

PROTECTIVE EFFECT OF CURCUMIN AGAINST KAINIC ACID INDUCED SEIZURES AND OXIDATIVE STRESS IN RATS

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Abstract : The effect of curcumin, a dietary antioxidant was studied against kainic acid (KA)-induced seizures and on markers of oxidative stress. Rats were administered KA (10 mg/kg, ip) and observed for behavioral changes, incidence and latency of convulsions and mortality over four hours. The rats were thereafter sacrificed for estimation of oxidative stress parameters; malondialdehyde (MDA) and glutathione (GSH). Curcumin was administered 30 min before KA at doses of 50, 100 and 200 mg/kg, ip.

KA induced long-lasting seizures and associated symptoms. The brain level of MDA was significantly ($P < 0.05$) raised after KA administration (536 ± 44 nmol/g wet tissue) as compared to saline treated group (200 ± 36 nmol/g wet tissue) and significantly decreased the levels of GSH.

Pretreatment with curcumin (100 and 200 mg/kg, ip) significantly increased the latency of seizures (120 ± 20 min and 115 ± 5.7 min respectively) as compared to the vehicle treated KA group. Curcumin (100 and 200 mg/kg, ip) significantly prevented the increase in MDA levels and ameliorated the fall in glutathione. Curcumin at the dose of 50 mg/kg had no effect on any of oxidative stress parameters. The study reports the potential antiepileptic effect of antioxidant curcumin.

Key words : curcumin kainic acid oxidative stress seizures rat

INTRODUCTION

Epilepsy is a chronic, complex and dynamic neurological disorder associated with ongoing neuronal damage, particularly when uncontrolled. The current antiepileptic treatment provides almost complete seizure control in, 54% to 82% of patients with

primary (idiopathic) generalized epilepsy syndromes, however, there remains a substantial group, which is refractory (1).

Oxidative injury produced by free radicals may play a role in the initiation and progression of epilepsy. Hence, therapies aimed at reducing Oxidative stress may

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ameliorate tissue damage and favorably alter the clinical course (2).

Kainic acid (KA) is an analogue of glutamic acid, which produce convulsions by activation of the kainite excitatory amino acid receptors (3) and initiates neuronal injury and death by producing reactive oxygen species (ROS) (4, 5).

These increased ROS in turn may further release glutamate thus forming, a loop. This 'vicious' cycle not only causes long lasting seizure formation but if not arrested may lead to the neuronal death (6, 7). Status epilepticus is an emergency condition where seizures last for a long time and if not controlled neuronal injury occurs (8, 9).

In various experimental studies, drugs with antioxidant properties (eg melatonin, trans resveratrol, vineatrol and adenosine) have prevented seizures induced by different chemoconvulsants: PTZ (10), FeCl₃ (11, 12) and kainic acid (13, 14) and also excitotoxicity induced by agents like glutamate and domoic acid (15–17). Thus antioxidants may have a potential role in preventing excitotoxicity-induced seizures.

In this respect, quenching of free radicals by exogenous antioxidants seems to be tire most plausible. This is due to the fact that many different nonenzymatic/exogenous antioxidants without major side effects are available and there is currently clinically no therapeutic approach known to increase levels of enzymatic/endogenous antioxidants in humans (18).

Curcumin is the active constituent of *CURCUMA LONGA* (turmeric). It is used as

a spice, food preservative and herbal medicine in India. It is a phenolic antioxidant and several times more potent than vitamin E (19) and has shown to protect the brain against lipid peroxidation (20). Recent studies have demonstrated the neuroprotective effect of curcumin in animal models of cerebral ischemia. The protective effect has been attributed to its antioxidant activity (21, 22). However, the effect of curcumin on seizures is unknown.

The present study was therefore designed to evaluate the effect of curcumin against kainic acid-induced status epilepticus and oxidative stress.

METHODS

Animals

Albino male Wistar rats weighing 200–250 g were used for the study. The animals were procured from the central animal facility at All India Institute of Medical Sciences, New Delhi. The rats were group housed in polypropylene cages (38×23×10 cm) with not more than 6 animals per cage. They were maintained under standard laboratory conditions with natural dark-light cycle and were allowed free access to standard dry rat diet (Golden Feeds, India) and tap water *ad libitum*. All experimental procedures in rats described were reviewed and approved by the Institutional Animal Ethics Committee.

