Antiepileptogenic effect of curcumin on kainate-induced model of temporal lobe epilepsy

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Abstract

Context: Temporal lobe epilepsy (TLE) is an intractable neurological disorder. Curcumin is the bioactive component of turmeric with anti-epileptic and neuroprotective potential.

Objective: The beneficial effect of curcumin on the intrahippocampal kainate-induced model of TLE was investigated.

Materials and methods: Rats were divided into sham, curcumin-pretreated sham, kainate and curcumin-pretreated kainate groups. The rat model of TLE was induced by unilateral intrahippocampal injection of 4 μg of kainate. Rats received curcumin p.o. at a dose of 100 mg/kg/d starting 1 week before the surgery. Seizure activity (SE) and oxidative stress-related markers were measured. Furthermore, the Timm index for evaluation of mossy fiber sprouting (MFS) and number of Nissl-stained neurons were quantified.

Results: All rats in the kainate group had SE, while 28.5% of rats showed seizures in the curcumin-pretreated kainate group. Malondialdehyde and nitrite and nitrate levels significantly increased in the kainate group (p < 0.01 and p < 0.05, respectively), and curcumin significantly lowered these parameters (p < 0.05). Superoxide dismutase activity significantly decreased in the kainate group (p < 0.05) and curcumin did not improve it. Rats in the kainate group showed a significant reduction of neurons in Cornu Ammonis 1 (CA1) (p < 0.05), CA3 (p < 0.005) and hilar (p < 0.01) regions, and curcumin significantly prevented these changes (p < 0.05–0.005). The Timm index significantly increased in the kainate group (p < 0.005), and curcumin significantly lowered this index (p < 0.01).

Discussion and conclusion: Curcumin pretreatment can attenuate seizures, lower some oxidative stress markers, and prevent hippocampal neuronal loss and MFS in the kainate-induced model of TLE.

Introduction

Epilepsy is considered as one of the most prevalent neurological disorders, which affects about 1.5% of the population (Majores et al., 2004). Temporal lobe epilepsy (TLE) is the most frequent form of epilepsy in adults (Jokeit & Schacher, 2004), characterized by recurrent seizures (Acharya et al., 2007) and attenuates oxidative stress (Gupta et al., 2012) and preserves nigrostriatal dopaminergic neurons in a 6-hydroxydopamine hemiparkinsonian model (Tripanichkul & Jaroensuppaperch, 2012). Curcumin administration can prevent hippocampal neuronal death and reduce seizures in mice with systemic administration of kainate (Shin et al., 2007) and attenuates oxidative stress (Gupta et al., 2009). This study was undertaken to investigate whether curcumin could attenuate intrahippocampal kainate-induced
seizures, hippocampal neurodegeneration and mossy fiber sprouting (MFS).

Materials and methods

All experiments were performed on adult male Wistar rats (300–340 g; n = 56) (Pasteur’s Institute, Tehran, Iran). They were housed three to four per cage in a temperature-controlled colony room under light/dark cycle with food and water available ad libitum. Procedures involving animals were conducted in conformity with National Institutes of Health guidelines for the care and use of laboratory animals. In this study, all efforts were made to minimize the number of animals used and their suffering.

