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The Role of Quercetin in Gene Expression of GluR1 Subunit of AMPA Receptors, and NR2A and NR2B Subunits of NMDA Receptors in Kainic Acid Model of Seizure in Mice

Sahar Moghbelinejad,[1] Ghazaleh Mohammadi,[2] Fatemeh Khodabandehloo,[2] Reza Najafipour,[1] Taghi Naserpour,[1] Zahra Rashvand,[1] and Marjan Nassiri-Asl[1,*]

[1]Cellular and Molecular Research Centre, School of Medicine, Qazvin University of Medical Sciences, Qazvin, Iran
[2]Departmant of Molecular Medicine, Qazvin University of Medical Sciences, Qazvin, Iran

[*]*Corresponding author*: Marjan Nassiri-Asl, Cellular and Molecular Research Centre, School of Medicine, Qazvin University of Medical Sciences, Box: 341197-5981, Qazvin, Iran. Tel: +98-2833336001, Fax: +98-2833324971, E-mail: marjannassiriaslm@gmail.com

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Abstract

Background: Flavonoids are recently being recognized for their important biological effects. Quercetin is a flavonoid that has several pharmacological properties including anti-inflammatory, neuroprotective, and anticonvulsant effects.
Objectives: This study was designed to elucidate the role of quercetin in the gene expression of ionotropic glutamate receptor subunits in kainic acid (KA)-induced seizure in mice.
Methods: This experimental study was conducted on 56 male BALB/c mice. Quercetin (50 and 100 mg/kg, intraperitoneally) was administered 7 days before administration of KA. The hippocampi of the animals were removed and used for molecular analysis 2 hours and 7 days after KA administration.
Results: Pretreatment of mice with quercetin (100 mg/kg) significantly enhanced the gene expression of GluR1 subunit of AMPA and NR2A and NR2B subunits of NMDA only 7 days after KA administration compared to the control and KA groups in the mice hippocampus ($P < 0.001$, $P < 0.001$, and $P < 0.05$, respectively).
Conclusions: Increment of gene expression of subunits of AMPA and NMDA receptors by quercetin might explain its protective effect on synaptic plasticity and memory.

Keywords: Quercetin, Kainic Acid, AMPA Receptors, NMDA Receptors

1. Background

Epilepsy is one of the most common neurological disorders affecting approximately 1% of the general population (1). About one-third of the patients have refractory epilepsy, and temporal lobe epilepsy (TLE) is a form of partial epilepsy in adults (2). Most of the available antiepileptic drugs could control or reduce the occurrence of seizure. Seizures induced by kainic acid (KA) exhibits neuropathological and electroencephalographic manifestations in patients with TLE (3). Fast excitatory glutamatergic neurotransmission in the central nervous system is mediated by ligand-gated or ionotropic glutamate receptors, primarily the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subtype. AMPA receptors have a critical role in excitatory glutamatergic neurotransmission. There are four AMPA receptor subunits including GluA1 - 4 that are encoded by separate genes (4, 5).

The N-methyl-D-aspartate (NMDA) receptors are among the other ionotropic glutamate receptors. They are tetrameric ligand-gated ion channels comprising GluN1, GluN2, and GluN3 subunits. Two different GluN2 subunits (GluN2A, GluN2B) have been identified in most NMDA receptor-expressing cells (6). In the adult mouse hippocampus, NR2A and NR2B are the predominant NR2 subunits of NMDA receptor (7).

Quercetin is a flavonoid that is found in vegetables and fruits with several biological effects (8) including antioxidative (9) anti-inflammatory (10), and neuroprotective (11) activities. We have earlier shown that quercetin has anticonvulsant activity in acute and chronic models of pentylenetetrazole (PTZ) (12, 13). Pretreatment with quercetin (100 mg/kg) had a modulatory effect on β1 and β3 subunits of GABAA receptor gene expression in a KA model of epilepsy (14). It is important to know that at anticonvulsant doses, quercetin causes molecular changes and could modulate glutamate receptors. This led us to find new target for treatment of epilepsy.

