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SHORT COMMUNICATION

The effects of quercetin on the gene expression of the $GABA_A$ receptor α5 subunit gene in a mouse model of kainic acid-induced seizure

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Abstract The flavonoid quercetin has recently been reported to have neuroprotective effects, and the role of the gamma-aminobutyric acid A alpha 5 subunit ($GABA_A$ α5) receptor has been determined in some nervous system disorders. The aim of this study was to identify the molecular mechanism of the effect of quercetin administered at anticonvulsive doses on the expression of the $GABA_A$ α5 receptor gene in kainic acid (KA)-induced seizures in mice. The experimental animals were divided into four groups: control, KA, and KA + quercetin at 50 or 100 mg/kg, respectively. The results showed a dose-dependent reduction in the behavioral seizure score with quercetin pre-treatment in the KA mouse model. Two hours after the end of the 7-day treatment regimen, expression of the $GABA_A$ α5 receptor gene in the hippocampus was found to be increased in the KA group, but this increase was reduced in the KA + quercetin 50 or 100 mg/kg treatment groups. These results suggest that expression of the $GABA_A$ α5 receptor could be a mechanism for reducing seizure severity or may be a marker of seizure severity. Further studies are necessary to clarify quercetin's mechanism of action and the relation of $GABA_A$ α5 receptor gene expression to seizure severity.

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Introduction

Epilepsy is one of the most common neurological disorders and affects approximately 1 % of the general population [1]. Most of the antiepileptic drugs currently available either control or reduce the occurrence of seizure. However, about one-third of patients with epilepsy have a refractory form of the disease. Temporal lobe epilepsy (TLE) is a form of partial epilepsy in adults [2]. Kainic acid (KA) is used to induce TLE in model systems and causes neuropathological and electroencephalographic manifestations that are observed in patients with TLE [3]. KA is a potent excitotoxin [4], and its effects are mediated through changes in the expression of the gamma-aminobutyric acid A alpha 5 subunit ($GABA_A$ α5) receptor in the hippocampus [5].

γ-Aminobutyric acid (GABA) is one of the major inhibitory neurotransmitters in the central nervous system. It acts on receptors coupled to chloride channels [6], controlling neuronal excitability by activating $GABA_A$ receptors on neurons by two major modes—phasic and tonic. Phasic inhibition is mediated by synaptic receptors, and tonic inhibition is mediated by extrasynaptic receptors [7]. Tonic inhibitory conductance is predominantly mediated by the $GABA_A$ α5 receptor [8], which is expressed in a number of areas of the brain but expressed at a higher level in the dendritic membrane of the principal cells of the hippocampus [9].

Quercetin is a flavonoid found in vegetables and fruits that has several biological effects [10], including antioxidative [11] and anti-inflammatory [12, 13] activities.

Various studies have shown that quercetin has neuroprotective properties in central nervous system disorders, including memory impairment [14–16], seizure [17, 18], Huntington [19], and Parkinson's disease [20]. In an earlier study we showed that quercetin has anticonvulsant activity in acute and chronic models of chemical kindling induced by pentylenetetrazole (PTZ) [15, 18]. More recently, Schipper et al. suggested that tonic $GABA_A$ receptors are a potential target for the treatment of TLE [21].

The aim of this study was to determine the molecular mechanism of the antiseizure effects of quercetin on the expression of the $GABA_A$ α5 receptor gene in a KA model of epilepsy in mice.

Materials and methods

A total of 48 male BALB/c mice (body weight 20–25 g) were obtained from the Razi Institute (Karaj, Iran) and housed under standard laboratory conditions. The mice were maintained at constant room temperature (21 ± 2 °C) under a 12:12 h light:dark cycle with free access to food and water. All animal experiments were performed in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) to minimize the number of animals used and their suffering.

Quercetin and KA were purchased from Sigma (St. Louis, MO). Other drugs used in the study included xylazine (Loughrea Co., Galway, Ireland) and ketamine (Rotexmedica GmbH, Trittau, Germany). Quercetin was dissolved in Tween 80 (0.8 % v/v) and KA was dissolved in saline.

The mice were divided into four groups of 12 animals each. The control group was given an intraperitoneal (i.p.) injection of saline + Tween 80 (10 ml/kg) daily for 7 days; on the last day, saline was injected 30 min after the administration of saline + Tween 80. The KA group was given an i.p. injection of saline (10 ml/kg) daily for 7 days; on the last day, KA (10 mg/kg, i.p.) was injected 30 min after the administration of saline. In the two treatment groups, the mice were given an i.p. injection of quercetin at either 50 and 100 mg/kg daily for 7 days; on the last day, KA (10 mg/kg, i.p.) was injected 30 min after the administration of quercetin.

