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(–)-Epigallocatechin-3-Gallate Protects Against Lithium-Pilocarpine-Induced Epilepsy by Inhibiting the Toll-Like Receptor 4 (TLR4)/ Nuclear Factor-κB (NF-κB) Signaling Pathway

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Background: Temporal lobe epilepsy (TLE) is the most common type of intractable epilepsy in humans, and it is often accompanied by cognitive impairment. In this study, we examined the effects of (–)-Epigallocatechin-3-gallate (EGCG) after SE on behavior in the rat lithium-pilocarpine model of TLE.

Material/Methods: The rats were randomly divided into 3 groups: (1) the control group, in which 12 rats received no treatment; (2) the epilepsy (EP) group, in which 15 rats were treated with saline after status epilepticus (SE); and (3) the EP+EGCG group, in which 15 rats were treated with EGCG (25 mg/kg/d, intraperitoneal) after SE. The SE model was induced with lithium chloride-pilocarpine, and electroencephalography and a high-definition camera were used to monitor SRS. The Morris water maze test and hippocampal late-phase long-term potentiation (L-LTP) recordings were used to evaluate cognitive impairment, and TLR4, NF-κB, and IL-1β levels were determined using Western blot analysis.

Results: We concluded that EGCG treatment after SE (1) markedly reduced SRS frequency in pilocarpine-treated rats, (2) improved epilepsy-induced cognitive impairment and reversed epilepsy-induced synaptic dysfunction in L-LTP *in vivo*, (3) protected hippocampal neurons from damage after SRS, and (4) significantly attenuated the increase in TRL-4 and IL-1β hippocampal levels. The above findings clearly show that EGCG exerts antiepileptogenesis and neuroprotective effects on pilocarpine-induced epilepsy.

Conclusions: We found that EGCG can suppress seizures and inhibit hippocampal neuronal apoptosis, as well as improving cognitive function of epileptic rats. Our findings suggest that EGCG may a novel adjuvant therapeutic approach in epilepsy by improving epileptic behavior and cognitive dysfunction.

MeSH Keywords: Epilepsy, Temporal Lobe • Long-Term Potentiation • Toll-Like Receptor 4

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Background

Epilepsy is a common chronic disease of the nervous system, characterized by recurrent spontaneous seizures [1]. It affects approximately 50 million people worldwide [2]. Although much progress has been made in antiepileptic drug research, and various new antiepileptic drugs have continuously been identified, approximately 1/3 of patients with epilepsy are not sensitive to drug treatment [3]. Moreover, many antiepileptic drugs have adverse effects, such as cognitive dysfunction [4]. In addition, the currently used antiepileptic drugs are mostly symptomatic, that is, they only control seizures, having no effect on the pathological changes that cause epilepsy and no ability to block the epileptic process, meaning that they do not have effects on the origin of the disease [5]. Therefore, the search for effective measures to prevent and cure epilepsy is still an important research focus.

Temporal lobe epilepsy (TLE) is the most common type of intractable epilepsy in humans, and is often accompanied by cognitive impairment. The behavioral and pathological features of the lithium-pilocarpine-induced epilepsy rat model are similar to those in humans caused by an inciting injury, such as hypoxia, status epilepticus (SE), traumatic brain injury, stroke, tumor, or febrile seizures, followed by spontaneous recurrent seizures (SRSs) after a period of silence [6]. The process leading from the initial injury to the subsequent SRSs is called epileptogenesis [7]. The latent period offers an opportunity to prevent epilepsy and modify the disease progression.

There is growing evidence that neuroinflammation plays a major role in the mechanism of epileptic seizures in clinical trials and animal experiments. In clinical studies, interleukin (IL)-17 expression was higher in specimens of focal cortical dysplasia patients [8]. Inflammatory mediators, such as tumor necrosis factor (TNF)-α, IL-1β, IL-6, and IL-17, may play an important role in epilepsy disorders [9]. Recent studies have shown activation of the Toll-like receptor (TLR) signaling pathway in epilepsy [10]. High expression of TLR4 protein was found in surgical resection specimens of drug-resistant TLE, focal cortical dysplasia (FCD), and TSC patients. Meanwhile, the application of HMGB1/TLR4 protein inhibitor can inhibit the formation of epilepsy and improve the susceptibility of seizures [11]. In addition, animal experiments have shown that TLR4 knockout animal models are not susceptible to experimental seizures [12]. In an animal model of acquired epilepsy, inhibiting the IL-1R1/TLR4 pathway mediates disease-modification therapeutic effects [13]. In conclusion, inflammatory factors provide a new target for disease modification.