Drugs

Kainic acid (KA) (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in normal saline. Curcumin (Sigma-Aldrich, St. Louis,

MO, USA) was prepared freshly by dissolving in dimethylsulphoxide (DMSO) and administrated at the doses of 50, 100 and 200 mg/kg, ip in rats.

Experimental seizure model

Rats were administered KA at a dose 10 mg/kg intraperitoneally (ip), pH adjusted to 7.2 ± 0.1 . Animals were than observed for behavioral changes (grooming, rearing, hind limb scratching, urination, defecation, wet dog shakes, jaw movements, salivation, head nodding), incidence and latency of convulsions and mortality over a total period of 4 hours (13).

Experimental protocol and drug

The animals were divided in to six different groups of seven rats each. In the first group the rats were administered with KA at a dose of 10 mg/kg, ip (control). In the second group the rats were injected with normal saline intraperitoneally instead of KA (This group was used in biochemical studies). In the third group the rats were treated with vehicle for curcumin (50% DMSO), 30 min before KA administration. The control and vehicle treated groups were run parallel to the drug treated groups. In the next three groups, the rats were treated with three graded doses of curcumin (50, 100 and 200 mg/kg, ip), 30 min prior to KA administration.

The rats in all the groups were sacrificed under ether anesthesia after 4 hours (after observing behavioral changes) for the estimation of brain malondialdehyde (MDA) and glutathione (GSH) levels. The brains were quickly removed, cleaned with chilled saline and stored at -70°C until biochemical

analysis, which was carried out within 2 days. The dose of curcumin was selected on the basis of earlier reports in which significant antioxidant property was demonstrated in doses ranging from 8–300 mg/kg, ip (22–24).

Measurement of lipid peroxidation

Malondialdehyde which is a measure of lipid peroxidation, was measured spectrophotometrically (25). Briefly, brain tissues were homogenized with 10 times (w/v) 0.1 sodium phosphate buffer (pH 7.4). The reagents acetic acid 1.5 ml (20%), pH 3.5, 1.5 ml thiobarbituric acid (0.8%) and 0.2 ml sodium dodecyl sulfate (8.1%) were added to 0.1 ml of processed tissue sample. The mixture was then heated at 100°C for 60 min. The mixture was cooled with tap water and 5 ml of n-butanol: pyridine (15:1% v/v), 1 ml of distilled water was added. The mixture was shaken vigorously. After centrifugation at 4000 rpm for 10 min, the organic layer was withdrawn and absorbance was measured at 532 nm using a spectrophotometer.

Measurement of glutathione

GSH was measured spectrophotometrically (26). Briefly, brain tissues were homogenized with 10 times (w/v) 0.1 M sodium phosphate buffer (pH 7.4). This homogenate was then centrifuged with 5% trichloroacetic acid to centrifuge out the proteins. To 0.1 ml of this homogenate, 2 ml of phosphate buffer (pH 8.4), 0.5 ml of 5'5 dithiobis (2-nitrobenzoic acid) (DTNB) and 0.4 ml of double distilled water was added. The mixture was vortexed and the absorbance read at 412 nm within 15 min.

Statistical analysis

The data is represented as mean±SEM. One way (ANOVA) with 'post hoc comparison' was used for statistical analysis. $P < 0.05$ represents level of significance.

RESULTS

Effect of KA on behavioral symptoms and convulsions

KA when administered per se at the dose of 10 mg/kg, ip (control), exhibited behavioral signs i.e., grooming, rearing, hind limb scratching, urination, defecation, wet dog shakes, jaw movements, salivation, head nodding in all the rats within 5 min. 100% of the rats exhibited convulsions with the mean latency of 53 ± 10.2 min.