Experimental procedure

Rats were divided into equal-sized sham operated (sham), curcumin-treated sham-operated (sham + curcumin), kainate and curcumin-treated kainate (kainate + curcumin) groups. For intrahippocampal injection, rats were anesthetized with chloral hydrate (350 mg/kg) and placed into the stereotaxic frame (Stoelting Co., Wood Dale, IL) with the incisor bar set at 3.3 mm below the interaural line. The dorsal surface of the skull was exposed, and a burr hole was drilled in the skull using the following stereotaxic coordinates according to the atlas of Paxinos and Watson (1986): anteroposterior, 4.1 mm caudal to bregma; 4 mm lateral to the midline (right side), and 4–4.2 mm ventral to the surface of the skull. A 5 μl microsyringe filled with normal saline containing 0.8 μg/μl of kainate was placed over the burr hole, and kainate solution was injected at a rate of 1 μl/min in order to induce the experimental model of TLE. Kainic acid (kainate; Sigma-Aldrich, St. Louis, MO) was dissolved in cold normal saline just prior to surgery. The sham group received an equivalent volume of normal saline at the same stereotaxic coordinates. The microsyringe was slowly withdrawn after 5 min, and the rat scalp was sutured. The sham + curcumin group received curcumin (Sigma-Aldrich, St. Louis, MO) p.o. using a gavage needle at a dose of 100 mg/kg/d starting 1 week before the surgery, and the last treatment was 1 h before surgery. Curcumin was dissolved in 10% cremophor (Sigma-Aldrich, St. Louis, MO). The dose of curcumin was chosen according to previous reports on its antiepileptic activity (Agarwal et al., 2011; Gupta et al., 2009).

Behavioral assessment of seizure

All animals were assessed for seizure activity (SE) during the first 24 h post-surgery according to Racine’s classification: 0, no reaction; 1, stereotypic mounting, eye blinking and/or mild facial clonus; 2, head nodding and/or multiple facial clonus; 3, myoclonic jerks in the forelimbs; 4, clonic convulsions in the forelimbs with rearing and 5, generalized clonic convulsions and loss of balance (Racine et al., 1972).

Assessment of oxidative stress markers

Determination of hippocampal malondialdehyde concentration

Rats were anesthetized with diethyl ether and decapitated. Hippocampi were isolated and blotted dry and then weighed and prepared as a 5% tissue homogenate in ice-cold 0.9% saline solution. After centrifugation (1000 × g, 4°C, 10 min), the supernatant was stored aliquots as at −70°C until assayed. The concentration of malondialdehyde (MDA), used as a marker of lipid peroxidation index, was calculated by measuring thiobarbituric acid reactive substances (TBARS) in the supernatant as described previously (Roghani & Baluchnejadmojarad, 2009). Briefly, trichloracetic acid and TBARS reagent were added to aliquots of the supernatant, which were subsequently mixed and incubated at 90°C for 80 min. After cooling on ice, the samples were centrifuged at 1000 × g for 10 min, and the absorbance of the supernatant was read at 532 nm. The results of TBARS measurements were expressed as MDA equivalents, using tetraethoxypropane as standard.

Assay of hippocampal nitrite concentration

Supernatant nitrite and nitrate content was assayed by the Griess method as described before (Baluchnejadmojarad & Roghani, 2011). The compound nitric oxide has a short half-life and is rapidly converted to the stable end products nitrate and nitrite. In the assay used in this study, nitrate is converted to nitrite by cadmium, followed by color development with Griess reagent (sulfanilamide and N-naphthyl ethylenediamine) in acidic medium. The absorbance was determined using a spectrophotometer at 540 nm.

Measurement of hippocampal superoxide dismutase activity

The supernatant of hippocampal homogenate was obtained as described above. Superoxide dismutase (SOD) activity was measured as previously reported (Baluchnejadmojarad & Roghani, 2011). Briefly, supernatant was incubated with xanthine and xanthine oxidase in potassium phosphate buffer (PB; pH 7.8, 37°C) for 40 min and then nitro blue tetrazolium (NBT) was added. Thereafter, blue formazan was monitored spectrophotometrically at 550 nm. The amount of protein that inhibited NBT reduction to 50% maximum was defined as 1 nitrite unit of SOD activity.

Protein assay

The protein content of the supernatant was measured by the Bradford (1976) method, using bovine serum albumin (Sigma Chemical, St. Louis, MO) as the standard.

