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Neuroprotective Effect of Electric Conduction Treatment on Hippocampus Cell Apoptosis in KA Induced Acute Temporal Lobe Epileptic Rats

Shuli Liang [a,b,*,1], Limin Zhang [c,1], Xiaoman Yu [b,1], Shaohui Zhang [b], Guojun Zhang [c], Ping Ding [d]

[a] Department of Neurosurgery, Chinese People's Liberation Army (PLA) General Hospital, Beijing, China
[b] Department of Neurosurgery, First Affiliated Hospital of PLA General Hospital, Beijing, China
[c] Laboratory Diagnosis Center, Beijing Tiantan Hospital, Capital Medical University, Beijing, China
[d] Medical School of Chinese PLA, Beijing, China

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ABSTRACT

Background: Electronic conduction, a new treatment approach for epilepsy, has been confirmed to reduce epileptiform discharge on EEG and convulsive behaviors, particularly epileptic discharge propagation and serious behavioral seizures, in rats with kainic acid (KA)-induced acute temporal lobe epilepsy (TLE).
Objective: Hippocampal cell apoptosis was examined to confirm the neuroprotective effect of electronic conduction therapy in rats with KA-induced acute TLE.
Methods: Rats were divided into four groups: control group (right CA3 injection of saline), KA group (right CA3 injection of KA), sham conduction group (KA rats with sham conduction), and conduction group (KA rats with electric conduction). Apoptotic cells were evaluated by flow cytometry, TUNEL staining, and mRNA expression levels of caspase-3, tumor necrosis factor-alpha, and glial fibrillary acidic protein measured by real-time quantitative PCR (qRT-PCR).
Results: The frequency of convulsive behaviors in the conduction group decreased significantly compared with the KA group and the sham conduction group. Significantly fewer apoptotic cells were detected in rats with conduction based on flow cytometry and TUNEL staining results. The qRT-PCR results indicated that KA-induced up-regulation of hippocampal caspase-3 mRNA expression was reduced 24 hours after KA injection in rats that received conduction treatment.
Conclusion: Electronic conduction treatment can reduce seizure frequency and hippocampal cell apoptosis in rats with KA-induced acute TLE.

Introduction

There are approximately 50 million people with epilepsy worldwide [1], and more than 30% of them have poor seizure control despite the use of optimal AEDs [2,3]. Although some patients benefit from resective surgery, a substantial proportion of patients are not suitable candidates for conventional surgical management. Such patients require alternative therapeutic strategies.

Electrical stimulation has been proposed as an alternative therapy for patients with medically refractory epilepsy who are not candidates for surgical resection. Several neuro-modulation therapies are currently being investigated, e.g., vagus nerve stimulation [4], stimulation of the anterior nucleus of the thalamus [5,6], stimulation of the hippocampus, and direct stimulation of the epileptic focus [7], and they demonstrate significant seizure reduction and neuroprotection [8,9]. Those studies have indicated that neural electrical activity can be abrogated by afferent input from an extracellular stimulus. The mechanism underlying all available therapies, including AEDs, neuro-modulation, and craniotomy surgery, is thought to involve the removal, inhibition, or blocking of the synchronization and excessive excitation of neural electrical activity. Thus, the basic idea is "inhibition". It is well known that the voltage in the seizure focus is very high during epileptiform activity, and an electrical current can be transmitted from the focus with high electrical

Abbreviations: AEDs, antiepileptic drugs; EEG, electroencephalogram; GFAP, glial fibrillary acidic protein; KA, kainic acid; qRT-PCR, real-time quantitative PCR; TLE, temporal lobe epilepsy; TUNEL, terminal deoxynucleotidyl transferase-mediated biotinylated UTP nick end labeling.

* Corresponding author. Fax: +0086-10-68989063.
E-mail address: 301_1sjwk@sina.cn (S. Liang).
[1] Co-first author.

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potential to the region with low electrical potential. Therefore, we proposed a novel conduction treatment, in which the epileptiform activity is transmitted from the focus with high electrical potential to somewhere outside the brain with low electrical potential using a specially made conduction electrode [10]. In contrast to an electrical stimulus, this treatment consists of an electronic output. In a previous study, we have confirmed that electronic conduction can clearly reduce epileptiform discharge on EEG and convulsive behaviors, particularly epileptic discharge propagation and serious behavioral seizures in rats with KA-induced TLE [11]. In the present study, we evaluated the neuroprotective effects of electrical conduction treatment on hippocampal cell apoptosis in rats with KA-induced TLE.

Materials and methods

Ethics statement

All animal procedures complied with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Animal Ethics Board of the First Affiliated Hospital of PLA General Hospital. All surgeries were performed under chloral hydrate anesthesia, and all efforts were made to minimize suffering.