Following the administration of KA, mice were observed for behavioral changes over a period of 2 h. The behavioral scores were as follows: 0, no response; 1, immobility; 2, rigid posture; 3, scratching/circling/head bobbing; 4, forelimb clonus/rearing/falling; 5, repetitive pattern of 4; 6, severe tonic–clonic seizures [22]. Two hours after the administration of KA, all animals were anesthetized with an i.p. injection of ketamine (60 mg/ml)/xylazine (6 mg/kg) and sacrificed. The hippocampus each animal was immediately removed, cleaned with chilled saline, and frozen until used for the molecular analysis.

In the molecular analysis, frozen hippocampus tissues were homogenized and total RNA was extracted using the Total RNA Extraction kit of Jena Bioscience GmbH (Jena, Germany). The extracted RNA was then reverse transcribed using the Revert Aid First Strand cDNA Synthesis kit (Thermo Scientific–Fermentas, Waltham, MA).

The primers used were GABRA5 (F: AGTTGGAGGCAAGAACAGTT; R: AAGGAGGGTTTGGGTCATG) for the target gene and β-actin (F: TTACTGAGCTGCGTTTTACAC; R: ACAAAGCCATGCCAATGTTG) for the β-actin gene (internal control). All primers were designed using Gene Runner software (version 3.05). Quantitative reverse transcription (RT)-PCR was used to detect $GABA_A$ α5 RNA content in hippocampal tissues. A multiplex real-time PCR assay using SYBR Green I was performed in final reaction volumes of 20 µl containing 10 µl of SYBR Green I Master Mix (Bioneer, Korea), 10 pmol of forward and reverse primers, and 20 ng total RNA-derived cDNAs. Thermal cycling was performed using the ABI-7500 Sequence Detection System (Applied Biosystems, Foster, CA) with cycling conditions of 10 min at 95 °C for the first denaturation step, followed by 40 cycles at 95 °C for 20 s and 58 °C for 45 s; dissociation was run at 95 °C for 15 s, 60 °C for 1 min and 95 °C for 15 s. The $2^{-\Delta\Delta Ct}$ method was used to quantify data [23].

Data were expressed as the mean ± standard error of the mean. The data reported were analyzed by using a one-way analysis of variance accompanied by post hoc Turkey test for multiple comparisons. The analysis was completed using GraphPad Prism software (v5.04; GraphPad Software, Inc., La Jolla, CA). $P < 0.05$ was considered to represent a statistically significant difference.

Results and discussion

In the mice model used in our study, KA administered at a dose of 10 mg/kg caused seizures. Quercetin administered at 50 and 100 mg/kg, respectively, reduced the seizure score in animals in a dose-dependent manner compared to the KA group ($P < 0.05$ and $P < 0.001$, respectively) (Fig. 1).

Expression of the $GABA_A$ α5 receptor gene was increased in the KA group compared to the control group at 2 h after the administration of KA ($P < 0.001$). The administration of quercetin doses at both 50 and 100 mg/kg significantly decreased the expression of the $GABA_A$ α5 receptor gene compared to the KA group ($P < 0.001$). The difference in expression of the $GABA_A$ α5 receptor gene in the quercetin and control groups was not significant (Fig. 2).

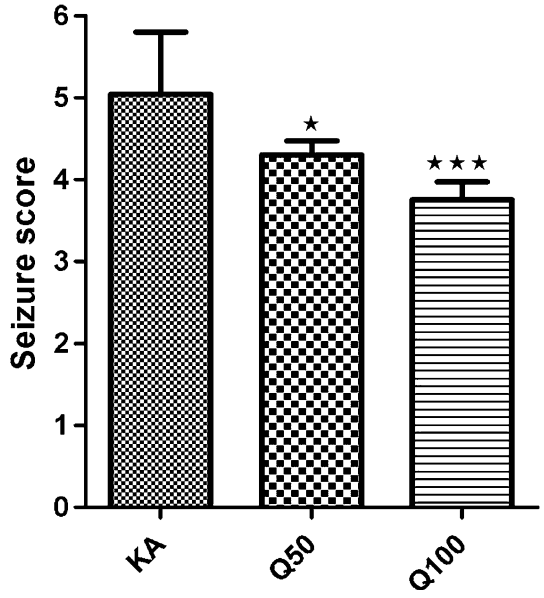

Fig. 1 The effects of quercetin (doses 50 and 100 mg/kg) on seizure scores in mice with kainic acid (*KA*)-induced seizures. The KA group was administered an intraperitoneal (i.p.) dose of KA for 7 consecutive days, and on the last day, KA (10 mg/kg, i.p.) was injected 30 min after the administration of saline. The treatment groups were administered an i.p. dose of quercetin, either 50 (*Q50*) or 100 (*Q100*) mg/kg, for 7 days, and on the last day KA (10 mg/kg, i.p.) was injected 30 min after the administration of quercetin. Data are expressed as the mean ± standard error of the mean (SEM). *Asterisks* indicate a significant difference ($*P < 0.05$, $***P < 0.001$) compared to the KA group according to the Tukey–Kramer test. $n = 12$ mice in each group

