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Salvianolic Acid Improves Status Epilepticus and Learning and Memory Deficiency in Rat Model of Temporal Lobe Epilepsy

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ABSTRACT

Background and Objective: Epilepsy is a long-lasting central nervous system disorder that is accompany with spontaneous seizures and insufficiency in learning and memory. Now drug treatment is the most common therapy but some patients do not research to suitable control of their seizures with current drugs. Hence, new treatment is needed to help those patients that are unaffected to existing drugs. In present study, we evaluate the protective effect of pretreatment with salvianolic acid B in experimental model of temporal lobe epilepsy in male rats.

Materials & Methods: In the present study, the effect of intraperitoneal administration of salvianolic acid B (10 mg/kg) on induced seizures and learning and memory impairment induced by intrahippocampal injection of kainic acid (KA, 4 μg) were assessed in rats**.**

Results: Obtained data showed that the epileptic rats display kainic acid-induced seizures. On the other hand, in epileptic rats, spontaneous alternation score in Y maze tasks lowered ($p<0.001$) and retention and recall capability in the passive avoidance test impaired ($p<0.05$). Treatment of kainate rats with salvianolic acid B decreased the scale of induced seizures ($p<0.01$), improved alternation score ($p<0.005$) and retention and recall capability ($p<0.01$).

Conclusion: Taken together, this study reveals that treatment of kainate rats with salvianolic acid B could attenuates kainic acid-induced seizures and impairment of short-term spatial memory in rats.

Key Words:
Kainic acid
Salvianolic acid B
Passive avoidance
Y maze
Seizure
Rat

1. Introduction

Epilepsy is the one of the most important neurodegenerative disease. It upsets about 50 million people in the world (1). Temporal lobe epilepsy is the most common form of epilepsy (2, 3), that is characterized with impulsive seizures that cause to brain neuronal injury. Studies show that rat epilepsy model that induced by kainic acid (KA) has the most clinical characteristics of human TLE (4, 5). In adult rats, the injection of KA causes an acute status epilepticus that followed by a chronic period of spontaneous seizures (6-8).

Stimulation of KA receptors causes nitric oxide synthase (NOS) activation, free radical production and mitochondrial dysfunction that result in inflammatory responses, cytokine expression and oxidative stress (9, 10).

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Inflammatory responses resulted from the reactive oxygen species (ROS) production play key role in cell death in some neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson disease (PD), amyotrophic lateral sclerosis, and epilepsy (11-13). Since, after over-stimulation of glutamate receptors, production of ROS scavengers and the preservation of low ROS levels for protection from neurons are necessary (14), thus, agents with antioxidant and anti-inflammatory attributes are recommended to be helpful in this circumstances.

One of major polyphenolic constituents of Salvianolic acids derived from danshen (*Salvia miltiorrhiza*) is salvianolic acid B (Sal B). Within half an hour, Sal B reaches to maximum plasma concentration and 180 min after oral administration, it is detectable in plasma (15, 16). It has been demonstrated that the protective role of salvianolic acids in the liver, nervous system and cardiovascular system (17-20). In spite of antioxidant (21) and anti-inflammatory (22) effects of salvianolic acid B, it could exert potential neuroprotective effect. However, there is no information on its protective effects in animal model of epilepsy. Therefore, this study was designed to determine the possible protective effect of salvianolic acid B against kainic acid-induced seizure and learning and memory deficiency in rat model of epilepsy by determining some behavioral examination.

2. Materials & Methods

2.1. Animals

Adult male Wistar rats (n=40) (Pasteur's Institute, Tehran), weighing 200-250 g at the start of the experiment were housed three per cage in a temperature-controlled colony room under natural light/dark cycle. Animals were given free access to tap water and standard rat chow. Procedures involving animals were made to minimize animal suffering.

2.2. Experimental Procedure

Rats were randomly divided into four equal (n=10) groups: Sham-operated (SH); salvianolic acid B (10 mg/Kg)-treated SH; Kainate; and salvianolic acid B (10 mg/Kg)-treated kainate rats. For stereotaxic surgery, rats were anesthetized with a combination of ketamine (100 mg/Kg, i.p.) and xylazine (5 mg/Kg, i.p.), placed in a Stoelting stereotaxic apparatus (incisor bar -3.3 mm, ear bars positioned symmetrically). The scalp was cleaned with iodine solution and incised on the midline, and a burr hole was drilled through the skull. Animals in the kainate group were unilaterally injected in the dorsal hippocampus with 10 µl of normal salin containing 0.4 µg/µl kainic acid (Sigma Chemicals, USA). Salvianolic acid B (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. Status epilepticus

The steps of kainic acid-induced seizures were scored according to Racine's standard classification: 0, no reaction; 1, stereotype mounting, eye blinking, and/or mild facial clonus; 2, head nodding and/or several 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 (23). Status epilepticus was recorded during 24 hours after stereotaxic surgery.

