Inhibitory Effect of High Dose of the Flavonoid Quercetin on Amygdala Electrical Kindling in Rats

Tourandokht Baluchnejadmojarad[1,*], Mehrdad Roghani[2], Homayoun Homayounfar[1]

1.Department of Physiology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.
2.Department of Physiology and Medicinal Plant Research Center, School of Medicine, Shahed University, Tehran, Iran.

Article info:
Received: 30 March 2009
First Revision:15 April 2009
Accepted: 16 June 2009

Key words:
Kindling,
Epilepsy,
Quercetin,
Rat.

ABSTRACT

Background & Objective: Epilepsy is a chronic neurological disorder in which patients experience spontaneous recurrent seizures. Although the most commonly recommended therapy is drug treatment, some patients do not achieve adequate control of their seizures on existing drugs. New medications with novel mechanisms of action are needed to help those patients whose seizures are resistant to currently-available drugs. Therefore, the anti-convulsant effect of a high dose of quercetin was evaluated in amygdala kindling model in male rats.

Materials & Methods: Rats were divided into sham-operated group, quercetin-treated SH, kindled, and quercetin-treated kindled rats. Quercetin was administered i.p. one day before amygdale kindling for 3 weeks (40 mg/kg/day). The parameters seizure stage, AD duration, the latency to the onset of stage 4, and the duration of stage 5 were analyzed.

Results: The results showed that quercetin pretreatment causes a lower seizure intensity in treated kindled rats ($p<0.05$-0.01), a lower after-discharge duration ($p<0.05$-0.01), and a higher latency to stage IV ($p<0.05$) as compared to untreated kindled ones.

Conclusion: To conclude, chronic administration of quercetin inhibits amygdala electrical kindling and more studies are warranted to clarify its underlying mechanisms.

Introduction

Epilepsy is a group of heterogeneous neurological disorders characterized by spontaneous and recurrent seizures and is one of the most common that affects 1% of the population, of whom as many as 40% may have complex partial epilepsy, in particular, temporal lobe epilepsy (Shorvon, 1996; Jallon, 1997). Despite optimal drug treatment, seizures persist in 35% of patients with partial epilepsy (Devinsky, 1999). Although patients with drug-resistant forms of epilepsy can undergo resective

**** Corresponding Author:***
Dr. Tourandokht Baluchnejadmojarad
Department of Physiology, School of Medicine, Iran University of Medical Sciences, Shaheed Hemmat Expressway,
P.O. Box: 14155-6183, Tehran, Iran.
Tel: +98-21-88058709- Fax: +98-21-88058719
E-mail: tmojarad@yahoo.com

surgery, many patients cannot be treated surgically because of unacceptable risks for loss of brain functions (Ojemann, 1997).

In the search for new therapeutic approaches, quercetin, a natural flavonoid, is a strong antioxidant and radical scavenger and a polyphenol component which is abundant in fruits and vegetables (Choi, 1996). It has been reported that quercetin has anti-inflammatory, anti-blood coagulation, anti-ischemic effects, and anti-MMP action (Moon, Cho, Jung, Gal, Kwon, Lee, Madamanchi, & Kim, 2003). Quercetin also has been known to have neuroprotective effect. In *in vitro* study with PC12 cell line, quercetin showed inhibitory effect against cell damage (Gelinas, & Martinoli, 2002). Quercetin also attenuated neuronal damage following focal brain ischemia in in vivo model. Youdim et al (Youdim, Qaiser, Begley, Rice-Evans, & Abbott, 2004) reported quercetin can pass blood-brain barrier. Several studies demonstrated quercetin can inhibit MMP activity. Quercetin treatment has been shown to attenuate UV irradiation-induced increase of MMP-1 in fibroblast (Moon, Lee, Zee, & Chung, 2005). In addition, MMP-9 increments in various types of disease model were decreased by quercetin administration (Huang, Hwang, Lee, Ke, Huang, Huang, Kandaswami, Middleton, & Lee, 1999; Moon, Cho, Jung, Gal, Kwon, Lee, Madamanchi, & Kim, 2003).

The animal model used in our study was the rat amygdala kindling model. This model is an animal model of human complex partial seizures, secondarily generalized (Albright, & Burnham, 1980). Employing this model, it is possible to assess the anticonvulsant effects of a compound against both the focal and the secondarily generalized components of the seizure. Drugs that suppress the amygdala focal seizure may be effective in the treatment of human complex partial seizures. Complex partial epilepsy is the most common type of epilepsy in adults, and is often drug resistant (Lonsdale, & Burnham, 2003). In the amygdala kindling model, most standard anticonvulsants suppress the generalized convulsive seizures at low doses. Higher doses, however, are required for even partial suppression of the amygdala focal seizure (Albright, & Burnham, 1980; Lonsdale, & Burnham, 2003). Therefore, the present experiment was designed to determine the anticonvulsant effect of quercetin at a high dose in male rats in amygdala kindling model.