Animals

A total of 255 adult male Sprague–Dawley (SD) rats (The Laboratory Animal Center, Academy of Military Medical Science) weighing 220–260 g were included in the study. The rats were divided into four groups: control group, KA group with sham surgery, sham conduction group, and conduction group. The KA group, sham conduction group and conduction group were divided equally into 24-hour, 3-day, and 7-day subgroups due to the time of sacrifice. Each rat was housed in a separate cage under environmentally controlled conditions (20 °C–23 °C, 12-hour light/12-hour dark cycle, and 50% relative humidity) with food and water provided ad libitum (Fig. 1).

Establishment of a rat TLE model

We established a rat epilepsy model as previously described [11]. All rats were anesthetized with 10% chloral hydrate (3 ml/kg) delivered intraperitoneally while they were fixed in a stereotaxic frame (David Kopf Instruments, USA) at cranial level. The scalp was shaved and sterilized, a midline skin incision was created, and the calvaria was exposed. For unilateral injection of KA (Sigma-Aldrich, St. Louis, MO, USA) into the CA3 region of the right hippocampus, the coordinates were AP = 5.6 mm, R = 4.5 mm, and V = 5.5 mm, according to the stereotactic atlas by Paxinos and Watson. A small burr hole was generated with an electric drill. Sodium kainate, at a dose of 0.6 μg/0.3 μl (in phosphate buffer) for a 250-g rat (adjusted according to body weight), was injected for 5–10 minutes with a 0.5-μl Hamilton microsyringe. The injection needle was held in the region for 3 minutes after completion of the injection to allow diffusion

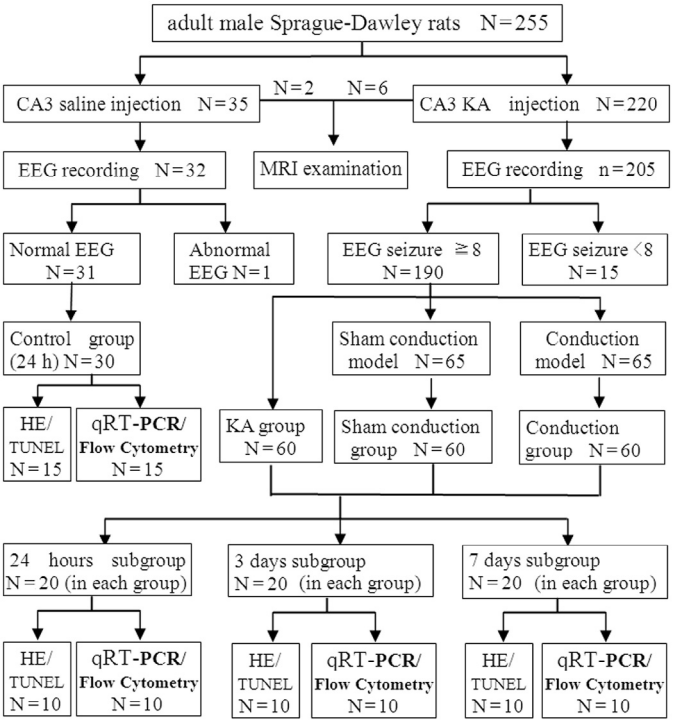

Figure 1. Flow diagram of the study. This diagram shows the construction of the animal model, groups, and examination.

of the KA. An EEG was recorded for each rat for 2 hours. Stainless steel screw electrodes were implanted in the frontal sinus and occipital bone as reference and ground electrodes, respectively. Depth electrodes for the EEG recording were inserted into the CA3 region of the right hippocampus. EEG seizures were recognized against background by their large amplitude (more than three times the baseline amplitude) and high-frequency EEG activity (≥5 Hz) lasting for at least 2 s [11]. EEG seizures observed at least eight times demonstrated the success of the KA model. Control group rats were stereotaxically injected with normal saline instead of KA. Two rats that received saline injection and six rats that received KA injection underwent a 7.0T MRI scan (German, Bruker Inc.) to confirm the location of the injection.

Conduction electrode implantation

After the rat was judged to be a successful KA model by EEG, a stainless steel cannula (0.5 mm in diameter) was inserted into the right side of the CA1 region of the hippocampus via a burr hole under chloral hydrate anesthesia. A Teflon-insulated silver conduction electrode (Fig. 2) (0.3 mm in outer diameter, 0.2 mm in silver wire diameter, 25 mm in length, <5 Ω) was inserted into the target region through the guide cannula by an electrode manipulator until the electrode tips were 2 mm beyond the end of the guide cannula. The coordinates were AP = 5.6 mm, R = 5.2 mm, and V = 7.5 mm. The

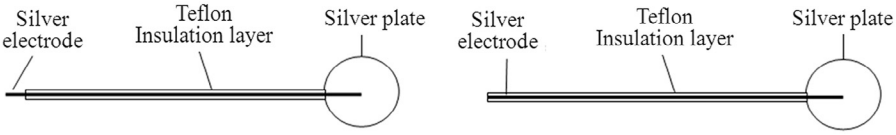

Figure 2. Diagram of the structure of the conduction electrode (left) and sham conduction electrode (right). The conduction electrode had a naked head, but the head of the sham conduction electrode was covered with a Teflon layer of insulation.