Effect of graded doses of curcumin on KA induced seizures

Curcumin at the dose of 50 mg/kg, ip 30 min prior to KA administration produced convulsions in 100% of the rats with a mean latency of 59 ± 5.4 min. However, when the dose of curcumin was increased to 100 mg/kg, ip, the % of convulsions was significantly ($P < 0.05$) decreased to 75% with the mean latency of 120 ± 20 min. At a dose of 200 mg/kg, 50% of the rats exhibited convulsions with a significant ($P < 0.05$) increase in mean latency of 115 ± 5.7 as compared to the vehicle (50% DMSO) treated KA group, which showed 100% convulsions, and with a mean latency of 56 ± 6.1 min (Figs. 1 and 2).

Effect of KA, 50% DMSO (vehicle) and curcumin on brain MDA levels

The brain levels of MDA were

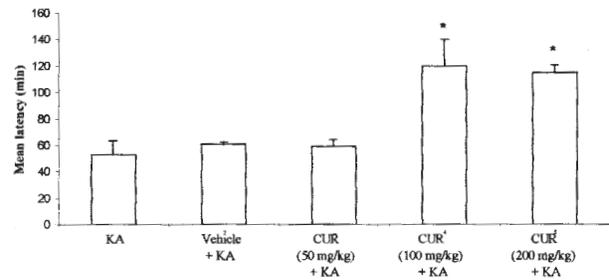


Fig. 1: Effect of graded doses of curcumin on kainic acid induced latency of seizures. * $P < 0.05$ Vs vehicle treated KA. 'KA' represents 'kainic acid' and 'CUR' represents 'curcumin' in the figure.

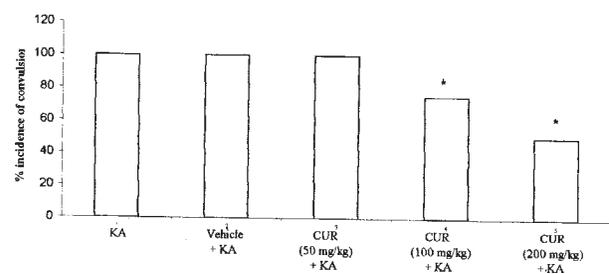


Fig. 2: Effect of graded doses of curcumin on percent incidence of convulsions induced by kainic acid in rats. * $P < 0.05$ Vs vehicle treated KA. 'KA' represents 'kainic acid' and 'CUR' represents 'curcumin' in the figure.

significantly raised after KA administration (536 ± 44 nmol/g, wet tissue) as compared to the saline treated rats (200 ± 36 nmol/g wet tissue) ($P < 0.05$). There was an insignificant change in the vehicle (50% DMSO) treated KA group (524.1 ± 36.1 nmol/g, wet tissue) as compared to the control (KA *per se* group).

In the rats treated with curcumin (50, 100 and 200 mg/kg, ip), the values of MDA were 450 ± 40 , 308.3 ± 48.8 and 280 ± 38.3 nmol/g, wet tissue respectively. The values of MDA were significantly ($P < 0.05$) decreased in the pretreated curcumin group (100 and 200 mg/kg, ip) as compared to the KA group (Fig. 3).

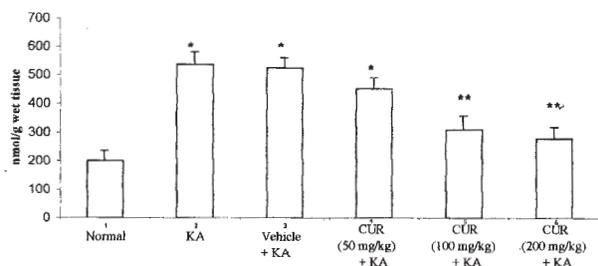


Fig. 3 : Effect of graded doses of curcumin on the levels of MDA in kainic acid induced seizures in rats. *P<0.05 Vs Normal (saline treated); **P<0.05 Vs vehicle treated KA. 'KA' represents 'kainic acid' and 'CUR' represents 'curcumin' in the figure.

Effect of KA, 50% DMSO (vehicle) and curcumin treatment on brain glutathione levels

The brain glutathione levels were estimated in KA (control), saline treated, vehicle-treated KA (50% DMSO) and curcumin treated rats. The brain levels of glutathione showed significant (P<0.05) decrease in KA (66.1±9.2 µg/g, wet tissues) and vehicle treated KA group (64.1±8.4 µg/g, wet tissues) as compared to the saline treated rats (115±15.2 µg/g, wet tissues). There was insignificant difference between the KA *per se* group and 50% DMSO (vehicle) KA treated group.

