

The protective effect of carvacrol on kainic acid-induced model of temporal lobe epilepsy in male rat

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ABSTRACT

Background and Objective: Temporal lobe epilepsy (TLE) is a chronic neurological disorder with spontaneous recurrent seizures and abnormal intracranial waves. Since the role of oxidative stress in the occurrence of epilepsy is inevitable, it seems that the use of antioxidants can prevent some of the complications resulting from this disease. This study was designed to assess the protective effect of carvacrol on seizure behavior and intracranial electroencephalographic (iEEG) recordings.

Materials and Methods: In this study, male Wistar rats were randomly allocated into four groups: Sham-operated, carvacrol (10 mg/kg) pretreated-sham-operated, kainic acid (0.8 µg/µl), and carvacrol (10 mg/kg) pretreated-kainic acid. In this study, we evaluated the status epilepticus and seizures according to Racine's scores and recorded iEEG for investigating the antiepileptic effect of carvacrol in kainite-injected rats.

Results: The seizures behavior (status epilepticus and spontaneous seizures) appeared in kainate-injected rats and iEEG amplitude increased as compared to sham group ($p < 0.01$). Pretreatment of kainate-injected rats with carvacrol significantly reduced their Racine's scores of seizures behavior and intracranial waves amplitude ($p < 0.05$).

Conclusion: Collectively, the results of this study indicate that carvacrol is able to prevent some of the epilepsy disease complications in an experimental model of temporal lobe epilepsy.

1. Introduction

Epilepsy is a series of sudden, transient and repeated disturbance of central nervous system caused by abnormal discharges of brain neurons (1). These spontaneous discharges lead to sensory impairment, loss of consciousness, abnormal psychological function and seizure. During a seizure, simultaneous discharge of a population of neurons leads to paroxysmal depolarization shifts (PDS) (2). Temporal lobe epilepsy is the most common epileptic disorder in

adults. At least 30 percent of seizures in patients with epilepsy not controlled and millions of people around the world still in need of more effective drugs for treatment (3). Therefore, understanding the mechanisms involved in human epilepsy has long been one of the research topics. According to a large body of studies, there is a direct correlation between the pathophysiology of epilepsy and neurotoxicity, increase of the production of free radicals and the routes leading to neurodegeneration and apoptosis (4).

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Studies have shown that the free hydroxyl and reactive nitrogen species, especially nitric oxide (NO), increase after seizures induced by PTZ, kindling and other animal models of epilepsy (5). So, it seems that the use of substances with antioxidant properties can prevent or alleviate behavioral and histological complications resulting from the epilepsy. In recent years, due to the obvious side effects of chemical drugs, the use of herbal medicines has increased in the world.

Carvacrol (5-isopropyl-2-methyl phenol-CAR) is a monoterpenoid phenol with antioxidant, anti-inflammatory, antitumoranalgesic, antihepatotoxic, antimicrobial, and insecticidal activities (6). Due to antioxidant effects, carvacrol may be has main role in prevention and inhibition of several diseases (7, 8). The presence of hydroxy group in structure of carvacrol gives it antioxidant effects (9, 10). Administration of carvacrol following induction of ischemia-reperfusion in the brain reduces neuronal apoptosis and stroke volume. Even 6 hours after induction of ischemia-reperfusion in the rat brain, a wide therapeutic effect of the carvacrol has been observed on histological and behavioral complications. Its therapeutic effects attributed to its antioxidant properties, anti-apoptotic and also in relation to its effects on neuronal signaling pathways (11). Regarding the protective effects of carvacrol, in this study, we examined its effects in preventing the occurrence of behavioral and electrophysiological symptoms associated with temporal lobe epilepsy in rats.

2. Materials and Methods

2.1. Animals

In this study, we used 40 adult male Wistar rats (local animal house), weighing 200-250 g. At the start of trials, rats were kept three or four per cage in a temperature-controlled colony room (21-23°C) with free access to tap water and standard rat chow. This study was conducted in accordance with the policies stipulated in the Research Council of Iran University of Medical Sciences (Tehran, Iran).