Materials & Methods

Animals

Adult male Wistar rats (n = 28) (Pasteur's Institute, Tehran), weighing 300-350 g at the start of the experiment were housed two per cage in a temperature-controlled colony room under light/dark cycle. Animals were given free access to water and standard rat chow. All behavioral experiments were carried out between 11 a.m. and 4 p.m. This study was carried out in accordance with the policies set forth in the Guide for the Care and Use of Laboratory Animals (NIH) and those of the Research Council of Iran University of Medical Sciences (Tehran, Iran).

Experimental Procedure

Rats were randomly divided into the following equal groups: 1. Sham-operated group (SH), 2. quercetin-treated SH, kindled, and quercetin-treated kindled rats. SH group had electrode implantation and received daily saline (i.p.) one day before stimulation experiments for two weeks. Quercetin-treated SH received quercetin (Sigma)(i.p.; 50 mg/kg/day dissolved in propylene glycol (Merck)) for three weeks. Kindled and treated-kindled rats received the vehicle and the quercetin respectively for the same period. For stereotaxic surgery, rats were anesthetized with a combination of ketamin (100 mg/Kg, i.p.) and xylazine (5 mg/Kg, i.p.), placed in a Stoelting stereotaxic apparatus (incisor bar -3.3 mm, ear bars positioned symmetrically). The scalp was cleaned with iodine solution, incised on the midline and after final steps of preparation, the animal was implanted with bipolar stimulation and monopolar recording electrodes (twisted into a tripolar configuration) terminating in the basolateral amygdala of right hemisphere (coordinates: AP, -2.6 mm; L, 4.8 mm from Bregma and 7.5 mm below dura). Electrodes (stainless steel, teflon coated) were insulated except at the tips. In all groups, two other electrodes were connected to skull screws, placed above the left cortical surface as earth and differential electrodes.

Kindling Procedure

One week after surgery and animal recovery, using stimulus isolated and constant current units, afterdischarge (AD) threshold was determined in the amygdala by a 1 s, 60 Hz monophasic square wave stimulus of 1 ms per wave. The stimulations were initially delivered at 10 μA and then at 5 min intervals increasing stimulus intensity in increments of 10 μA until at least 5 s of AD

recorded. Animals were stimulated daily at AD threshold until first stage 5 seizure was elicited.

Behavioral Assessment

Behavioral seizure severity was classified according to the classification described by Racine (Racine, 1972): stage 1, facial clonus; stage 2, head nodding; stage 3, bilateral forelimb clonus; stage 4, rearing; stage 5, rearing and falling. Animals which displayed 5 consecutive seizures on stage 5 were defined as kindled.

Kindling Parameters

The recorded parameters were seizure stage, amygdala AD duration, the latency to the onset of stage 4, and the duration of stage 5.

Histological Verification

Electrode location was determined at the end of study. For this goal, each animal was deeply anesthetized with ketamine and sacrificed by perfusion-fixation with 4% paraformaldehyde by gravity feed through the left ventricle for 15 min. The brains were removed and sectioned on a cryostat (Leica). Coronal sections were cut 50µ m thick and examined under microscope for electrode position and the presence of any tissue damage. In case of any abnormality, the data from that particular animal were not included in the results.

Statistical Analysis

All data are expressed as the means ± S.E.M. Comparison of data within control or kindled groups was carried out by unpaired student's t-test. A p-value less than 0.05 considered to be a significant difference.

Results

In this study, 40 rats were initially used. During the study, 7 and 5 rats were excluded for electrode displacement and inappropriate location of electrode respectively. As a result, a total of 28 rats were used.

Quercetin pretreatment at a dose of 40 mg/kg/day one day before kindling experiments for 3 weeks caused lower seizure intensity as determined by Racine staging in treated kindled rats as compared to untreated kindled ones ($p<0.05$-0.01) (Fig 1). Regarding afterdischarge duration, there was also a lower score in quercetin-treat-

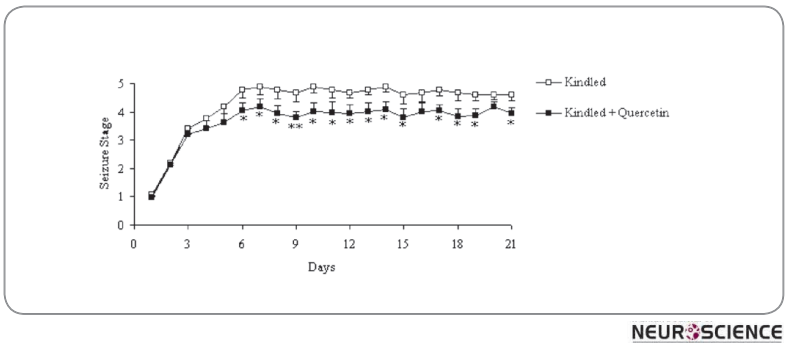

Fig. 1. Time-dependent changes in seizure intensity in kindled groups
* p<0.05, ** p<0.01

