The effect of Vitamin E on learning and memory deficits in intrahippocampal kainate-induced temporal lobe epilepsy in rats

Zahra Kiasalari, Mohsen Khalili, Samaneh Shafiee, Mehrdad Roghani

Neurophysiology Research Center, Shahed University, Department of Physiology, School of Medicine, Shahed University, Tehran, Iran

Received: 21-04-2014
Revised: 17-11-2015
Accepted: 20-12-2015

Correspondence to:
Dr. Mehrdad Roghani,
E-mail: mehjour@yahoo.com

ABSTRACT

Objective: Since temporal lobe epilepsy (TLE) is associated with learning and memory impairment, we investigated the beneficial effect of Vitamin E on the impaired learning and memory in the intrahippocampal kainate model of TLE in rats.

Materials and Methods: Rats were divided into sham, Vitamin E-treated sham, kainate, and Vitamin E-treated kainate. Intrahippocampal kainate was used for induction of epilepsy. Vitamin E was injected intraperitoneal (i.p.) at a dose of 200 mg/kg/day started 1 week before surgery until 1 h presurgery. Initial and step-through latencies in the passive avoidance test and alternation behavior percentage in Y-maze were finally determined in addition to measurement of some oxidative stress markers.

Results: Kainate injection caused a higher severity and rate of seizures and deteriorated learning and memory performance in passive avoidance paradigm and spontaneous alternation as an index of spatial recognition memory in Y-maze task. Intrahippocampal kainate also led to the elevation of malondialdehyde (MDA) and nitrite and reduced activity of superoxide dismutase (SOD). Vitamin E pretreatment significantly attenuated severity and incidence rate of seizures, significantly improved retrieval and recall in passive avoidance, did not ameliorate spatial memory deficit in Y-maze, and lowered MDA and enhanced SOD activity.

Conclusion: Vitamin E improves passive avoidance learning and memory and part of its beneficial effect is due to its potential to mitigate hippocampal oxidative stress.

KEY WORDS: Kainate, learning and memory, oxidative stress, temporal lobe epilepsy, Vitamin E

Introduction

Temporal lobe epilepsy (TLE) is the most prevalent kind of epilepsy in adults, usually characterized by hippocampal sclerosis and neurodegeneration. A well-characterized model of TLE is through intrahippocampal unilateral injection of the excitotoxic glutamate analog kainate, with subsequent retarded acquisition and impaired retention of visual-spatial information in memory tasks. Cognitive deficits and learning and memory impairment also represent a serious neuropsychological problem and the most common neuropsychological hallmark in TLE patients.

Vitamin E family comprises potent antioxidants that could act as chain-breaking antioxidant and protects cell membrane against oxidative damage. Vitamin E inhibits seizures induced by ferrous chloride, hyperbaric oxygen, and penicillin, where oxidative stress may have an important role in seizures development. Vitamin E has also a protective effect against pentylenetetrazole-induced toxicity by the inhibition of free radicals and support of antioxidant systems. Depletion of energy metabolites due to KA-induced seizures leads to oxidative stress and Vitamin E could attenuate this depletion.
Vitamin E could improve cognitive dysfunction under conditions of enhanced oxidative stress. Therefore, we decided to evaluate whether Vitamin E could attenuate learning and memory deficits in this model of TLE.

Materials and Methods

All experiments were performed on adult male Wistar rats (250–300 g; n = 32) (Pasteur’s Institute, Tehran, Iran). They were housed 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 NIH guidelines for the care and use of laboratory animals, and its protocol was approved by the Ethics Committee of Shahed University (Tehran, Iran).