2. Objectives

Thus, in the present study, we investigated the effect of quercetin on the expression of genes encoding GluR1 subunit of AMPA receptor as well as NR2A and NR2B subunits of NMDA receptors in KA-induced seizure in hippocampus.

3. Methods

3.1. Animals

A total of 56 male BALB/c mice (20 - 25 g) were obtained from the Razi Institute (Karaj, Iran) and housed in groups of four per cage under standard laboratory conditions. They were maintained at constant room temperature (21 $\pm$ 2°C) under a 12: 12 hour's light-dark cycle with free access to food and water. All animal experiments were performed in accordance with the European communities council directive of November 24, 1986 (86/609/EEC), so as to minimize the number of animals used and their suffering.

3.2. Drugs

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

3.3. KA Administration and Experimental Design

In this experimental study, mice were divided into four groups. The control group (n = 8) was administered an intraperitoneal (i.p.) injection of saline + Tween 80 (10 ml/kg) daily for 7 days, and on the last day, saline (10 mL/kg, i.p.) was injected 30 minutes after administration of saline.

The kainic group (n = 16) was administered saline (10 ml/kg, i.p.) daily for 7 days, and on the last day, KA (10 mg/kg, i.p.) was injected 30 minutes after administration of saline. In the two treatment groups (n = 16 in each group), quercetin (50 and 100 mg/kg, i.p.) was administered daily for 7 days, and on the last day, KA (10 mg/kg, i.p.) was injected 30 minutes after administration of quercetin.

Then, half of the mice in each group after 2 hours and another half after 7 days of KA administration were anesthetized with i.p. injection of ketamine (60 mg/mL)/ xylazine (6 mg/kg) and were sacrificed. The hippocampi of the animals were removed, cleaned with chilled saline (0.9%), and used for molecular analysis.

3.4. Quantitative Real-Time Polymerase Chain Reaction

Total RNA from the hippocampus was extracted using the total RNA extraction kit (Jena Bioscience, GmbH, Jena, Germany). Then, the RNA was reverse transcribed using the Revert Aid First Strand cDNA Synthesis Kit (Thermo Scientific Fermentas, Waltham, MA, USA). The beta-actin gene was used as an internal control for the quantification of target genes expression. All primers for Gria2, Grin2A, and Grin2B genes were designed using Gene Runner software (version 3.05) as follows: Gria2 F: TGGCTTCCTCTATGGATGCT, R: TTGCTATGGCAGGTGAAGAG; Grin2A F: GGGATGACCAACGCTTAGTT R: CCTCAAGGATGACCGAAGAT; Grin2B F: CCCACAGGGAAATCTGTCTT R: CCCACAGGGAAATCTGTCTT; beta-actin F: TTACTGAGCTGCGTTTTACAC R: ACAAAGCCATGCCAATGTTG. Quantitative real-time PCR was used to detect the mRNA content of these genes in the hippocampal tissues. The SYBR Green I real-time PCR assay was performed in final reaction volumes of 20 μL with 10 μL of SYBR Green I Master Mix (Bioneer, Korea), 10 pmol of forward and reverse primers, and 20 ng of total RNA-derived cDNAs. Thermal cycling was performed using the calibrated ABI-7500 (Applied Biosystems, Foster, CA, USA) Sequence Detection System under the following cycling conditions: 10 minutes at 95°C for the first denaturation step, followed by 40 cycles at 95°C for 20 seconds and 60°C for 45 seconds. Each complete amplification stage was followed by dissociation stage at 95°C for 15 seconds, 60°C for 1 minute, and 95°C for 15 seconds. The values of cycle threshold (Ct) obtained in quantification were used to calculate fold changes in mRNA abundance according to the $2^{-\Delta\Delta Ct}$ method (15).

3.5. Statistical Analysis

Data were expressed as mean $\pm$ SEM and tested with analysis of variance (ANOVA), followed by the multiple comparison test of Tukey-Kramer. The analysis was completed using Prism software (5.04). $P < 0.05$ was considered to be statistically significant.

