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Brief report

Modulation of the Expression of the GABAA Receptor β_1 and β_3 Subunits by Pretreatment with Quercetin in the KA Model of Epilepsy in Mice

-The Effect of Quercetin on $GABA_A$ Receptor Beta Subunits-

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Key Words
beta subunits, GABAA, gene expression, kainic acid, quercetin

Abstract

Objectives: Quercetin is a flavonoid and an important dietary constituent of fruits and vegetables. In recent years, several pharmacological activities of quercetin, such as its neuroprotective activity and, more specifically, its anti-convulsant effects in animal models of epilepsy, have been reported. This study evaluated the role of quercetin pretreatment on gene expression of γ-amino butyric acid type A (GABAA) receptor beta subunits in kainic acid (KA)-induced seizures in mice.

Methods: The animals were divided into four groups: one saline group, one group in which seizures were induced by using KA (10 mg/kg) without quercetin pretreatment and two groups pretreated with quercetin (50 and 100 mg/kg) prior to seizures being induced by using KA. Next, the messenger ribonucleic acid (mRNA) levels of the GABAA receptor β subunits in the hippocampus of each animal were assessed at 2 hours and 7 days after KA administration. Quantitative real-time polymerase chain reaction (RT-PCR) assay was used to detect mRNA content in hippocampal tissues.

Results: Pretreatments with quercetin at doses of 50 and 100 mg/kg prevented significant increases in the mRNA levels of the β_1 and the β_3 subunits of the GABAA receptor at 2 hours after KA injection. Pretreatment with quercetin (100 mg/kg) significantly inhibited β_1 and β_3 gene expression in the hippocampus at 7 days after KA injection. But, this inhibitory effect of quercetin at 50 mg/kg on the mRNA levels of the β_3 subunit of the GABAA receptor was not observed at 7 days after KA administration.

Conclusion: These results suggest that quercetin (100 mg/kg) modulates the expression of the GABAA receptor β_1 and β_3 subunits in the KA model of epilepsy, most likely to prevent compensatory responses. This may be related to the narrow therapeutic dose range for the anticonvulsant activities of quercetin.

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1. Introduction

Systemic administration of kainic acid (KA) induces seizures and brain damage associated with epilepsy [1]. γ-amino butyric acid (GABA) is the main inhibitory neurotransmitter in the mammalian brain, and it is composed of five subunits that form a chloride channel. The messenger ribonucleic acid (mRNA)

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molecules of the three β subunits have been shown to be distributed throughout the principal cell layers of the hippocampal and the parahippocampal areas in rats [2]. In addition, an association between mutations in the GABRB3 gene, encoding the β_3 subunit of the GABA receptor (GABR), and families from Honduras and Mexico with no incidents of childhood epilepsy has been established [3].
The association between mutations in the GABRB3 gene, encoding the β_3 subunit of the GABAR, has been established in families with childhood absence epilepsy (CAE) from Honduras and Mexico [3].

Quercetin (3,3′,4′,5,7-pentahydroxyflavone) is a flavonoid and an important dietary constituent of fruits and vegetables [4]. It has several biological effects [5], including anti-oxidative [6] anti-inflammatory [7], and neuroprotective [8-10] activities. Quercetin at a concentration of 30 μM has been shown to have an inhibitory effect on the $\alpha_1\beta_1\gamma_2$ GABAA and the ρ1 GABAc receptors in *Xenopus laevis* oocytes [11]. $GABA_{A_{Ap}}$ receptors are known to have low affinity for allosteric modulators of GABA such as benzodiazepine and barbiturates, and quercetin has been shown to antagonize $GABA_{A_{Ap1}}$ receptors in a dose-dependent way via a redox-independent allosteric mechanism [12].

Our previous studies demonstrated that quercetin had an anticonvulsant activity in acute and chronic models of pentylenetetrazole (PTZ) in rats [13, 14]. Furhtermor, the expressions of the GABAA receptor β subunits were reported to have been altered at early time points during or soon after acute seizures induced by KA injection, [15]. Thus, this study aimed to assess the effects of quercetin by determining the variations in the mRNA levels in the β subunits of GABAA receptors in the KA model of epilepsy in mice.

2. Materials and Methods

A total of 70 male BALB/c mice (20 — 25 g) were maintained at constant room temperature (21°C ± 2°C) under a 12:12 hour light/dark cycle and had 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).

The 70 mice were divided into 4 groups as follows: Each mouse in the saline group (group 1, n = 10) received a daily intraperitoneal (i.p.) injection of saline and Tween 80 (10 mL/kg) for 6 days and two i.p. injections of saline 30 minutes apart on the 7th day. Each mouse in the KA group (group 2, n = 20) received a daily i.p. injection of saline for 6 days and an i.p. injection of saline 30 minutes prior to the i.p. injection of KA (10 mg/kg) on the 7th day. The mice in groups 3 (n = 20) and 4 (n = 20) received daily i.p. injections with quercetin at doses of 50 and 100 mg/kg, respectively, for 6 days and, on the 7th day i.p. injections of KA (10 mg/kg, i.p.) were administered 30 minutes after the quercetin injections. Fifty percent of mice in each group were anesthetized with i.p. injections of ketamine (60 mg/mL)/xylazine (6 mg/kg), and then sacrificed at 2 hours after KA administration, with the other fifty percent undergoing the same procedure at 7 days after KA administration.