(–)-Epigallocatechin-3-gallate (EGCG) is the most abundant of the tea polyphenols, which display many beneficial properties, such as antioxidative, antiinflammatory, and antiapoptotic effects [14,15]. A neuroprotective effect has been found in Alzheimer's disease, Parkinson's disease, ischemic stroke, and spinal cord injury. EGCG inhibits the TLR4-nuclear factor (NF)-κB signaling pathway[16], rescues lipopolysaccharide (LPS)-impaired neurogenesis[17], and attenuates inflammatory-induced cognitive impairment [18]. Some studies have confirmed that EGCG inhibits the TLR4/NF-κB pathway and subsequently reduces inflammatory factors such as TNF-α, IL-1β, and IL-6 [17]. Our previous experiments confirmed that EGCG suppressed the progression of kindling in a pentetrazol-induced epilepsy model [19]. However, it is unclear whether EGCG after SE protects against epilepsy. In this study, we examined the effects of EGCG after SE on behavior in a rat lithium-pilocarpine model of TLE.

Material and Methods

Animals and grouping

Eighty male Sprague-Dawley (SD) rats (weighing 180–200 g, 6–8 weeks old) were obtained from Beijing Vital River Laboratory Animal Technology Co., and were placed in comfortable environmental conditions (12 h light-dark cycle, room temperature 25±1°C and humidity 50–60%). All experiments conformed to the National Institutes of Health guidelines for the care and use of laboratory animals and with the European Communities Council Directive of 24 November 1986 (86/609/EEC). The study was approved by the Research Ethics Committee of the Second Hospital of Hebei Medical University (approval No. 2017-P036, dated 2017.02.23).

The animals were randomly divided into 3 groups: (1) the control group (n=12), (2) the epilepsy (EP) group (n=15), and (3) the EP+EGCG group (n=15). The EP+EGCG group was treated with EGCG (25 mg/kg/d, intraperitoneal (i.p.), purity >98%, Meilun Biotechnology, China) beginning on the first day after SE daily for 28 days, while the control and EP groups were given corresponding vehicle (saline) injections.

Induced status epilepticus (SE) model

The SE model was induced in the EP and EP+EGCG groups (n=30) with lithium chloride-pilocarpine, according to the methods of Cavalheiro [20]. Briefly, lithium chloride (127 mg/kg; i.p. Sigma, USA) was injected 20 h prior to the administration of pilocarpine (50 mg/kg i.p. Sigma, USA). To reduce the peripheral reaction of pilocarpine, atropine sulfate (1 mg/kg, i.p. Tianjin, China) was administered 30 min before pilocarpine injection. Seizures were evaluated on the 5-stage Racine scale [21] as follows: level 0: no convulsion; level I: chewing movement; level II: rhythmic nodding; level III: unilateral forelimb clonus; level IV: bilateral forearm clonus with rearing; and level V: all of the

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above with a fall. When the rats showed a sustained level IV or above, they were marked as beginning SE. After 60 min of continuous generalized seizure activity, seizures were terminated with 6% chloral hydrate (300 mg/kg, i.p. Tianjin, China). The control rats were treated with lithium-atropine sulfate and saline instead of pilocarpine and were injected with only a single chloralhydrate injection 2 h after saline injection. During the first day of SE, 6 rats died. The remaining SE rats were randomly divided into 2 groups, the EP group (n=12) and the EP+EGCG group (n=12).