2.2. Experimental procedure

Rats were randomly divided into four groups: sham-operated (SH); carvacrol (10 mg/kg)-treated SH; Kainate; and carvacrol (10 mg/kg)-treated kainate rats. For stereotaxic surgery, rats were anesthetized with a combination of ketamine (80 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) and then placed into a stereotaxic apparatus (Stoelting Co., USA) with incisor bar set at 3.3 mm below the interaural line. After exposing the dorsal surface of the skull and drilling a burr hole, kainic acid (Sigma-Aldrich, USA) was injected into CA3 area of right hippocampus at coordinates of anteroposterior, 4.3 mm caudal to bregma, 4.1 mm lateral to the midline, and 4–4.2 mm ventral to the surface of the skull. For inducing of rat model of TLE, 5 µl of normal saline containing 0.16 µg/µl of kainate was injected into CA3 area at a rate of 1 µl/min. Carvacrol (Sigma Chemicals, USA) was dissolved in propylene glycol and administered daily (10 mg/kg body weight; i.p) for one week before surgery.

2.3. Behavioral assessment

During the first 24 h post-kainic acid or vehicle injection, animals were evaluated for status epilepticus (SE). Also, four weeks after surgery (at fifth week), all animals were examined for kainate-induced spontaneous seizures 4 h/day for five consecutive days. Behavioral assessment was scored 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 (12).

2.4. Recording of intracranial electroencephalograph (iEEG)

For iEEG recording, first rats were anesthetized with urethane (1.5 g/kg) and placed into the stereotaxic frame. Then, two monopolar electrodes made of stainless steel (0.125 mm diameter, Advent, UK) with teflon cover, positioned bilaterally into CA3 area of hippocampus. The electrical signals from the CA3 area were amplified 500-fold by EXT-02F (NPI Electronics, Germany), digitized at 200 Hz, and

band-pass filtered at 0.3 Hz–70 Hz. The signals were recorded by A/D board (PCI 6024e, National instruments, USA) connected to computer. Data analysis was conducted by Spike program (2010 version). iEEG was recorded for each animal for a period of 2 hours, and the average amplitude were ultimately reported.

2.5. Statistical analysis

All results were expressed as mean ± SEM. The behavioral results analyses by the nonparametric Kruskal-Wallis test and if necessary followed by the Mann–Whitney U-test. In all trials, a difference at $p < 0.05$ was regarded as significant.

3. Results

3.1. Seizure activity and behavior

Sham and sham + carvacrol groups exhibited no signs of seizure behavior during the first 24 h or 4 weeks after intrahippocampal injection of kainic acid or vehicle. But, in kainate group all rats (except for one, 90%) showed the class 5 seizures (status epilepticus) and 66.6 % of them had spontaneous seizures. Pretreatment of kainate rats with carvacrol caused the scores of seizure activity to be lower as compared to the kainate group. So that, 44.4 and 50% of pretreated rats showed signs of SE and spontaneous seizures, respectively. The difference of status epilepticus between kainate and pretreated kainate groups was statistically significant ($p < 0.05$) (Table 1).

Table 1. Numbers and rates of status epilepticus and spontaneous seizures in each group

Group	Number (status epilepticus)	Rate (%)	Number (spontaneous seizures)	Rate (%)
Sham (n=7)	0	0	0	0
Carvacrol (10 mg/Kg)-treated SH (n=7)	0	0	0	0
Kainate (10) (n=10)	9	90	5	66.6
Carvacrol (10 mg/kg) (n=10)	4	44.4*	2	50

χ^2 test, * $P < 0.05$ compared to Kainate group

3.2. iEEG recording

After behavioral monitoring, recording of iEEGs was started. No obvious epileptiform waves were seen in sham and sham+ carvacrol groups. In contrast, in kainate group, a lot of epileptiform waves were recorded in hippocampus. The amplitude and frequency of kainate rats iEEG was significantly more than that of sham rats ($p < 0.01$). Pretreatment of kainate rats with carvacrol reduced amplitude and frequency of hippocampal epileptiform waves as compared to untreated kainate group ($p < 0.05$) (Fig.1).

