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EXPERIMENTAL STUDY

Effect of quercetin on the brain-derived neurotrophic factor gene expression in the rat brain

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ABSTRACT

INTRODUCTION: Quercetin is a ubiquitous flavonoid found in many plants. Neuroprotective effects of quercetin have been shown in several in vitro and in vivo studies, but its mechanism of action has not been fully defined yet. Brain-derived neurotrophic factor (BDNF) is a fundamental neurotrophin with vital functions in the survival of neuronal cells. In the present study, we aimed to investigate the effects of quercetin on expression of BDNF mRNA in the hippocampus of rat brain.

METHODS: Male rats were daily gavaged with quercetin (10, 20 or 50 mg/kg·bwt) for 30 days. Hippocampal levels of the BDNF transcripts were assessed using quantitative (q) RT-PCR.

RESULTS: Quercetin at doses of 20 and 50 mg/kg caused a significant increase in the mRNA expression of BDNF as compared with the control group. Quercetin treatment at a dose of 10 mg/kg failed to cause any significant changes in the levels of BDNF mRNA

CONCLUSION: Our findings suggest that the neuroprotective effects of quercetin may be at least partly due to its inducing effects on the expression levels of the BDNF mRNA *(Fig. 1, Ref. 40)*. Text in PDF *www.elis.sk.*

KEY WORDS: BDNF, quercetin, hippocampus, rat.

Introduction

Neurodegenerative diseases are mostly age related diseases, resulting from deterioration of neurons or their myelin sheaths that will lead to dysfunction and disability (1). Growing body of evidence suggests that neuro-inflammation and oxidative stress are implicated in the pathophysiology of neurodegenerative diseases such as Parkinson's disease (PD) and Alzheimer's disease (AD) (2).

Plant metabolites including flavonoids and alkaloids being powerful antioxidants, play protective roles in several oxidative stress-mediated diseases (3, 4). A growing body of evidence from animal models to clinical studies indicates that dietary flavonoids protect neurons against injury and promote memory, learning and cognitive function (3, 5). Neuroprotective effects of flavonoids are mainly explained by their free radical scavenger properties, involvement in intracellular signaling pathway and mitochondrial dysfunction, as well as control of processes as inflammation, apoptosis, and production of BDNF (6). In our previous studies, we showed that the polyphenol stilbene, resveratrol, increased the expression of BDNF transcripts in the hippocampus of rats (7).

Quercetin (3,5,7,30,40-pentahydroxyflavone), is a member of the flavonoid family of polyphenols that is generally found in fruits and vegetables such as onions, apples and blueberries. Quercetin attracted intense interest for its potentially beneficial effects on human health. The protective effects of quercetin against various diseases such as pulmonary diseases, cardiovascular diseases, cancer, and neurodegenerative disorders have been shown by many researchers in epidemiological studies (8, 9).

Quercetin is shown to modulate the intracellular signaling pathways. The effects of quercetin on the expression of pro-survival proteins appear to be an important mechanism for its neuronal protection properties. Quercetin also regulates the activity of kinases, changing the phosphorylation state of target molecules, resulting in modulation of cellular function and gene expression (10, 11).

A growing body of evidence indicates that neurotrophic factors such as BDNF play key roles in the development and survival of neurons (12). BDNF is the most abundant and widely distributed neurotrophin in the mammalian CNS and is expressed throughout the brain, with the highest levels in the neurons of hippocampus (13). In the adult brain, neurotrophins, in particular BDNF, are involved in learning and memory, but they can also support the survival and regenerative sprouting of damaged neurons in the brain. Alterations in BDNF expression in specific neuron subpopulations contribute to various pathologies, including depression, epilepsy, Alzheimer's, Huntington and Parkinson's disease (14, 15). Increased expression of neurotrophic factors in vitro and in vivo is one of the mechanisms of neurotrophic action of flavonoids and is a good therapeutic candidate for neurodegenerative diseases (6).

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Therefore, we hypothesized that the neuroprotective effects of quercetin may be due to increased expression of the BDNF gene. Here, we have investigated BDNF expression in hippocampal tissues of rats treated by oral quercetin.

Materials and methods

Subjects

A total of 21 Male Sprague–Dawley rats weighing 250 ± 20 g were provided by the Animal Breeding Center of Shiraz University of Medical Sciences (Shiraz, Iran). Rats were housed in a temperature-controlled room (22 ± 2 °C) and fed with food and water ad libitum on a 12-h light/dark cycle. Ethics Committee of Shiraz University of Medical Sciences approved these animal experiments.