2.4. Y-maze task

The assessment of spatial recognition memory was performed through recording of spontaneous alternation behavior in a single-session Y-maze on the 14^{th} day post-surgery, as described elsewhere (24). The maze was made of black Plexiglas. Each arm was 40 cm long, 30 cm high and 15 cm wide. The arms converged in an equilateral triangular central area that was 15 cm at its longest axis. The procedure was as follows: each rat, naive to the maze, was placed at the end of one arm and was allowed to move freely through the maze during an 8-min session. The series of arm entries were recorded visually. Entry was considered to be complete when the base of the animal's tail was entirely within the arm. Alternation was defined as successive entries into the three arms on overlapping triplet sets. The maximum number of possible spontaneous alternations was determined as the total number of arms entered - 2, and the percentage was calculated as the ratio of actual to possible alternations × 100

2.5. Single-trial passive avoidance test

The apparatus (40 cm long - 20 cm wide - 30 cm high) consisted of an illuminated chamber connected to a dark chamber by a guillotine door. Electric shocks were delivered to the grid floor by an isolated stimulator. On the first and second days of testing, each rat was placed in the apparatus for 15 min to habituate. On the third day, an acquisition trial was performed. Rats were placed individually in the illuminated chamber. After a habituation period (5 min), the guillotine door was lifted, and, after the rat had entered the dark chamber, the door was lowered and an inescapable scrambled single electric shock (1 mA, 1 s) was delivered. In this trial, the initial latency (IL) of entrance into the dark chamber was recorded, and all rats had ILs greater than 60 s and were included in the study. Twenty-four hours later, each rat was placed in the illuminated chamber for retention trial. The interval between placement in the illuminated chamber and entry into the dark chamber was measured as step-through latency (STL, up to a maximum of 300 s) (25).

2.6. Statistical analysis

All results were expressed as mean ± SEM. The nonparametric Kruskal-Wallis test was used to analyze the behavioral tests, and if a difference was found to be significant, pair-wise comparison was done using the Mann–Whitney U-test. In all calculations, a difference at $p< 0.05$ was regarded as significant.

3. Results

3.1. Behavior observation

According to Racine's standard classification, the class 5 seizures were observed during the acute period (24 hours after kainic acid administration) in 100% of rats treated with kainic acid. Obtained results showed that salvianolic acid B could significantly decrease the scale of induced seizures ($p<0.05$) (Fig. 1). The SH and salvianolic acid B-treated SH rats showed no spontaneous seizures.

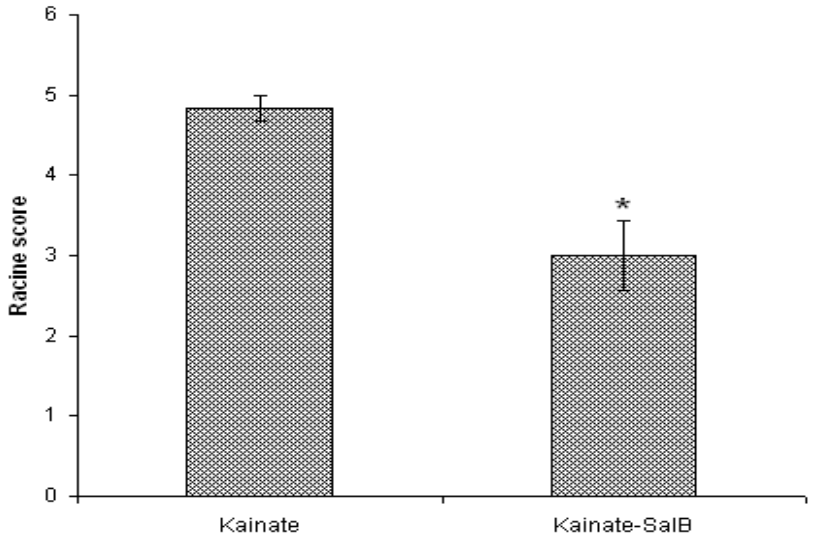

Fig. 1: Seizure scores according to Racine index. As shown, the seizure scores of Sal b-pretreated kainate rats decreased as compared to untreated kainate rats. Values are means ± SEM. *$p<0.01$ (vs. KA)

