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The Effects of Ellagic Acid on Hippocampal Cell Damage in Pentylenetetrazole Induced Kindling Seizure Model

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Abstract

Introduction: Epilepsy is a chronic neurological disease that is characterized by recurrent, spontaneous brain seizures. The aim of this study was to evaluate the effect of ellagic acid (EA) on hippocampal cell damage in penthylentetrazole (PTZ) induced kindling model.

Methods: In this experimental study, 50 male Wistar rats were randomly divided into 5 groups (n= 10): control, PTZ (40 mg/kg, ip), PTZ+EA12, PTZ+EA25 and PTZ+EA51 (which received 12.5, 25 & 50 mg/kg, respectively) groups. After investigation of seizure delay in five consecutive days, the brains of rat's skulls were removed and histopathological evaluation were performed. Comparison between different groups was assessed by one way ANOVA followed by Tukey's post hoc test ($p< 0.05$).

Results: Intraperitoneally injection of PTZ induced kindling seizure in animal model ($p< 0.001$). The data showed the seizure delay following the administration of EA increased ($p< 0.001$). In addition, after administration of EA the density of hippocampal cells increased in experimental groups ($p< 0.001$).

Conclusion: The results of this study showed that EA decreases the severity of seizure in kindling seizures. Also, EA increased the neuronal density following the administration of PTZ. Therefore, EA possesses neuroprotective effects that enhance the hippocampal damages in animals and also perhaps in patients suffering from seizure.

Keywords: Ellagic Acid, Penthylentetrazole, Hippocampus, Rat

Introduction

Epilepsy is a brain disorder which is characterized by unpredictable seizures (1). One percent of the worldwide people suffer from epilepsy (2). The epilepsy incidence is age- related and mostly affecting young children and middle-aged people (>50 years old) (1). Most of the anti-seizure medications and drugs have unwanted side effects in most of people. Therefore, we focused on naturally neuroprotective components. Ellagic acid (EA) is a polyphenolic compound that possesses many pharmacological activities such as anti-allergic (3), anti-malarial (4), anti-inflammatory (5) and antioxidant (6) and neuroprotective activities (7). Naturally, Ellagic acid exists in pomegranate, grapes, raspberries, blackberries, strawberries and walnuts (8). Pentylenetetrazole (PTZ) administration is one of the most common methods for investigating the antiepileptic drugs (9). Administration of pentylenetetrazole (PTZ) is used for investigating of brain excitability (10). PTZ induced chemical kindling seizures, myoclonic and primary generalized tonic-clonic seizure in rodent models (11). Kindling is suitable for epilepsy model for investigating the neurochemical and structural changes in the brain (12). Also, PTZ is used for atrophy of the hippocampus in rats. Studies showed that following the administration of PTZ the cerebellum volume decreased and selective neuronal loss and astrocytosis appeared in rats (12). Kindling induced by PTZ relates with permanent reduction of the GABAergic system inhibitory function in the brain (13). There is no study about the protective effect of EA on PTZ-

kindeling seizure. Therefore, the aim of this study was investigating the effect of EA on hippocampal cell damage in seizure model.

Methods

Fifty male Wistar rats weighing 200- 220 g were used. Animals were kept on a regular dark/light cycles (12 h/12 h) and 22±2°C temperature. Also, all animals had free access to enough food and water (ad libitum). All of the testing and training procedures were performed with insights from the recommendations and policies of Iran. The protocols pointed out by The National Institutes of Health for using animal experiments were approved by the Ethics Committee of Shiraz Branch, Islamic Azad University. Animals were randomly divided into five main groups with 10 rats in each group: Control (without treatment), PTZ (40 mg/kg, *i.p.*), and experimental groups (PTZ+EA12.5, PTZ+EA25 & PTZ+EA50) received Ellagic acid (EA, Sigma, USA) intraperitoneally (12.5, 25 & 50 mg/kg, respectively) from two hours before receiving PTZ (40 mg/kg, ip) to five days later (14). For the kindling seizure induction, the rats were injected by 40 mg/kg of PTZ (sigma, USA) in five consecutive days. Following each injection the rats were placed in a Plexiglas cage separately, and observed for 60 min. The resultant seizures were classified according to a modified Racine scale as follows: 1- Mouth and facial movements; 2- Head nodding; 3- Forelimb clonus; 4- Rearing; 5- Rearing and falling. The latencies to the first sign of seizure were also recorded (14). After completion of PTZ-kindling, the animals were terminally anesthetized with an *i.p.* injection of mixture of ketamine hydrochloride (100 mg/kg) and xylazine (5 mg/kg) and intracardially perfused with 0.9% saline followed by 10% phosphate-buffered formalin. After fixation, the brains were removed, weighed and further post-fixed in 10% phosphate-buffered formalin for 7 days. The brains were then paraffin-embedded and sectioned on a microtome (Leitz 1512; Leica Microsystems, Wetzlar, Germany). The brains were sectioned at 5 µm, and deparaffinized, serially stained with hematoxyline-eosin, then cover-slipped for tissue analysis. All sections were visualized with a light microscope (BX51, Japan). The digital photographs were taken from hippocampal CA1, CA2, CA3 and dentate gyrus (DG) areas of both hemispheres. For quantitative analysis of dark neurons, the physical dissector method was used (15). Data were expressed as mean±SEM (standard error of mean). Also, the comparison between different groups was assessed by one way ANOVA followed by Tukey's post hoc test in SPSS software version 22. The P-values less than 0.05 were considered as statistically significant.