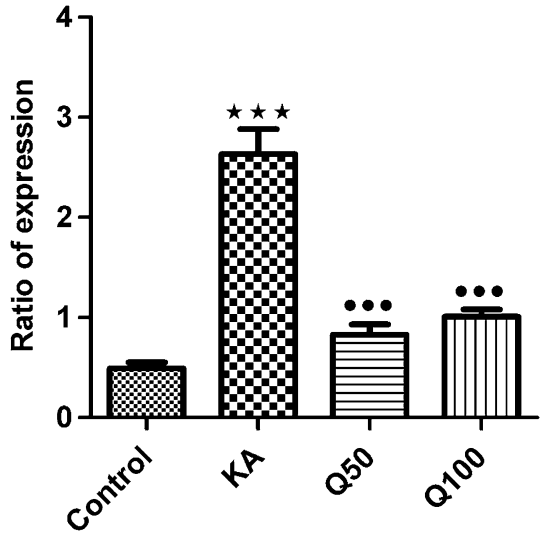

Fig. 2 Effect of quercetin on the mRNA ratio of expression of the $GABA_A$ α5 receptor gene in a KA model of seizure in the hippocampus of mice. Data are expressed as the mean ± SEM. ***Significant difference at $P < 0.001$ compared to controls, •••significant different at $P < 0.001$ compared to the KA group according to the Tukey–Kramer test. $n = 12$ mice in each group

The results of our study show a dose-dependent reduction in the behavioral seizure score with quercetin pretreatment in a KA mouse model of epilepsy, as well as a reduction in the expression of the $GABA_A$ α5 gene. In a previous study, we found that quercetin administered at anti-convulsive doses provided protection against memory impairment caused by PTZ-induced chemical kindling [17]. Other studies have also reported an increase in $GABA_A$ α5 expression in KA and pilocarpine models [24–26].

Increased expression of GABA receptors has been reported in epilepsy, suggesting that compensatory mechanisms are involved in disease pathogenesis [26]. Long-lasting decreases in the mRNA levels of the α5 and α2 $GABA_A$ receptor subunits in the CA1 area of the hippocampus and increases in α5 in the dentate granule cell layer have been observed in a pilocarpine model of epilepsy [27].

In a model of febrile seizures, the administration of lipopolysaccharide/KA to rat pups increased the protein levels of the $GABA_A$ α5 receptor concomitant with increasing interictal-like hippocampal activity and excitation of the Shaffer collateral–CA1 pathway in adult rats [28]. The authors of this study suggested that increased levels might be related to a compensatory mechanism [28]. It has also been shown that status epilepticus (SE) induced by KA causes early pyramidal neuron loss in the hippocampus with a transient increase in $GABA_A$/central benzodiazepine (cBZR) density. These results suggest that an increase in the GABA/cBZR expression for each neuron might be a neuroprotective mechanism against the excitotoxic effects of SE to protect cells against further seizures [29]. Pretreatment with quercetin (100 mg/kg) has been found to have a modulatory effect on the expression of the $GABA_A$ subunits β1 and β3 receptor genes in a KA model of epilepsy [30]. However, flavonoids have also been shown to have an effect on ionotropic GABA receptors by acting as a positive, neutralizing allosteric modulator, suggesting that they could act on a variety of modulatory receptors [31]. Quercetin administered at a dose of 30 μM inhibited α1β1γ2 $GABA_A$ and ρ1 GABAc receptors in *Xenopus laevis* oocytes [32].

In conclusion, the expression of $GABA_A$ α5 may be a mechanism for reducing seizure severity or may, as a response to seizure activity, be a marker of seizure severity. A better understanding of the time course of changes in the expression of the $GABA_A$ α5 gene and quantification of seizure severity (e.g., by electroencephalography) are necessary to clarify quercetin's mechanism of action and the relation of α5 gene expression to seizure severity.

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Compliance with ethical standards

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Ethical approval All applicable international national, and/or institutional guidelines for the care and use of animals were followed.

Conflict of interest All of the authors declare that they have no conflicts of interest.

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