The β-actin gene was used as an internal control for quantifying the expressions of the target genes. Primers for the Gabrb1 and the Gabrb3 genes of subunits of the GABAA receptors were designed by using Gene Runner software (version 3.05). Primer sets were Gabrb1 F: 5′CCTGGCTCTACTGGAATATG3′, R: 5′ATTGGCACTCTGGTCTTG3′; Gabrb3 F: 5′CTCCTACGCTGGTGTCTGAA3′, R: 5′CACTGTGTTCCCACATGACA3′; β-actin F: TTACTGAGCTGCGTTTTACAC, R: ACAAAGCCATGCCAATGTTG.

Quantitative real-time polymerase chain reaction (RT-PCR) assays were conducted with SYBR Green I Master Mix (Bioneer, Korea). Thermal cycling was performed using the ABI-7500 Sequence Detection System (Applied Biosystems, Foster, CA, USA) as follows: 10 minutes at 95°C for the first denaturation step, followed by 40 cycles at 95°C for a total of 20 seconds and 40 cycles at 60°C for a total of 45 seconds. The values of the cycle threshold (Ct) were used to quantify mRNA fold changes. In this regards, we used the $2^{-\Delta\Delta Ct}$ calculating method by normalizing the Ct of the target mRNAs to the Ct of housekeeping gene β-actin in the treated and the control samples.

Data were expressed as means ± standard errors of the mean (SEMs). Statistical analyses were performed using the one-way analysis of variance (ANOVA), followed by the post-hoc Turkey test for multiple comparisons. For the analyses, Prism software was used. $P < 0.05$ was considered statistically significant.

3. Results

Compared to the mRNA levels of the GABAA receptor β_1 subunit in the saline group, those levels in the KA group were significantly increased in the hippocampi of the mice at 2 hours and 7 days after KA administration (Figs. 1A, 1B). In contrast, no statistically significant changes were observed in the quercetin groups compared to the saline at those times. However, statistically significant differences were observed when comparing the KA group and the 100-mg/kg quercetin group at 7 days after KA administration.
The mRNA levels of the GABAA receptor β_3 subunit were significantly higher in the KA group at 2 hours and 7 days after KA administration than they were in the saline group (Figs. 2A, 2B). In contrast, no significant differences were observed in the mRNA levels of the GABAA receptor β_3 subunit between the 50-mg/kg quercetin group at 2 hours after KA administration and the saline group (Fig. 2A). However, mRNA levels were increased in the quercetin 50-mg/kg group at 7 days after KA administration compared to the saline group (Fig. 2B).

Furthermore, the mRNA levels of the GABAA receptor β_3 subunit observed in the 100-mg/kg quercetin group at 2 hours and 7 days after KA administration were similar to those observed in the saline group at those times (Figs. 2A, 2B). However, significant differences were found when comparing the KA group and both quercetin groups at 2 hours after KA administration (Fig. 2A). Moreover, significant differences were observed between the KA group and the quercetin groups at 7 days after KA administration (Fig. 2B).

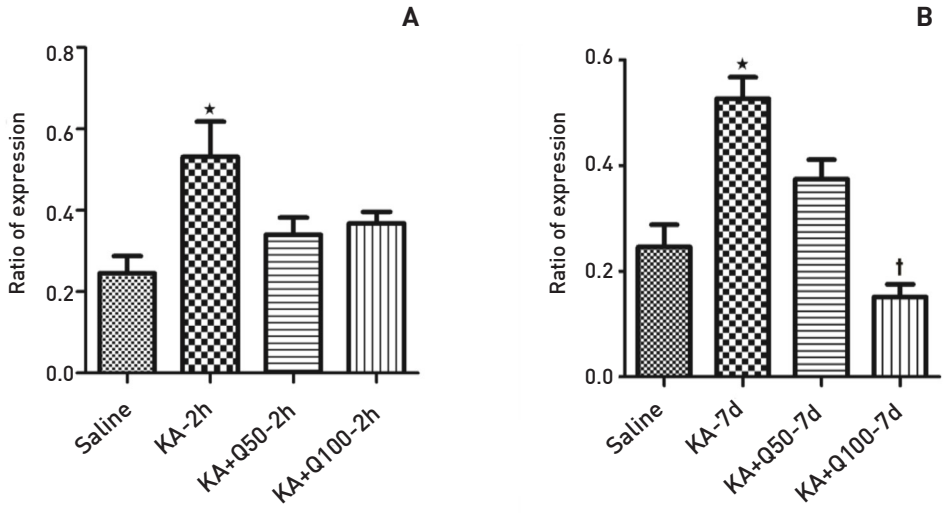

Figure 1 Effect of quercetin on the mRNA ratio of the expression of the β_1 subunit of the GABAA receptor in KA-induced seizure in mice at (A) 2 and (B) 7 hours after KA administration.