Experimental Procedure

Rats were randomly divided into equal-sized sham-operated (sham), Vitamin E-treated sham-operated (sham + Vitamin E), kainate, and Vitamin E-treated kainate (kainate + Vitamin E) groups. For intrahippocampal injections, rats were anesthetized with chloral hydrate (350 mg/kg), placed into the stereotaxic frame (Stoelting Co., USA) 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: Anteroposterior; 4.1 mm caudal to bregma; 4.2 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 to induce an experimental model of TLE. Kainic acid (KA, Sigma-Aldrich, USA) 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. Vitamin E-treated groups received this vitamin (alpha tocopherol) (Sigma-Aldrich, USA) i.p. at a dose of 200 mg/kg/day starting 1 week before surgery and the last treatment was 1 h before surgery. Vitamin E was diluted in propylene glycol (Merck, Germany). The dose of Vitamin E was chosen according to previous reports on its antiepileptic activity.

Behavioral Assessment of Seizure

During the 24 h postsurgery, all animals were assessed and scored for the progression of seizures 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 associated with loss of balance.

Y-maze Task

Short-term spatial recognition memory performance was assessed by recording spontaneous alternation behavior in a single-session Y-maze as described before. Each rat was placed at the end of one arm and allowed to move freely through the maze during an 8-min session. The series of arm entries was recorded visually. Alternation was defined as successive entries into the three arms on overlapping triplet sets. The number of maximum spontaneous alternation was then the total number of arms entered-2 and the percentage is calculated as the ratio of actual to possible alternations (defined as the total number of arm entries-2).

Single-trial Passive Avoidance Test

This test was conducted 2–3 days after Y-maze task and was conducted as described before. On the 1st and 2nd day of testing, each rat was placed on the apparatus and left for 5 min to habituate to the apparatus. On the 3rd day, an acquisition trial was performed. Rats were individually placed in the illuminated chamber. After a habituation period (5 min), the guillotine door was opened and after the rat entered the dark chamber, the door was closed and an inescapable scrambled electric shock (1 mA, 1 s once) was delivered. In this trial, the initial latency (IL) of entrance into the dark chamber was recorded, and rats with ILS > 60 s were excluded from the study. Twenty-four hours later, each rat was placed in the illuminated chamber for retention trial. The interval between the placement in the illuminated chamber and the entry into the dark chamber was measured as step-through latency (STL up to a maximum of 400 s as cut-off).

Determination of Hippocampal Malondialdehyde Concentration

The rats were anesthetized with ketamine (150 mg/kg) 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 aliquoted and stored at −70°C until assayed. The concentration of malondialdehyde (MDA) was measured as described previously.

Assay of Hippocampal Nitrite Concentration

Supernatant nitrite (NO−) content was assayed by the Griess method as described before.

Measurement of Hippocampal Superoxide Dismutase Activity

Superoxide dismutase (SOD) activity was measured as previously reported.

Protein Assay

The protein content of the supernatant was measured by the Bradford method.

Statistical Analysis

Values were expressed as means ± standard error mean. To compare the groups, nonbehavioral data were analyzed using one-way ANOVA followed by Tukey’s post-hoc test. Seizure-related and learning and memory data were analyzed using the nonparametric Kruskal–Wallis test. Percentage of rats with spontaneous seizure was examined by Chi-square test. In all analyses, the null hypothesis was rejected at a level of 0.05.

Results

Seizure Activity and Behavior

Sham and sham + Vitamin E groups showed no seizure activity during the first 24 h postsurgery. In contrast, all rats (100%) in kainate group exhibited high scores of seizures. In contrast, rats injected with KA and pretreated with Vitamin E exhibited only mild behavioral signs (lower seizure scores), as compared to kainate group. In this respect, only 37.5% of such
rats had signs of seizures and this difference was statistically significant versus kainate group ($P < 0.05$).

**Spatial Recognition Memory in Y-maze**

Figure 1 shows the results of the performance of rats in Y-maze task. Both kainate and Vitamin E-pretreated kainate groups had a significantly lower alternation score as compared to sham-operated rats ($P < 0.01$) and Vitamin E treatment of kainate group did not improve it. Locomotor activity of the animals was also assessed by counting the total number of arms visited by each rat [Figure 1]. In this regard, there were no significant differences among the groups.