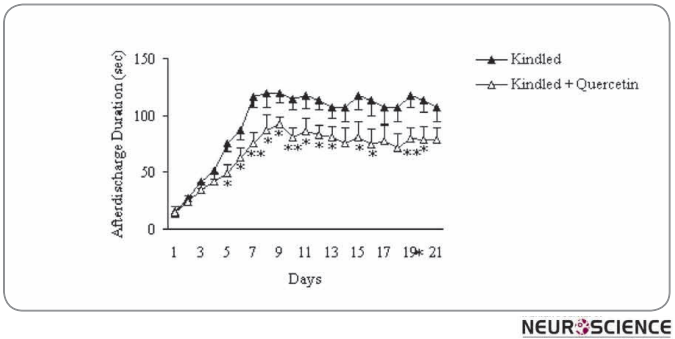

Fig. 2. Time-dependent changes in after-discharge duration in kindled groups
* p<0.05, ** p<0.01

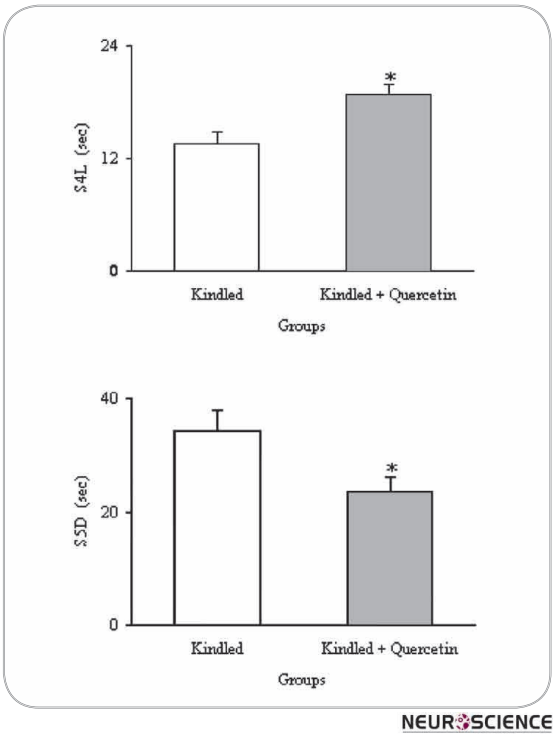

Fig. 3. Latency to onset of stage IV seizure (top) & stage V seizure duration (bottom) in kindled groups * $p<0.05$

ed kindled rats relative to kindled ones in most of days tested ($p<0.05$-0.01) (Fig. 2). In a similar manner, quercetin pretreatment caused a higher latency to stage IV seizure in treated kindled rats as compared to untreated kindled ones ($p<0.05$) (Fig. 3).

Discussion

Amygdala electrical kindling model is considered a commonly used model for assessment of epilepsy in experimental animals and is similar to human epilepsy with regard to seizure activity, EEG, and the epileptiform discharge (Albertson, Joy, Stark, 1984). The kindling model in rats is considered to be a good model of human chronic epilepsy (Albertson et al., 1984).

The present study showed that quercetin administration (40 mg/kg/day; i.p.) inhibited amygdala electrical kindling in all respects including its seizure severity and duration. Neuroprotective effect of quercetin against neurotoxin-induced damage has already been reported in central nervous system (Zbarsky, Datla, Parkar, Rai, DK., Aruoma, Dexter,). In addition, its systemic administration could protect hippocampal neurons against global ischemic consequences (Cho, Kim, Jang, Kim, Lee, 2006). There is some evidence that following lesions and repetitive electrical stimulation of neuronal circuits, expression of matrix metalloproteinase (MMP) increases (Cho et al., 2006). This pathway may be one candidate for beneficial effect of quercetin in the present study and in this way the flavonoid could reduce neuroplastic changes in neural circuits and augmented excitability in certain sited involved in epilepsy. On the other hand, quercetin and its derivatives in the body can selectively inhibit NMDA receptor functionality (in some ways acting as an antagonist) (Wagner, Fachinetto, Dalla, Corte, Brito, Severo, de Oliveira Costa Dias.Morel, Nogueira, Rocha, 2006) and in this way exert their beneficial effect in some animal model of neural diseases like epilepsy.

In conclusion, chronic administration of quercetin inhibits amygdala electrical kindling and more studies are warranted to clarify its underlying mechanisms.

Acknowledgement

This research study was supported by a grant from the Iran University of Medical Sciences (IUMS; Tehran, Iran).

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