Treatment schedules and tissue preparation

Quercetin (Sigma Chemical, USA) was uniformly dispersed in saline containing 10 % ethanol. Rats were randomly assigned to five groups of three animals each. Experimental groups were treated as follows: Saline (C), Ethanol 10 % (vehicle), quercetin in doses of 10 mg/kg (Q10), 20 mg/kg (Q20) and 50 mg/kg (Q50). Quercetin treatment was administered orally once a day using a gavage syringe for 30 days. The same procedure was used for treating the control group and vehicle group with saline and ethanol, respectively. At the end of the experimental period, animals were sacrificed by decapitation and hippocampal tissues were separated and then stored at –70 °C until analysis.

RNA isolation and cDNA synthesis

Hippocampal tissues were subjected to RNA extraction using Tripure Isolation Reagent (Roche Applied Sciences, Indianapolis, IN), according to manufacturer protocol. The concentrations of extracted RNA were determined by measuring the absorbance at 260 and 280 nm and the quality of RNA was determined from the absorbance ratio of A260/A280 (A260/A280 > 1.8). 4µg of total RNA was used for cDNA synthesis in a total volume of 20 µL by using RevertAid ™ First Strand cDNA Synthesis Kit (Fermentas, EU). Synthesized cDNA was kept at –70 °C until used.

Real-time qRT-PCR

Real-Time qRT-PCR was performed with a Bio-Rad multicolor real time PCR detector, and iQ5 thermal cycler system (Bio-Rad). The level of expression of the BDNF gene was normalized to the level of expression of the GAPDH gene. Primers for forward 5'-GTGACARTATTAGCGAGTGGG-3' and reverse 5'-GGGTAGTTCGGCATTGC-3' were used to detect BDNF, producing a 212 bp fragment (16). GAPDH primers for forward 5'-TCACCCAACACTGTGCCCATCTACGA-3' and for reverse 5'-TCGGTGAGGATCTTCATGAGGTA-3' were used to produce a 380 bp fragment (17). PCR reaction was performed under the following conditions: An initial denaturation at 95 °C for 10 min and then 45 cycle of denaturation at 95 °C for 40 s seconds, annealing at 58 °C for 45 s, extension at 72 °C for 40 s, and a final extension at 72 °C for 10 min. All experiments were run in duplicate. $2^{-\Delta\Delta CT}$ method was used to calculate the relative expression level (fold changes) of the BDNF gene in the hippocampal tissues of test rats to those of control animals.

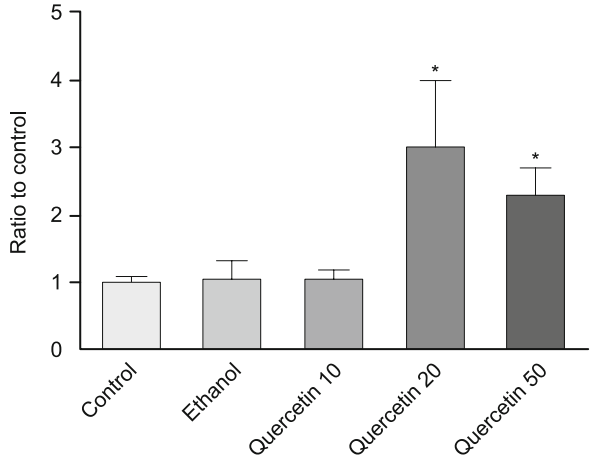

Fig. 1. Comparison of BDNF gene expression between test and control groups in the hippocampus of rats fed with different doses of quercetin (10, 20 and 50 mg/kg bwt) using quantitative real-time PCR. The relative expression level of each sample was calibrated by the comparative threshold cycle method, using GAPDH as an endogenous control. Data are presented as the ratio of levels of mRNA for BDNF gene in treated rats to the average of those in the saline treated animals. One-way ANOVA and a post hoc LSD test were performed for analyzing the data. Data are mean ± SEM. The asterisk represents a significant increase in the relative expression of BDNF transcript ($p < 0.05$) as compared with the control group.

Statistical analysis

All data are represented as mean ± SEM. One-way ANOVA with LSD post-hoc test was used for comparison between ratios of BDNF mRNA expression in the test and control samples. A p value of < 0.05 was considered as statistically significant. Data were analyzed with SPSS10 analytic software (SPSS, Inc.,Chicago).

Results

To obtain more detailed information about neuroprotective mechanisms of quercetin in brain, we investigated effects of various doses (10, 20 and 50 mg/kg bwt) of this compound on the mRNA expression of BDNF in the brain. As shown in Figure 1, treatment of rats by quercetin at doses of 20 and 50 mg/kg bwt caused a significant ($p < 0.05$) upregulation of mRNA expression of BDNF as compared to control groups. To test the possible interference of ethanol with our data, we orally treated a group of rats by ethanol 10 % for the same period of time as described in the methods section. Our data showed that the levels of BDNF mRNA in the hippocampus of ethanol group of rats were not significantly different from those of saline treated animals. Therefore, ethanol 10 % did not affect our results.