**Learning and Memory in Passive Avoidance Test**

Figure 2 shows the performance of rats in passive avoidance paradigm as indicated by initial (IL) and STL. Kainate group developed a significant impairment in retention and recall in passive avoidance test ($P < 0.01$) in comparison with sham group, as it was evident by a lower STL, and Vitamin E pretreatment of kainate group significantly improved it ($P < 0.05$).

**Oxidative Stress Markers**

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 Vitamin E significantly lowered MDA ($P < 0.05$) and increased the activity of SOD ($P < 0.05$) [Figure 3].

**Discussion**

TLE is a neurological disorder with recurrent seizures. Intrahippocampal injection of kainate causes the development of seizures and a pattern of cell loss that is similar to that observed in TLE patients. Deficits of spatial hippocampus-dependent memory in experimental models of TLE have been previously reported. Mice with an intrahippocampal injection of KA show deficits of spatial learning and short- and long-term memories in a hippocampus-dependent large diameter pool Morris water maze task (MWM), which is rather consistent with our Y-maze task data. However, Y-maze task evaluates spatial recognition memory which does not involve a learning component and
Although the etiology of epilepsy plays a significant role in the development of seizures, astrocytic oxidative stress, and development of seizures is also associated with such condition. An increased production of reactive oxygen species could reduce cognitive function. On the other hand, deficiency of antioxidant redox systems could exacerbate the etiology of epilepsy. Increased lipid peroxidation in the brain has also been reported in pentylenetetrazole-induced epilepsy in rats. Vitamin E as a lipophilic antioxidant protects membranes from being oxidatively damaged by free radicals and attenuates lipid peroxidation in the brain. Although the role of inflammation in this model of TLE was not evaluated in the present study, Vitamin E may also exert a protective effect against cognitive impairment due to its anti-inflammatory activity.

**Conclusion**

To conclude, Vitamin E could attenuate seizures and improve passive avoidance learning and memory, and part of its beneficial effect is due to its potential to mitigate hippocampal oxidative stress.

**Acknowledgments**

This study was a MD thesis project, approved and financially supported by Shahed University (Tehran, Iran) in 2010. The authors would also like to appreciate Miss Fariba Ansari for her great technical assistance.

**Financial Support and Sponsorship**

Financially supported by Shahed University (Tehran, Iran) in 2010.

**Conflicts of Interest**

There are no conflicts of interest.