Results

The seizure delay of PTZ group showed significant difference in comparison with PTZ+EA50 on the second, third, fourth and fifth days. Also, the mean of seizure delay in PTZ group showed significant difference between PTZ+EA25 and PTZ+EA12.5 on the fourth & fifth days (Figure 1). The results showed that the mean density of CA1, CA2, CA3 and DG in PTZ group significantly decreased in comparison with control group (p<0.001). In addition, the mean density of CA1 and CA2 in PTZ+EA 12.5& 25 groups significantly decreased in comparison with control group (p<0.001). Also, the mean density of CA3 and DG in PTZ+EA12.5, 25 & 50 groups significantly increased in comparison with PTZ group (p<0.001) (Figure 2).

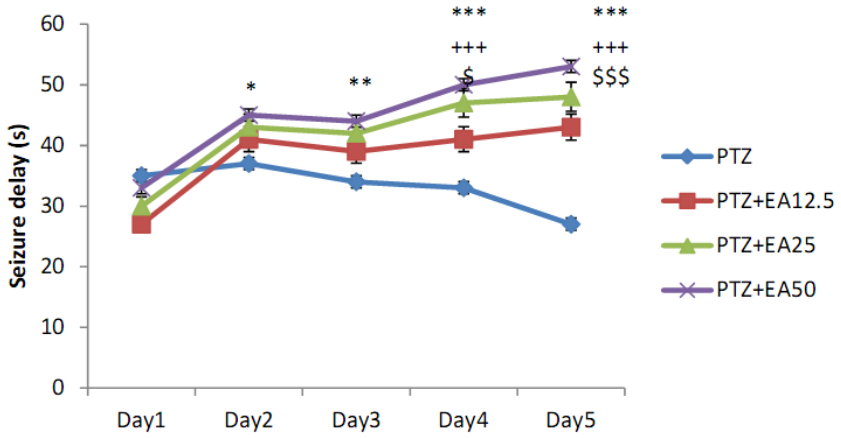

Figure 1. The comparison of seizure delay in different groups

The seizure delay of PTZ group showed significant difference in comparison with PTZ+EA50 on the second, third, fourth and fifth days ($^{*}p<0.05$, $^{**}p<0.01$ & $^{***}p<0.001$). Also, the mean of seizure delay in PTZ group showed significant difference between PTZ+EA25 ($^{+++}p<0.001$) & PTZ+EA12.5 ($^{\$}p<0.05$ & $^{\$\$\$}p<0.001$) on the fourth & fifth days.