Discussion

In the present study, to investigate the possible mechanisms of neurotrophic actions of quercetin (3), we determined the expression of BDNF using real-time PCR in the hippocampus of rats treated with this compound. Our results showed that quercetin could increase the expression of BDNF. Significantly increasing effects of oral quercetin on expression levels of BDNF mRNA were observed when rats received 20 or 50 mg/kg bwt of quercetin, but at a dose of 10 mg/kg bwt quercetin failed to induce expression of BDNF.

The bioflavonoid quercetin which is widely distributed in fruits and vegetables (9) has drawn much attention for its key biological functions, such as anti-oxidant properties as well as neurotrophic and anti-carcinogenic activities (18). In vitro and in vivo studies have shown that neuroprotective effects of quercetin are exerted by different molecular mechanisms (19). Our study, in converge with these works, suggested a new possible mechanism for neuroprotection by quercetin. BDNF and its signaling pathway is one of the most important of factors involved in neuroprotection (20). Results of the present study show that quercetin up-regulates the expression of BDNF mRNA in the hippocampus. Our observation is in line with a recent report showing that quercetin increased expression of BDNF and attenuated oxidative stress in the hippocampus of rats with high fat diets (21).

Quercetin has also shown protective effects against Aβ-induced toxicity in Alzheimer's disease (22). Indeed, quercetin destabilizes and increases the clearance of abnormal proteins such as beta-amyloid peptide and also hyperphosphorylated tau protein (23). Other neuroprotection mechanisms have been postulated for quercetin including regulation of synaptic plasticity, learning and neuronal adaptation via signaling pathways of the PGC-1α/FNDC5/BDNF, ERK/CREB/BDNF, PI3K/AKT/Nrf2 and activation of SIRT1 (22–24). Therefore, quercetin interactions with these pathways may be responsible for its role in the central nervous system. In contrast, another result suggests that acute administration of quercetin impairs cognitive function by suppression of pAkt, which, in turn, decreases PCREB expression in the hippocampus (25).

Many studies state that oxidative stress could be the major cause of neurodegenerative disorders such as Parkinson and Alzheimer's disease (26, 27). In neuronal culture, quercetin increases survival against oxidative insults and appears important for neuronal protection (28). Studies in animal models have also provided supportive evidence for neuroprotective effects of quercetin (3). These activities of quercetin have been attributed mainly to its antioxidant capacity, such as its free radical scavenger activity and by the induction of Nuclear factor (erythroid-derived 2)-like 2 and the consequent upregulation of antioxidant enzymes (28, 29). There are some studies on the in vivo effects of quercetin in patients suffering from a disease which is associated with oxidative stress such as high blood pressure (30), sarcoidosis (31) and rheumatoid arthritis (32).These studies suggest that quercetin improves antioxidant defense system and reduces pro-inflammatory cytokines such as TNFα and IL-8. Several studies have also demonstrated that various polyphenols with neuroprotective activity such as resveratrol, apigenin, luteolin and green tea increase expression of BDNF mRNA (33–35). In our previous studies we showed that oral resveratrol induced the expression of various BDNF transcripts in the hippocampus of rats (7, 36).

It is noteworthy that quercetin has also been reported to display some adverse health effects. Toxic effects of quercetin are attributed to oxidized products produced during its anti-oxidative activities. These oxidized products are able to attack thiol groups in proteins (29).

It is well established that neurotrophic family members are required for development and survival of neuron (37). BDNF is the most promising neurotrophic factors in the survival and regenerative sprouting of damaged neurons in the brain (38). Animals with reduced expression of BDNF showed deficits in learning and memory (39). Nowadays, increased attention is observed towards strategies aimed at inducing the expression of endogenous neurotrophic factors including BDNF as a way for preserving neurons or for restorative treatment of neurodegenerative diseases (40). Presently, we have observed that treatment with oral quercetin increased BDNF levels in the rat hippocampus. This finding suggests that quercetin has neurotrophic potential and may have beneficial effects on the survival of hippocampal neurons. Accordingly, agents like quercetin that induce the expression of BDNF are conceivable to mimic the biological effects of neurotrophin and have therapeutic values for neurodegenerative diseases.

In conclusion, the present findings are one of the earliest studies showing that oral quercetin increases the expression of BDNF gene in the hippocampus of rats. These results might explain the molecular basis of neuroprotective properties of quercetin.

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