**References**

1. Blümcke I, Coras R, Miyata H, Ozkara C. Defining clinico-neuropathological subtypes of mesial temporal lobe epilepsy with hippocampal sclerosis. Brain Pathol 2012;22:402-11.
2. Arida RM, Scorza FA, Scorza CA, Cavaleiro EA. Is physical activity beneficial for recovery in temporal lobe epilepsy? Evidences from animal studies. Neurosci Biobehav Rev 2009;33:422-31.
3. Ulatowski L, Manor D. Vitamin E trafficking in neurologic health and disease. Annu Rev Nutr 2013;33:87-103.
4. Ayüldüz M, Yildirim M, Agar E. The effects of Vitamin E on penicillin-induced epileptiform activity in rats. Exp Brain Res 2006;174:109-13.
5. Armagan A, Kılıçman S, Yılmaz M, Yılmaz N, Bülbü M, Vural H, et al. Topiramate and vitamin E modulate antioxidant enzyme activities, nitric oxide and lipid peroxidation levels in pentylenetetrazol-induced nephrotoxicity in rats. Basic Clin Pharmacol Toxicol 2006;103:166-70.
6. Gupta RC, Milatovic D, Zilvin M, Dettbarn WD. Seizure-induced changes in energy metabolites and effects of N-tet-buty1-alpha-phenylindotri (PNB) and Vitamin E in rats. Pflugers Arch 2000;440 5 Suppl. R160-2.
7. Joshi YB, Pratczi D. Vitamin E in aging, dementia, and Alzheimer’s disease. Biofactors 2012;38:90-7.
8. Tomé Ada R, Ferreira PM, Freitas RM. Inhibitory action of antioxidants (ascorbic acid or alpha-tocopherol) on seizures and brain damage induced by pilocarpine in rats. Arq Neuropsiquiatr 2010;68:355-61.
9. Tomé AR, Feng D, Freitas RM. The effects of alpha-tocopherol on hippocampal oxidative stress prior to in pilocarpine-induced seizures. Neurochem Res 2010;35:580-7.
10. Racine R, Okujava V, Chipashvili S. Modification of seizure activity by electrical stimulation 3. Mechanisms. Electroencephalogr Clin Neurophysiol 1972;32:295-9.
11. Baluchnejadmojarad T, Roghani M. Chronic epigallocatechin-3-gallate ameliorates learning and memory deficits in diabetic rats via modulation of nitric oxide and oxidative stress. Behav Brain Res 2011;224:305-10.
12. Jalal-Nadoushan M, Roghani M. Alpha-lipoic acid protects against 6-hydroxydopamine-induced neurotoxicity in a rat model of hemi-parkinsonism. Brain Res 2013;1505:68-74.
13. Ben-Ari Y, Cossart R, Kainate, a double agent that generates seizures: Two decades of progress. Trends Neurosci 2000;23:580-7.
14. Chauvière L, Rafrafi N, Thious Blanc C, Bartolomei F, Esclapez M, Bernard C. Early deficits in spatial memory and theta rhythm in experimental temporal lobe epilepsy. J Neurosci 2009;29:5402-10.
15. Mittadous P, Stamatakis A, Koutsoudaki PN, Tniakos DG, Stylinopoulou F. IGF-I ameliorates hippocampal neurodegeneration and protects against cognitive deficits in an animal model of temporal lobe epilepsy. Exp Neurol 2011;231:223-35.
16. Canas PM, Porciúncula LO, Cunha GM, Silva CG, Machado NJ, Oliveira JM, et al. Adenosine A2a receptor blockade prevents synaptotoxicity and memory dysfunction caused by beta-amyloid peptides via p38 mitogen-activated protein kinase pathway. J Neurosci 2009;29:14741-51.
17. Duarte JM, Agostinho PM, Carvalho RA, Cunha RA. Caffeine consumption prevents diabetes-induced memory impairment and synaptotoxicity in the hippocampus of NONZNO10/LTJ mice. PLoS One 2012;7:e21899.
18. Grötlick I, Hoffmann K, Löscher W. Behavioral alterations in the pilocarpine model of temporal lobe epilepsy in mice. Exp Neurol 2007;207:329-49.
19. Shapiro LA, Wang L, Ribak CE. Rapid astrocyte and microglial activation following pilocarpine-induced seizures in rats. Epilepsia 2008;49 Suppl 2:S3-41.
20. Mansouri Z, Sabetkasaei M, Moradi F, Masoudnia F, Aitae A. Currucim has neuroprotection effect on homocysteine rat model of Parkinson 2012;47:234-42.
21. Li SY, Jia YH, Sun WG, Tang Y, An GS, Ni JH, et al. Stabilization of mitochondrial function by tetramethylpyrazine protects against kainate-induced oxidative lesions in rat hippocampus. Free Radic Biol Med 2010;48:597-608.
22. Liu SH, Chang CD, Chen PH, Su JR, Chen CC, Chaung HC. Docosahexaenoic acid and phosphatidylserine supplemetations improve antioxidant activities and cognitive functions of the developing brain on pentetylentetrazole-induced seizure model. Brain Res 2012;1451:19-26.
23. Verrotti A, Scardapane A, Franzoni E, Manco R, Chiarelli F. Increased oxidative stress in epileptic children treated with valproic acid. Epilepsy Res 2008;78:171-7.
24. Obay BD, Tasdemir E, Tümer C, Bilgin H, Atmaca M. Dose dependent effects of ghexolin on pentetylentetrazole-induced oxidative stress in a rat seizure model. Peptides 2008;29:448-55.
25. Zhu Q, Emanuele MA, LaPagnia N, Kovacs EJ, Emanuele NV. Vitamin E prevents ethanol-induced inflammatory, hormonal, and cytotoxic changes in reproductive tissues. Endocrine 2007;32:59-68.