4. Results

All mice survived and were used for molecular analyses. There were no significant changes in the hippocampal GluR1 subunit gene expression levels between the KA group after 2 hours and after 7 days of KA administration and those in the control group animals (Table 1) ($P > 0.05$). However, the level of GluR1 gene expression in the KA group decreased after 7 days. It was observed that only quercetin at a dose of 100 mg/kg could significantly increase GluR1 subunit gene expression compared to the control and KA groups 7 days after KA administration ($P < 0.001$), whereas

the expression level of the same gene at quercetin dose of 50 mg/kg was not significant in the hippocampus.

No significant difference was observed between the level of NR2A subunit gene expression in the KA group and that in the control group after 2 hours and 7 days of KA injection in the hippocampus (Table 1) ($P > 0.05$). However, quercetin (100 mg/kg) significantly increased the level of NR2A subunit gene expression when data were compared to those in the control and KA groups 7 days after KA administration in the hippocampus ($P < 0.001$).

There was also no significant difference between the control and KA groups in the expression levels of NR2B subunit gene after 2 hours and 7 days of KA injection (Table 1) ($P > 0.05$). Quercetin at a dose of 100 mg/kg significantly increased the level of NR2B subunit gene expression compared to that in the control and KA groups on 7 days after KA administration in the hippocampus ($P < 0.05$).

5. Discussion

This study demonstrated that the gene expression levels of GluR1 subunit of AMPA receptor and NR2A and NR2B subunits of NMDA receptors were not significantly different in the KA group than the control group after 2 hours or 7 days of KA administration. However, pretreatment with quercetin (100 mg/kg) increased the gene expression of GluR1 and NR2A and NR2B subunits of glutamate receptors 7 days after administration of KA.

Status epilepticus for 1 hour could inhibit the expression of GluR1 - 4 subunit in the hippocampus of immature rats and adult rats (16). However, the role of reduction of AMPA receptors in epileptogenesis is still not clear. Moreover, Solomonia et al. (2010) (17) reported that there was no change in the expression of GluR1 subunit of AMPA receptor after 28 - 30 hours of KA-induced status epilepticus. However, they observed that on the 28^{th} day of study, the GluR1 levels reduced in the hippocampus of KA-treated animals. Our results are consistent with their results, wherein the gene expression of GluR1 in the KA group did not change significantly after 2 hours or 7 days of KA administration. However, quercetin (100 mg/kg) increased the expression of this gene. As we had shown earlier that quercetin has anticonvulsant activities and inhibitory effects on lipid peroxidation (12, 13), we used KA to study any induced subclinical change.

Lee et al. (2010) (18) showed that reversible phosphorylation of AMPA receptor GluR1 subunit is important in the long-term potentiation (LTP) and long-term depression (LTD). It seems that GluR1 involves in mediating the synaptic plasticity in the Schaffer collateral synapses onto CA1. Therefore, it is possible that quercetin has an important role in LTP and synaptic plasticity.

In patients with focal cortical dysplasia associated with epilepsy, mRNA expression of GluR1 reduces in both dysplastic and heterotopic neurons. Furthermore, mRNA expression of NR2A and NR2B decreased and increased, respectively, in dysplastic compared to heterotopic and pyramidal neurons (19). On the other hand, an immediate increment in the expression of GluR1 and GluR2 was reported after seizure (20) and repeated administration of AMPA receptor antagonists 48 hours after seizures could prevent long-term increases in seizure susceptibility and seizure-induced neuronal injury in the hippocampus at postnatal days 28 and 30 (21). Experimental limbic seizure made changes in the protein expression of AMPA receptor subunits of GluR1 and GluR2 between 6 and 96 hours after systemic injection of KA (22).

One important reason for the controversial results in expression is the fact that the study populations (animal or human), the model of seizure and applied toxin, and the method for detection of changes are different. In addition, some studies have focused on mRNA and others on protein changes, which makes them difficult to compare.