Histological studies

Half of the animals were used for histological assessment. The rats were deeply anesthetized with ketamine (150 mg/kg) and perfused through the ascending aorta with 50 ml of heparinized normal saline followed by 100 ml of sulfide solution (1.2% Na₂S and 1.0% NaH₂PO₄) and then with 100–150 ml of fixative solution containing 4% paraformaldehyde in 0.1 M PB (pH 7.4). Following perfusion, the brains were removed from the skull, hippocampal blocks were prepared and immersed in 30% sucrose in PB at 4°C for 2–3 d. Then, sections were cut at a thickness of 40 μm on a freezing microtome (Leica, Wetzlar, Germany) and collected in PB (0.1 M). Every second section was Nissl-stained with 0.1% cresyl violet (Sigma Aldrich, St. Louis, MO), and alternate sections were used for...
Timm staining. In Nissl-stained sections, neuronal loss was quantified in Cornu Ammonis 1 (CA1), CA3 and hilar regions of the hippocampus in at least three sections at a level range between −3.6 and −4.3 mm from the bregma using an image capturing and analysis system (Bel Engineering, Milano, Italy). The process was repeated at least two times for each section, and its average was taken as the final value. Counting was done blind to the treatments received.

To visualize MFS in the inner molecular layer of the dentate gyrus (DG) that accompanies epileptogenesis, we employed a modified Timm histological procedure to label the zinc-containing axons of the granule cells (Karoly et al., 2011). The slices were immersed for 5 min in 100% alcohol, 5 min in 70% alcohol and 10 min in distilled water. The slices were then developed in the dark under continuous agitation for 60 min in Timm working solution with the following compositions: 60 ml of 50% gum arabic, 10 ml of 2 M sodium citrate buffer (pH 3.7), 30 ml of 5.6% hydroquinone and 0.5 ml of 17% silver nitrate solution. The staining process was terminated with 2% sodium acetate, and the unreacted silver ions were removed with 5% sodium thiosulfate. The sections were then dehydrated and cover slipped. Assessment of MFS (as Timm index) was obtained from the absolute value of the area of Timm granules divided by the length of DG (Wu et al., 2009). The Timm index for each animal was the mean of three sections. All procedures and analyses were done blind to the treatments.

Statistical analysis

Values were expressed as means ± SEM. To compare the experimental groups, non-behavioral data were analyzed using one-way analysis of variance followed by Tukey’s post hoc test. Seizure-related behavioral data were analyzed using the non-parametric Kruskal–Wallis test. Percentage of rats with seizure was examined by \( \chi^2 \) test. In all analyses, the null hypothesis was rejected at a level of 0.05.

Results

SE and behavior

Sham and sham + curcumin groups showed no signs of SE during the first 24 h post-surgery. In contrast, all rats (100%) in kainate group exhibited high scores of seizures. In addition, rats injected with kainic acid and pretreated with curcumin exhibited only mild behavioral signs (lower seizure scores) as compared to kainate group. In this respect, only 28.5% of such rats showed SE, and this was statistically significant versus kainate group (\( p < 0.01 \)).

Oxidative stress markers

Curcumin pretreatment of the sham group did not cause a significant change in hippocampal level of MDA and nitrite and nitrate and activity of SOD as compared to sham group. In contrast, kainate group showed a significant elevation of MDA (\( p < 0.01 \)) and nitrite content (\( p < 0.05 \)), and a significant reduction of SOD activity (\( p < 0.05 \)) and pretreatment of kainate group with curcumin significantly attenuated the increased MDA (\( p < 0.05 \)) and nitrite and nitrate (\( p < 0.05 \)) with no significant improvement of SOD activity (Figures 1–3).
Cytoarchitecture of the hippocampus in Nissl staining

In this study, the number of neurons per unit area in the CA1, CA3 and hilar regions was counted and compared among groups (Figure 4). Our results showed that curcumin pretreatment of the sham group did not produce any significant change in this regard. In contrast, intrahippocampal kainate induced a dramatic and significant degeneration and reduction of neurons in CA1 (p < 0.05), CA3 (p < 0.005) and hilar (p < 0.01) regions of the hippocampus versus sham group. In this regard, the neurodegeneration in the hippocampus was typified by an apparent cell loss in the dentate hilus and considerable thinning of cell layers in the CA1 and CA3 regions. Meanwhile, the DG of the kainate group typically showed granule cell dispersion and displacement, and it was 2- to 3-fold broader as compared to the contralateral side (non-injected side) in the upper border. Furthermore, curcumin pretreatment of kainate group significantly attenuated these changes in CA1 (p < 0.05), CA3 (p < 0.005) and hilar (p < 0.05) regions as compared to the kainate group. These data suggest that curcumin pretreatment can protect the neurons against kainate neurotoxicity.