In the rats treated with curcumin (50, 100 and 200 mg/kg, ip), the values of GSH were 70.5±10.7, 110.8±11.8 and 112.5±12.4

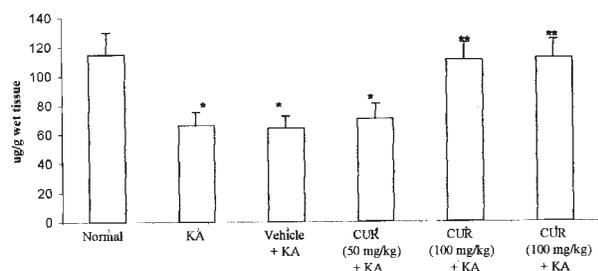


Fig. 4 : Effect of graded doses of curcumin on the levels of GSH in kainic acid induced seizures in rats. P<0.05 Vs Normal (saline treated); **P<0.05 Vs vehicle treated KA. 'KA' represents 'kainic acid' and 'CUR' represents curcumin' in the figure.

µg/g, wet tissues respectively. The values were significantly (P<0.05) higher at the dose of 100 and 200 mg/kg, ip, than the vehicle treated kainic acid group (Fig. 4).

DISCUSSION

Epilepsy is the most common neurological disorder worldwide, and its biochemical and molecular events are still unclear. Despite the availability of a large number of antiepileptic drugs, the treatment is unsatisfactory in about 20% refractory cases where neurodegeneration is inevitable (27). Over activation of excitatory amino acid receptors is an important pathogenetic factor that leads to seizure genesis and increased oxidative stress has been implicated in the mechanisms of excitotoxicity-induced neurodegeneration (28). Therefore, use of antioxidants could be a potential approach in arresting or inhibiting the seizure genesis caused by excitotoxic agents (29).

The phenolic yellow curry pigment curcumin has potent antioxidant activities (30, 31), anti-inflammatory (32) and chemoprotective properties (33). It has been used as a food additive in India for ages. It has shown a neuroprotective effect in models of cerebral ischemia (21, 22), ethanol induced brain damage (34) and reduces amyloid pathology in transgenic mice of Alzheimer's disease (35).

In the present study, KA produced behavioral changes as well as convulsions in all the animals. Curcumin pretreatment dose dependency increased the latency of onset of seizures as compared to the KA *per se* (control group). The effect was significant at doses of 100 and 200 mg/kg, ip.

Moreover, curcumin (100 and 200 mg/kg, ip) pretreatment prevented KA induced oxidative stress. In the KA *per se* group, there was a significant increase in levels of MDA and a significant decrease in the levels of GSH signifying oxidative stress.

MDA is an end product of lipid peroxidation, a measure of free radical generation. The significantly less increase in MDA levels in the groups treated with curcumin as compared to the vehicle treated KA group indicates attenuation of lipid peroxidation. Also, there was a simultaneous significant increase in the glutathione levels in the curcumin (100 and 200 mg/kg, ip) group as compared to control group. Glutathione is the most abundant intracellular thiol and low molecular weight tripeptide found in living cells. It reacts with the free radicals and can protect cells from singlet oxygen, hydroxyl radical and superoxide radical damage.

The increase in levels of glutathione by curcumin indicates its antioxidant property possibly by increasing the endogenous

defense of the brain to combat oxidative stress induced by KA.

Conclusion

KA seizures are associated with an increase in levels of extracellular glutamate and this appears to be associated with generation of lipid free radicals and with a decrease in residual antioxidant effects (36). In a vicious cycle, the increase in free radicals in turn will enhance glutamatergic activity. It is difficult to predict at what level curcumin acts. However, it is evident that it prevents this vicious chain, thus decreasing the excitotoxicity and thereby showing a protective effect. Recently, preliminary reports have shown that curcumin protects against NMDA receptor mediated excitotoxicity in rat retinal cultures (37). Hence, a dual effect of curcumin acting on both as an antioxidant and an anti-excitotoxic agent cannot be ruled out.

The present study demonstrates the beneficial effect of curcumin and suggests its potential use in status epilepticus.

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