$^{*}P < 0.01$ compared to the saline, $^{\dagger}P < 0.001$ compared to the KA group at D7, n = 10.
mRNA, messenger ribonucleic acid; GABAA, γ-amino butyric acid type A; KA, kainic acid.

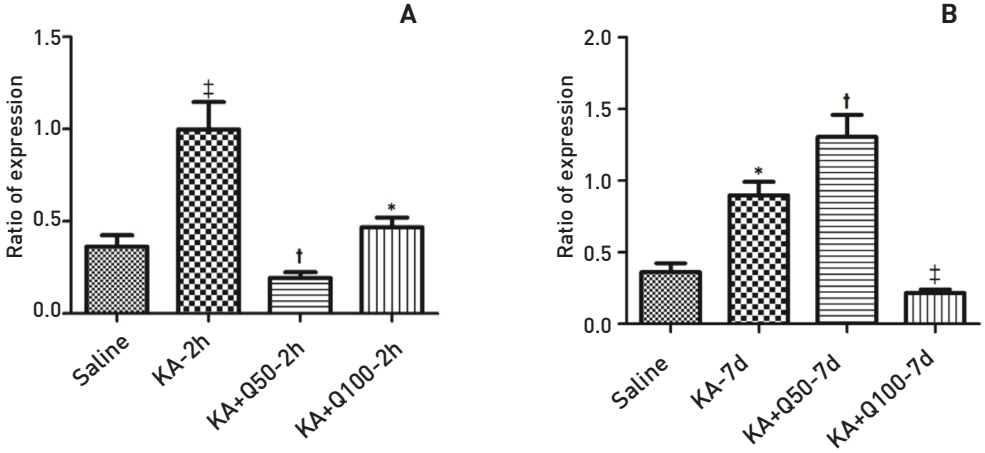

Figure 2 Effect of quercetin on the mRNA ratio of expression of the β_3 subunit of the GABAA receptor in the KA model in mice at (A) 2 and (B) 7 hours after KA administration. $^{\dagger}P < 0.001$ compared to the KA at 2 hours, $^{*}P < 0.01$ compared to the KA group at 2 hours, $^{\ddagger}P < 0.001$ compared to the saline at 2 hours, $^{*}P < 0.01$ compared to the saline at D7, n = 10, $^{\ddagger}P < 0.001$ compared to the KA group at D7, $^{\dagger}P < 0.001$ compared to the saline and $P < 0.05$ compared to the KA group at D7, n = 10.

mRNA, messenger ribonucleic acid; GABAA, γ-amino butyric acid type A; KA, kainic acid.

4. Discussion

This study aimed at determining the effects of quercetin pretreatment on the expressions of the β_1 and the β_3 subunits of the GABAA receptor in a mouse KA-induced seizure model. The doses of quercetin used in this study were the same as those employed in a previous study assessing quercetin's anticonvulsant activity [13, 14]. Compared to the saline group, pretreatments with quercetin at doses of 50 and 100 mg/kg prevented significant increases in the mRNA levels of the β_1 and the β_3 subunits of the GABAA receptor at 2 hours after KA injection. In addition, pretreatment with 100 mg/kg of quercetin inhibited increases in the mRNA levels of the β_3 subunit of the GABAA receptor at 7 days after KA administration. Our results showed similar mRNA levels of the β_1 and the β_3 subunits of the GABAA receptor both in the 100-mg/kg quercetin group at 2 hours and day 7 after KA administration and the saline group, suggesting that high doses of quercetin have inhibitory effects on the gene expression in the β_1 and the β_3 subunits of the GABAA receptor. However, this inhibitory effect of quercetin at 50 mg/kg on the mRNA levels of the β_3 subunit of the GABAA receptor was not observed at 7 days after KA administration. This may be related to the narrow therapeutic dose range for the anticonvulsant activities of

quercetin in different modes of seizure.

Of the three β subunits of the GABA$_A$ receptor, the mRNA level in the β_3 subunit is the one that is increased in animal models and human temporal lobe epilepsy (TLE), as well as in the KA-induced-seizure model [16]. In addition, significant increases in the mRNA levels in the β_1 and the β_3 subunits have been observed in the granule cells of the dentate gyrus at 12 hours to 30 days and at 7 to 30 days after KA injection. These changes are consistent with the presumed compensatory up-regulation of GABA$_A$ receptors observed in the dentate molecular layer in epileptic rats [17].

5. Conclusion

In conclusion, pretreatment with quercetin significantly inhibited the gene expression of the GABA$_A$ receptor β subunits in the KA model of seizure. Quercetin (100 mg/kg) seems to modulate the expression of the GABA$_A$ receptor β subunits to prevent any progress in neurodegenerative and compensatory responses.

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Conflict of interest

The authors declare that there are no conflict of interest.

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