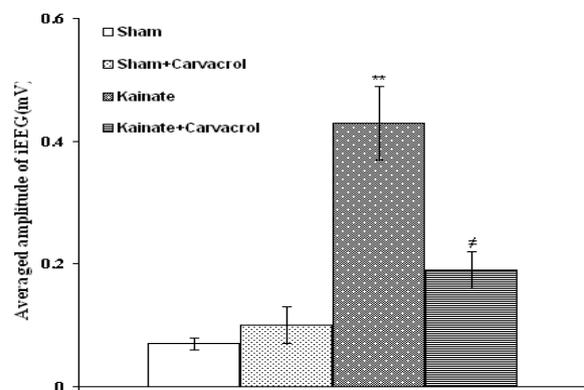


Fig. 1. The average amplitude of iEEG in different groups.

** $p < 0.01$ (vs. sham); # $p < 0.05$ (vs. kainate)

4. Discussion

The results of present study showed that induction of temporal lobe epilepsy using intrahippocampal injection of kainic acid is associated with a characteristic seizure behavior and abnormal hippocampal waves. Intrahippocampal kainic acid injection, due to destruction of CA3 neurons, that have a high density of glutamate receptors, intensifies hippocampal glutamatergic pathways. Finally, it occurs in the form of varying degrees of seizures regarding to the severity of the damage (13). Previous studies have shown that kainic acid injection into the brain cause seizures and brain damage through excitotoxicity (14). Also, kainite-induced seizures in rat alter the expression of the genes involved in synaptic plasticity and gliosis. On the other hand, kainic acid causes a significant increase in the levels of nitric oxide and oxidative stress in the brain. Since the brain uses the most amount of body oxygen compared to other organs, and it also takes a lot of unsaturated fatty acids that are susceptible to lipid peroxidation, so brain is highly impressionable to oxidative damage (14). In our research, with intrahippocampal injection of kainic acid, model of temporal lobe epilepsy was created and behavioral outcomes were consistent with previous studies.

In this study, pretreatment of kainate rats with carvacrol significantly reduced the severity of seizures and intracranial waves as compared to kainate rats. Other studies has been also shown that carvacryl acetate (CA), a derivative of monoterpene carvacrol, has anticonvulsant effects after seizures induced by pilocarpine (P400), picrotoxin (PIC) or pentylenetetrazol (PTZ) (16). In addition, carvacrol prevent s seizures in a partial seizure-psychomotor model (17). These results fits with the anti-epileptic effect of carvacrol in our study. In recent years, effects of carvacrol and its derivatives on voltage gated sodium channels in mammalian (18) and vertebrate neurons (19) were evaluated. These studies indicated that hydroxyl groups in the structure of carvacrol exerts compound action potential blocking effect. The attenuating effect of carvacrol on the intracranial waves (iEEG) in the present study may be attributed to its effect on voltage gated channels including sodium channels.

In conclusion, our results suggest that carvacrol pretreatment could improve kainic acid-induced seizures and intrahippocampal epileptic waves.