3.2. Spatial recognition memory in Y-maze

The short-term spatial recognition memory can be evaluated by alternation behavior in Y-maze task. According to data, the alternation percent of the kainic acid injected rats was found to be considerably lower (5.42 ± 2.27%) than that of the sham-operated group (82.85 ± 10.78%) ($p<0.001$). Salvianolic acid B-treated kainic acid injected rats showed a significant increase in alternation percent (16.3 ± 3.90%) as compared to kainate group ($p<0.001$). In addition, total number of arms entered as an index for locomotor activity showed no considerable difference between the kainic acid injected rats and the sham-operated group. Administration of salvianolic acid B caused no substantial change in total number of entered arms (Fig. 2).

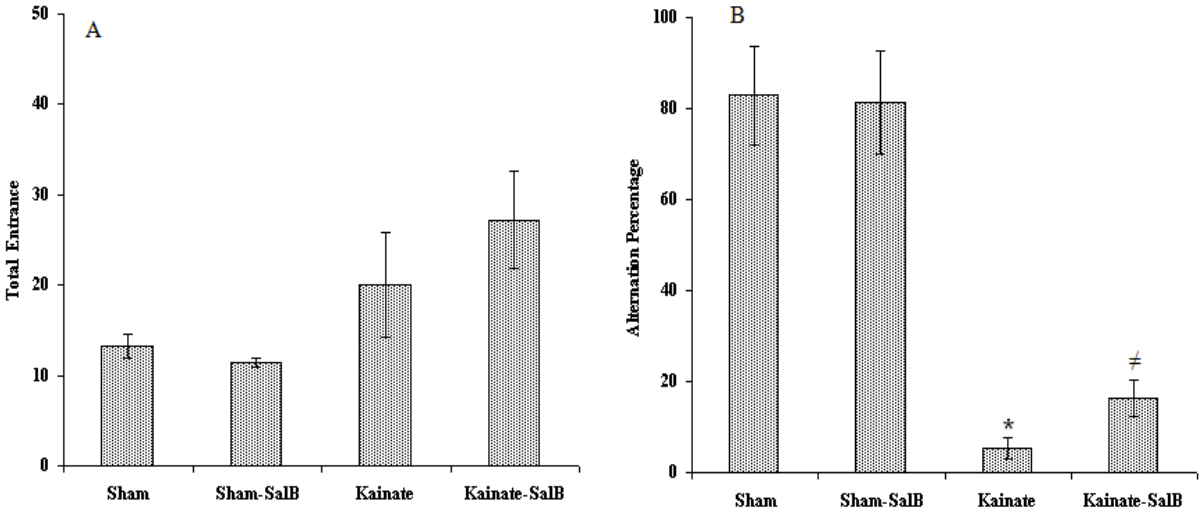

Fig. 2: Total entrance (A) and alternation behavior (B) displayed in the Y-maze by rats. Values are means ± SEM. * $p<0.001$ (vs. sham); ≠ $p<0.05$ (vs. KA).

3.3. Passive avoidance test

Figure 3 shows initial latency and step through latency as passive avoidance indices. As shown, initial latency had no significant difference among the groups. But, kainate rats exhibited a significant destruction in retention and recall in passive avoidance test ($p < 0.05$), as it is obvious by a lower STL. After pretreatment with salvianolic acid B at a dose of 10 mg/kg did generate an improvement in this respect ($p < 0.01$).