Timm histochemistry

Kainate-induced aberrant MFS was shown by the Timm method at sixth week post-lesion that selectively labeled synaptic terminals of mossy fibers due to their high zinc content. In the sham groups, little sprouting was present in the DG molecular layer. On the contrary, in the kainate group, Timm staining showed robust MFS that extended into the dentate supragranular layer and in the curcumin-pretreated group, supragranular MFS was less intense and more dispersed, though it was still denser than the sham group. We further compared the average width and Timm staining density (as indicated by the Timm index) between kainate and curcumin-pretreated kainate groups and found that curcumin pretreatment could significantly reduce MFS width and staining density (p < 0.01). These data indicate that curcumin could restrain kainate-induced aberrant MFS.

Discussion

TLE is a chronic and intractable neurological disorder with seizures due to development of excitatory or inhibitory circuits. Recurrent excitation and the development of seizures have also been associated with aberrant MFS in the hippocampus (Sharma et al., 2007). Intracerebral injection of kainate into the CA3 region of the hippocampus causes development of epileptic seizures. These seizures are followed by a pattern of cell loss that is similar to that seen in patients suffering from TLE (Sperk, 1994). For this reason, kainate-induced brain damage has been routinely used for modeling TLE and excitotoxic neurodegenerative disorders (Liu et al., 2007). Accumulating evidence indicates that hippocampal oxidative stress is involved in kainate-induced neurotoxicity (Weber et al., 1996). In this study, a massive neuronal loss was found in the CA1, CA3 and hilar regions of the kainate group. Furthermore, typical aberrant mossy fibers invading into the granule cell layer and granule cell inner molecular layer in the hippocampus of these rats were noticed, which was consistent with previous studies (Wu et al., 2009). Kainate injection into the hippocampus led to the degeneration of CA3 pyramidal neurons and dentate hilar cells. The granule cell axons (known as mossy fibers) originating from the DG lose their postsynaptic target cells and sprout into the inner molecular layer. These pathologic changes cause the formation of a functional recurrent excitatory circuit between granule cells that leads to recurrent seizures (Shetty & Hattiangady, 2007; Wu et al., 2009).
In our study, the CA2 area of the hippocampus was not evaluated for neuronal loss. Previous studies have shown that following kainate injection, no significant and noticeable change is observed in this area. The reason for this issue is that CA2 area being resistant to glutamate excitotoxicity (i.e., after kainate injection and similar analogues). This resistance resides in its high density of adenosine A1 receptors (Ochiishi et al., 1999). These receptors like those of γ-aminobutyric acid exert an inhibitory effect on the hippocampus and make CA2 area resistant to kainate (Mattson & Kater, 1989; Sharma et al., 2007; Young & Dragunow, 1995).

Following kainate injection, there is an increased oxidative stress, and development of seizures is also associated with such stressful condition (Liu et al., 2012). On the other hand, deficiency of antioxidant redox systems could exacerbate the etiology of epilepsy (VERROTTI et al., 2008). The imbalance between oxidant and antioxidant defense mechanism in the body may result into seizures. Following kainate receptor activation, intracellular calcium level in hippocampal neurons rises (CRESPO-BIEL et al., 2010), leading to activation of several injurious pathways and triggering oxidative stress that ultimately causes neuronal death (LI et al., 2010). Oxidative stress itself is accompanied with activation of a cascade of intracellular toxic events resulting in oxidation, lipid peroxidation, and elevation of intracellular calcium, ultimately leading to cell death (KANG et al., 2012).