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References

1. Dichter MA. Emerging concepts in the pathogenesis of epilepsy and epileptogenesis. *Archives of Neurology* 2009; 66(4): 443.
2. Aylward RL. Epilepsy: a review of reports, guidelines, recommendations and models for the provision of care for patients with epilepsy. *Clinical Medicine* 2008; 8(4):433-8.
3. Galanopoulou AS, Kokaia M, Loeb JA, Nehlig A, Pitkanen A, Rogawski MA, et al. Epilepsy therapy development: technical and methodologic issues in studies with animal models. *Epilepsia* 2013; 54 Suppl 4:13-23.
4. Gélinas S, Martinoli MG. Neuroprotective effect of estradiol and phytoestrogens on MPP⁺-induced cytotoxicity in neuronal PC12 cells. *Journal of Neuroscience Research* 2002; 70(1): 90-96.
5. Milatovic D, Gupta RC, Dettbarn WD. Involvement of nitric oxide in kainic acid-induced excitotoxicity in rat brain. *Brain Research* 2002; 957: 330-337.
6. Burt S. Essential oils: their antibacterial properties and potential applications in foods—a review,” *International Journal of Food Microbiology* 2004; 94(3): 223–253, 2004.
7. Melusova M, Slamena D, Kozics K, Jantova S, Horvathova E. Carvacrol and rosemary essential oil manifest cytotoxic, DNA-protective and pro-apoptotic effect having no effect on DNA repair. *Neoplasma* 2014; 61(6): 690–699.
8. Liang WZ, Lu CH. Carvacrol-induced [Ca²⁺]_i rise and apoptosis in human glioblastoma cells. *Life Sciences* 2012; 90(17-18): 703–711.
9. Aristatile B, Numair AKS, Assaf AHA., Veeramani C, Pugalendi KV. Protective effect of carvacrol on oxidative stress and cellular DNA damage induced by UVB irradiation in human peripheral lymphocytes. *Journal of Biochemical and Molecular Toxicology* 2015; 29(11): 497–507.
10. Guimaães AG, Oliveira GF, Melo MS. Bioassayguided evaluation of antioxidant and antinociceptive activities of carvacrol. *Basic and Clinical Pharmacology and Toxicology* 2010; 107(6): 949–957.
11. Yu H, Zhang ZL, Chen J, Pei A, Hua F, Qian X, et al. Carvacrol, a food-additive, provides neuroprotection on focal cerebral ischemia/reperfusion injury in mice. *PloS One* 2012; 7(3): e33584.
12. Racine R, Okujava V, Chipashvili S. Modification of seizure activity by electrical stimulation.3.Mechanisms. *Electroencephalography and Clinical Neurophysiology* 1972; 32: 295–299.
13. Polli RS, Malheiros JM, Dos Santos R, Hamani C, Longo BM, Tannus A. Changes in hippocampal volume are correlated with cell loss but not with seizure frequency in two chronic models of temporal lobe epilepsy. *Frontiers in Neurology* 2014; 5:111.
14. Kiasalari Z, Roghani M, Khalili M, Rahmati B, Baluchnejadmojarad T. Antiepileptogenic effect of curcumin on kainate-induced model of temporal lobe epilepsy. *Pharmaceutical Biology* 2013; 51(12):1572-8.
15. Bazan NG, Tu B, Rodriguez de Turco EB. What synaptic lipid signaling tells us about seizure induced damage and epileptogenesis. *Progress in Brain Research* 2002; 135:175-85.

16. Pires LF, Costa LM, de Almeida AA, Silva OA, Santos Cerqueira G, de Sousa DP, et al. Neuropharmacological effects of carvacryl acetate on δ -aminolevulinic dehydratase, Na⁺, K⁺-ATPase activities and amino acids levels in mice hippocampus after seizures. *Chemico-biological Interactions* 2015; 226: 49-57.
17. Mishra RK, Baker MT. Seizure prevention by the naturally occurring phenols, carvacrol and thymol in a partial seizure-psychomotor model. *Bioorganic & Medicinal Chemistry Letters* 2014; 24(23):5446-9.
18. Gonçalves JC, AlvesAdem, deAraújo AE, Cruz JS, Araújo DA. Distinct effects of carvone analogues on the isolated nerve of rats. *European Journal of Pharmacology* 2010; 645: 108–112.
19. Kawasaki H, Mizuta K, Fujita T, Kumamoto E. Inhibition by menthol and its related chemicals of compound action potentials in frog sciatic nerves. *Life Science* 2013; 92: 359–367.