Recurrent seizures induced by tetanus toxin or flurothyl in infancy downregulated the expression of NR2A and the associated scaffolding protein, PSD95, in both hippocampus and neocortex. An interesting matter in this study is that the postnatal day and numbers of seizures were important in the intensity of NR2A downregulation (23). However, recurrent seizures in adult mice could not alter NR2A expression. It was suggested that the developing brain has a compensatory mechanism to reduce the excitability of neurons against the hyperactivity of recurrent seizure. On the other hand, the role of NMDA receptors in learning and memory has been established, and reduction of NMDA receptors could disturb the maturation of synapses (23). In the knockout mice without NMDA receptors, spatial learning and memory were impaired (24).

Kinesin superfamily motor protein 17 (KIF17) is a candidate transporter of NMDA receptor NR2B subunit. It was shown that this protein is necessary to preserve NR2A/NR2B levels for memory process including acquisition, consolidation, and retrieval (7). In addition, it is clear that NR2B is essential to maintain NR2A levels in neurons (25) and parallel to this study, micro-RNA (miRNA) interference knockdown and pharmacological approaches showed that loss of NR2B function results in decreased levels of NR2A via an increase in the proteasome-mediated degradation of NR2A (7). The critical role of GluN2 subunits in increasing learning and memory has been established in the adult mouse brain. There is a relationship between GluN2 subunit motifs, synaptic plasticity, and memory enhancement (26).

Table 1. Effect of Quercetin on mRNA Ratio of Expression of GluR1, NR2A, and NR2B in KA-Induced Seizure in the Hippocampus of Mice[a,b]

Treatment	Glu	P Value	NR2A	P Value	NR2B	P Value	Statistical Test
Control	1.3 ± 0.21	> 0.05	0.28 ± 0.11	> 0.05	0.27 ± 0.09	> 0.05	Tukey-Kramer
KA-2 hours	1.29 ± 0.58	> 0.05	0.27 ± 0.09	> 0.05	0.24 ± 0.20	> 0.05	
KA-7 days	1.098 ± 0.41	> 0.05	0.3 ± 0.16	> 0.05	0.28 ± 0.15	> 0.05	
Q50-2 hours	0.96 ± 0.37	> 0.05	0.31 ± 0.21	> 0.05	0.24 ± 0.10	> 0.05	
Q50-7 days	0.82 ± 0.22	> 0.05	0.27 ± 0.11	> 0.05	0.19 ± 0.03	> 0.05	
Q100-2 hours	1.92 ± 0.33	> 0.05	0.72 ± 0.43	> 0.05	0.20 ± 0.12	> 0.05	
Q100-7 days	4.2 ± 1.6	< 0.001	1.68 ± 0.56	< 0.001	0.72 ± 0.25	< 0.05	

[a] Data are expressed as mean ± SEM.
[b] P < 0.05 and P < 0.001 for 100 mg/kg quercetin group compared to control and KA groups on day 7, Tukey-Kramer test, n = 8 mice.

Consistent with these reports, we observed that quercetin at 100 mg/kg dosage could increase the gene expression of NMDA receptor subunits, which might explain that pretreatment with quercetin before seizure could have an enhancing effect on synaptic plasticity and memory. This hypothesis may be supported by the evidence that quercetin improves memory in kindled rats (12). Furthermore, quercetin has inhibitory effect against PTZ in stimulated rat, because PTZ is a selective blocker of the chloride channel coupled to the GABA receptor complex. It was suggested that quercetin may have a modulatory effect on GABAergic system (12).

This study showed the alterations of mRNA expression in the subunits of AMPA and NMDA receptors following quercetin administration using KA model of epilepsy in mice. Further study is necessary to evaluate the effect of quercetin on the expression of different excitatory and inhibitory neurotransmitter at transcriptomic and protein levels probably involved in epilepsy.

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Footnote

Authors' Contribution: Sahar Moghbelinejad participated in the design of the study, carried out the molecular genetic studies, prepared and drafted the manuscript. Ghazaleh Mohammadi and Fatemeh Khodabandehloo conducted the experiments. Zahra Rashvand carried out the molecular genetic studies. Reza Najafipour and Taghi Naserpour participated in coordination and helped with drafting the manuscript. Marjan Nassiri-Asl designed the study and performed the statistical analysis, involved in drafting the manuscript, and revising it. All authors read and approved the final manuscript.

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