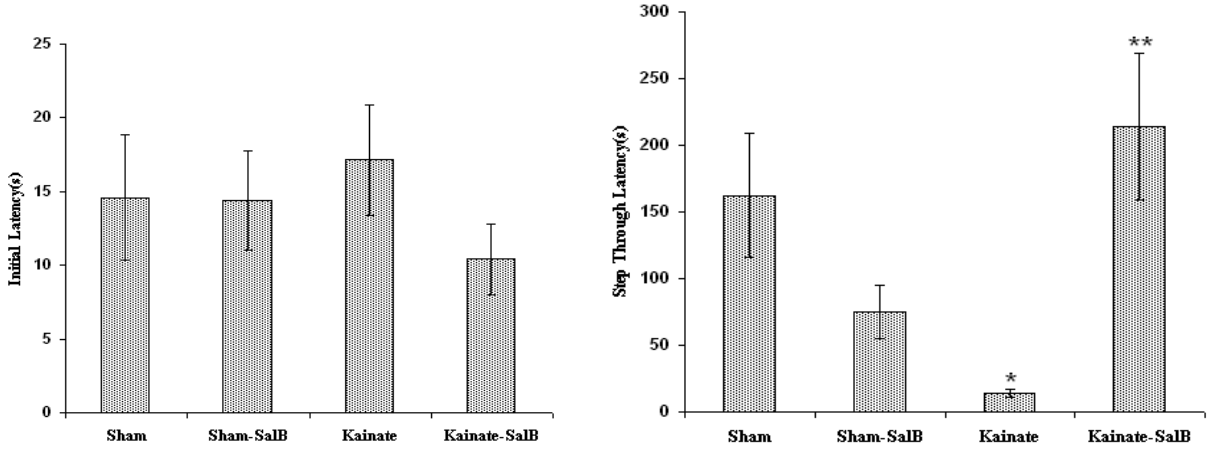

Fig. 3: Initial latency (A) and step-through latency (B) recorded in a single-trial passive avoidance test for rats. Values are means ± SEM * $p<0.05$ (vs. sham); ** $p<0.01$ (vs. KA).

4. Discussion

The results of this study showed that intrahippocampal administration of kainic acid caused development of acute induced seizures, disorder in animal performance in Y-maze and passive avoidance as were shown by a lower alternation percent and step through latency respectively. Second, pretreatment of kainate rats with salvianolic acid B at a dose of 10 mg/kg for one week caused attenuation of spontaneous seizures and improvement of short-term spatial recognition memory.

As the recent studies indicate, the KA- induced temporal lobe epilepsy is a useful method for the study of the concerned mechanisms of epileptic discharge in the limbic system. The activation pattern of KA receptors is similar to human TLE (26,27). Like human TLE, hippocampus, amygdala, and other limbic structures are involved in kainic acid–induced TLE and these structures play a central role in developing of spontaneous seizures subsequent intrahippocampal administration of KA.

Some studies indicate that at acute periods of post-KA, protein oxidation and lipid peroxidation elevates in the hippocampus (28). Also, it reveals that 8-hydroxy-2-deoxyguanosine (8-OHdG), an oxidative marker for DNA damage, may be elevated in the hippocampus and cerebral cortex 8 h after KA treatment (29). Moreover, in the CA1 and CA3 of hippocampus and in the dentate hilus, KA causes an increase in the intracellular calcium and depolarization of neuronal mitochondrial membrane potential. These effects lead to O2 utilization insufficiency, diminished ATP generation, extreme production of ROS, NO, and peroxynitrite. The production of these substances could damage structural cell component including lipids, proteins, and DNA. Meanwhile, KA also caused a decrease in reduced form of glutathione (GSH) levels in the hippocampus (30). With regard to above evidence, it seems that kainic acid exerts its epileptic effects through rising of oxidative stress (31). Since, oxidative damage is linked with cognitive malfunction (32, 33), therefore, treatment with antioxidants could be a beneficial approach in temporal lobe epilepsy.

It has been shown that salvianolic acid B reduces apoptosis through lowering the expression of tumor necrosis factor alpha receptor type 1, matching the expression of Bcl-2 family members, decreasing the release of mitochondrial cytochrome C into the cytosol and inhibiting activated caspase-3 (34), thus, salvianolic acid B may act by this mechanism in such studies.

Also, some studies detected that salvianolic acid B treatment could also satisfy the inflammation induced by some toxic agents (35), so that salvianolic acid B could suppress the expression of pro-inflammatory cytokines TNF-α and IL-1β, whereas enhance the expression of anti-inflammatory cytokines IL-10 and TGF-β1 (36). Therefore, part of its favorable effect in our study could be ascribed to its anti-inflammatory property (36). The same mechanism may have occurred in our study to produce the protective role of salvianolic acid B in kainic acid-induced TLE and reveal that its beneficial effect might be related to its anti-inflammatory activity.

On the other hand, as mentioned, overproduction of ROS and NO have essential roles in progress of kainic acid-induced epilepsy complications (37). Significant reports show that salvianolic acid B could show its anti-oxidant properties by decreasing of the free radicals-induced lipid peroxidation and increasing of neuronal antioxidant system (38), thus it is possible that salvianolic acid B has exerted its anti-seizure effect through its antioxidant attribute.

In conclusion, our results suggest that salvianolic acid B pretreatment could improve kainic acid-induced seizures and short-term spatial recognition memory.

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