The neuroprotective effect of curcumin is exerted via various intra- and extra-cellular mechanisms in the central nervous system, which are responsible for its antioxidant and anti-inflammatory properties (Bala et al., 2006; MYTHRI & BHARATH, 2012). Curcumin could reduce N-methyl-D-aspartate (NMDA)-mediated excitotoxic cell damage through modulation of NMDA receptor activity and attenuating NMDA receptor-mediated calcium rise, leading to a lower rate of apoptosis and enhancing cell viability (MATTEUCCI et al., 2005). In this respect, curcumin could reduce the detrimental action of neurotoxins and/or excitotoxic agents on neurons (MANSOURI et al., 2012). In our study, due to the neuroprotective effect of curcumin, there were lower degrees of neuronal loss and MFS in the kainate + curcumin group than those of kainate group. Consistent with our findings, previous studies have shown that curcumin administration can prevent hippocampal neuronal death and reduce seizures in mice with systemic kainate (SHIN et al., 2007). In addition, it has been shown that curcumin could ameliorate pentylentetrazol-induced SE by inhibiting free radicals and supporting the antioxidant redox system (NAZIROGLU et al., 2009). In our study, curcumin pretreatment of kainate group attenuated oxidative stress burden as was evident by significantly lower level of MDA and nitrite and nitrate in hippocampal tissue, and this have certainly protected hippocampal neurons against oxidative damage with subsequent lower MFS and less severe SE. This study clearly suggests that curcumin could protect against kainate-induced seizures by functioning as an antioxidant.

Kainate-induced seizure model in the rat accompanies inflammation with increased production of certain prostaglandin, such as prostaglandin E2 following an enhancement in messenger RNA levels of cyclooxygenase 2 and prostaglandin E2 synthase in the brain tissue, and anti-inflammatory agents could reduce the severity of the condition (CICERI et al., 2002). In parallel with this fact, it has been shown that kainate-induced excitotoxicity through induction of matrix metalloproteinases leads to selective neuronal death; and neuroinflammation in the hippocampus and inhibitors of such enzymes could attenuate the ensuing neuronal damage, and this could be therapeutically relevant in related neurological disorders (JOURQUIN et al., 2003). Although the role of anti-inflammatory activity of curcumin in this model of TLE was not evaluated in this study, curcumin possesses a potential anti-inflammatory effect under toxic conditions (SOOD et al., 2012) and it may exert a protective effect against seizures in kainate-injected rats through its anti-inflammatory activity. In this respect, curcumin attenuates the expression and secretion of inflammation-related proteins.
inflammatory mediators following spinal cord injury in vivo and lipopolysaccharide-induced astrocyte reactivation in vitro (Lin et al., 2011). However, some researchers have claimed that the neuroprotective effect of curcumin is not only related simply to its anti-inflammatory and/or antioxidant properties but also involves other mechanisms (Yu et al., 2010), which entail further investigation. In addition, the role of anti-inflammatory agents in epilepsy progression and prevention is itself a controversial issue (Akarsu et al., 2006; Dhir et al., 2006).

Although bioavailability of compounds like curcumin is lower when orally administered as compared to intraperitoneal route, most previous studies have administered curcumin orally (Agarwal et al., 2011; Ezz et al., 2011; Noor et al., 2012; Reeta et al., 2011). In addition, there are some reports that in vivo curcumin could cross the blood–brain barrier in sufficient amount to exert a direct neuroprotective effect in the brain (Garcia-Alloza et al., 2007; Yang et al., 2005). In this study, we used only one dose of curcumin (100 mg/kg). This selection has been according to previous studies on its anti-oxidant, anti epileptic and neuroprotective activity (Agarwal et al., 2011; Gupta et al., 2009). Even, some studies have used a single oral dose of the curcumin at a dose of 80 mg/kg. Since curcumin was to be administered daily for a week, overall, we selected the best dose as 100 mg/kg. Meanwhile, although curcumin is a rather safe compound, its toxic effect should not be ignored if administered at higher doses.

In conclusion, curcumin can attenuate seizures and prevent hippocampal neurodegeneration and aberrant MFS in the kainate-induced model of TLE and part of its beneficial effect is due to its potential to mitigate oxidative stress markers.

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Declaration of interest

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References