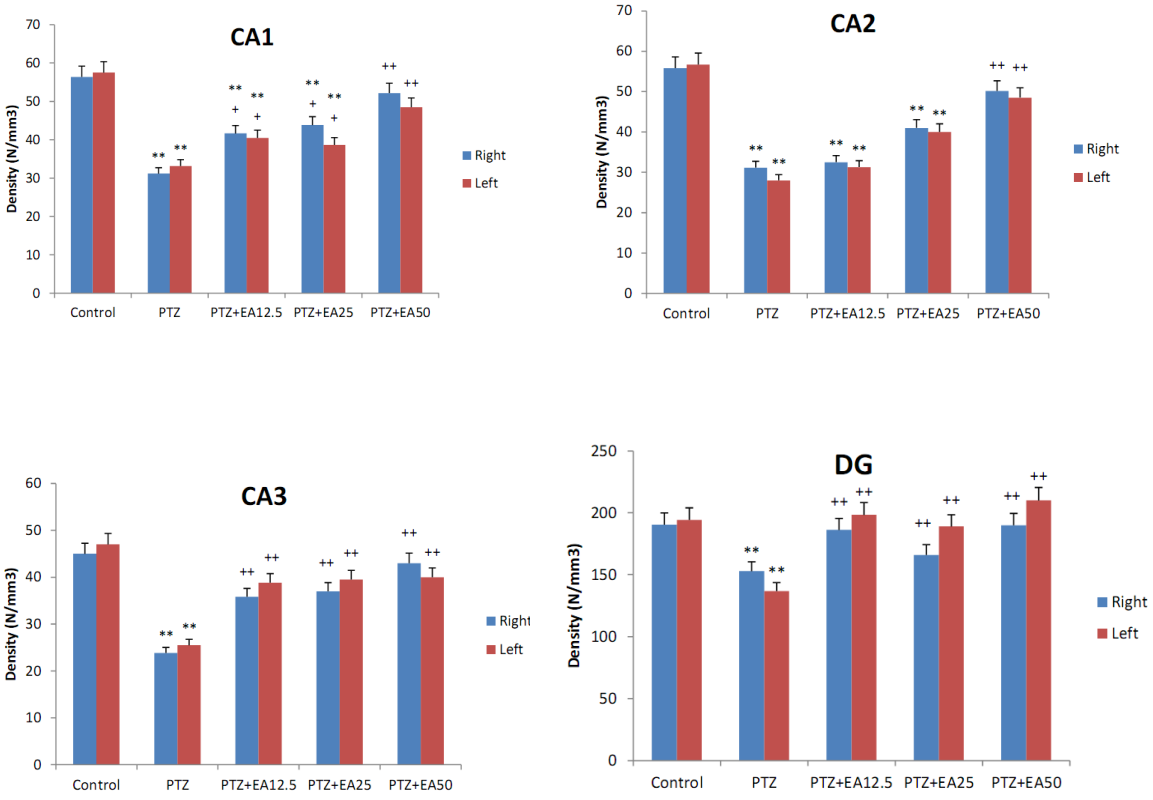

Figure 2. The comparison of neuronal density in the different hippocampal regions (CA1, CA2, CA3 and DG)

$^{**}p<0.001$: the significant differences between control and PTZ groups; $^{++}p<0.001$: the significant differences between PTZ and PTZ+EA12.5, 25, 50 groups.

Discussion

The pentylenetetrazol (PTZ)-induced epilepsy model seems similar to the primary tonic-clonic generalized epilepsy in humans. The results of this study showed that PTZ decreased the neuronal density in CA1, CA2, CA3 and DG regions. Previous study showed seizures increased neurogenesis in the dentate gyrus (DG) animal models (16). Previous study showed following kindled seizures increased neurogenesis (17). Also, another hypothesis showed the relationship between post-seizure neurogenesis and neuronal hyperactivity. Neuronal hyperactivity occurs during seizure increased neurogenesis following repeated brief seizures (18). Another study showed following epilepsy increased the young neurons and basal dendrites in hilus (19). Hadar *et al.* (20) showed that increased the excitability of CA3 pyramidal cells in the epileptic hippocampus. Hannesson *et al.* reported that hippocampal kindling impaired long-lasting of spatial learning and memory in the 8-arm radial maze and the Morris water maze task (21). EA has a different actions such as antibacterial, anti-inflammatory, immune regulatory, antitumor and antioxidant effects (8). Ellagic acid has a scavenger activity of free radicals (22) and inhibited the reactive oxygen species (ROS) effect such as lipid peroxidation (23). Study showed that EA could cross the blood-brain barrier to induced therapeutic effect on the central nervous system (24). One of the PTZ action mechanisms was the increased voltage at the voltage-gated potassium channel (25). Studies showed PTZ triggered activation of caspases-3 to induce the apoptotic neuronal death in the prenatal hippocampus of rats (26). Similarly, another study showed PTZ induced neuronal apoptosis and epileptic seizures in adult rats (27). Spencer *et al.* (28) showed that EA inhibited the damage of the dopamine and copper-mediated DNA (which due to prevents of oxidative stress and cell death) then confirmed the neuroprotective role of EA against oxidative stress (28). Also, administration of EA (50-100 mg/kg/day) inhibited accumulation of sorbitol in erythrocytes, lens and sciatic nerve of rats. These data suggested that EA could be used to central nervous system treatment (29).

Conclusion

In this study we showed that EA could decrease the severity of seizure in animal model of kindling seizure. On the other hand, EA ameliorate the PTZ-induced neuronal damage in hippocampus. Therefore, EA has a neuroprotective effects in animal model of seizure and maybe also in epilepsy.

Ethical issues

Not applicable.

Authors' contributions

All authors equally contributed to the writing and revision of this paper.

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