p<0.05) and treatment of LPS-injected rats with quercetin at a dose of 50 mg/kg significantly decreased MDA (p<0.05) as compared to LPS group.

Figure 2. MDA concentration as a marker of lipid peroxidation in hippocampal lysate from different groups. * p<0.05 (in comparison with control), # P<0.05 (in comparison with LPS). (n=6 for each group).

Figure 3 presents AChE activity in hippocampal lysate from different groups. * p<0.05, ** p<0.01 (in comparison with control), # P<0.05 (in comparison with LPS). (n=6 for each group).

4. Discussion

The obtained results from our study clearly confirmed that quercetin treatment dose-dependently improves LPS-induced cognitive deficits via attenuation of lipid peroxidation and AChE activity. Earlier investigations have shown that passive avoidance test using the shuttle box in rodents involves two independent memory types, short-term memory when retention test is conducted 90 min after training and long-term memory when retention test is conducted 24 h and 7 days after training (24, 25). Since retention and recall trial in passive avoidance task in our study was performed 24 h after the acquisition trial, thus, it is considered a long-term memory that needs protein synthesis process in related synapses. In our study, quercetin treatment may have improved retention and recall in passive avoidance task via affecting synaptic organization, however, there is still no report on this topic that itself warrants more researches. In contrast to our results, one study showed that quercetin targets cysteine string protein (CSPalpha) and could impair synaptic transmission (26).

LPS challenge is also associated with increased production of reactive oxygen species (ROS) and appearance of an imbalance between the oxidant and antioxidant status (27). In this study, we showed a marked elevation of hippocampal MDA level in LPS-challenged group. The increased MDA content of these rats clearly indicate that enhanced lipid peroxidation is involved in the development of cognitive impairment. MDA level significantly decreased in quercetin50-treated LPS group. This may indicate that quercetin at its higher dose may have exerted antioxidant activity and protected the hippocampal tissue against lipid peroxidation.
Brain cholinergic system has a pivotal role in learning and memory processes (28). Part of cognitive dysfunction in metabolic (29, 30) and/or inflammatory (31, 32) diseases is ascribed to reduced cholinergic transmission and its impact on retention and recall of stored information. One of the important regulators of cholinergic signaling pathway is the degrading enzyme AChE. There are conflicting and contrasting reports on the effect of LPS-induced inflammation on AChE activity, some showing its increase (31, 33) and some demonstrating its reduction (34, 35) in the brain region. In conclusion, quercetin at a dose of 50 mg/kg has a protective effect on learning and memory impairment due to LPS and part of its beneficial effect is mediated via attenuation of lipid peroxidation and AChE.

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Conflict of interest

The authors declare that they have no competing interests.

References