Electroencephalography (EEG) electrode implantation, EEG monitoring, and SRS recording

After 4 weeks of SE, rats were randomly divided into 2 subgroups, one for SRS behavior monitoring (n=6/group) with a high-definition camera and the other for EEG recording (n=6/group). Briefly, the rats were fixed on a stereotaxic apparatus (type: 68027 RWD China), and 3 screws with copper wires were fixed to the surface of the skull, with one inserted into the right frontal cortex to serve as the recording electrode (2 mm posterior and 2 mm lateral to bregma) and one in the left and right occipital cortices each to serve as ground and reference electrodes. Then, the electrodes were fixed with dental cement, and the electric wire was connected to the electroencephalograph (PL3516, AD Instruments, Australia; DP304, Warner Instruments, America). Animals were continuously recorded for 24 h/day from week 6 to 7 after SE by video-EEG recording. Recordings were analyzed and examined by 2 independent, blinded investigators.

Morris water maze (MWM) test

After 7 weeks, the Morris method, as described by Xie et al. [19], was applied, with minor modifications. Briefly, the MWM tests the following 2 skills. (1) Positioning navigation: the experiment generally lasted for 5 days. The latency of escape (i.e., the time from entering the water to finding the platform) was recorded. If the platform was not found within 2 min, the operator placed the rat on the platform to rest for approximately 30 s and recorded the latency as 2 min. (2) Spatial exploration: on the second day after the training sessions, the platform hidden under the water was removed, and the opposite quadrant was selected as the water entry point of the rat. The number of times the rat crossed the location of the platform and the distance swum in the platform quadrant were recorded.

Hippocampal late-phase long-term potentiation (L-LTP) recording

Hippocampal L-LTP has been widely accepted as an electrophysiological mechanism underlying memory. After MWM testing, the rats underwent electrophysiological study (n=6/group) and were anesthetized with urethane (UK 1.5 g/kg i.p. Sigma-Aldrich). Parallel recording/stimulating electrodes (Sequim, WA, USA) were inserted into the hippocampus. The recording electrode was placed at the stratum radiatum of the CA1 region (3.8 mm posterior to the bregma and 2.9 mm lateral to the midline), and the stimulating electrode was implanted at the Schaffer collateral pathway (4.2 mm posterior to the bregma and 3.8 mm lateral to the midline). The stimulus intensity was adjusted to elicit 50% of the maximum field excitatory postsynaptic potential (fEPSP) amplitude, and the stimulus interval was 30 s. Baseline fEPSPs were monitored at least 30 min prior to induction of L-LTP until the amplitude was stabilized. L-LTP was induced using 3 sets of high-frequency stimulation (HFS) with 5-min intervals. Every set of HFS protocol consisted of 20 pulses at 200 Hz and was repeated 3 times at an interval of 30 s. A ≥30% increase in fEPSP amplitude from the baseline was considered a successful induction of L-LTP [22]. L-LTP was recorded for at least 3 h. The averaged value of fEPSP amplitude during 30 min of baseline recording was taken as 100%, and all recoded fEPSPs were normalized to this baseline value.

Nissl staining

After L-LTP, the rats were anesthetized with 10% chloral hydrate and perfused with cold saline solution, and the brain tissue was extracted. The whole brain was divided into 2 hemispheres, with one immersed in 4% paraformaldehyde fixed with 0.1 mol/L PBS 24 h at 4°C for Nissl staining and the other used for Western blot analysis. Once the tissue was properly fixed with 4% paraformaldehyde, it was placed in 30% sucrose until it sank to the bottom. The tissue was transferred to O.C.T. embedding compound, frozen in liquid nitrogen, and stored at - 80°C. Coronal sections 8 mm thick were prepared using a frozen microtome (Leica, Germany) and placed on polylysine-treated slides for Nissl staining, with 1 out of every 5 sections retained (6 animals per group, 6 sections per animal). The dyeing process was as follows: 1) the specimen was soaked in 70% ethanol solution for degreasing overnight, 2) rinsed in double-distilled water for 3 min, and 3) incubated in 1% methyl violet for 5 min. 4) Color separation was controlled with 0.05% glacial acetic acid under the microscope. 5) The tissue was dehydrated in an alcohol gradient, cleared in xylene, and 6) sealed in neutral resin. The number of intact pyramidal neurons in the hippocampal CA1 and CA3 region in a 1-mm^2 field was observed and recorded under an Olympus BX51 microscope.

Western blotting

The hippocampus was isolated from the brain tissue retained on an ice plate, and the total protein was extracted (Applygen Technologies, Beijing, China) using a conventional bicinchoninic acid (BCA) kit to detect the protein concentration

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of each sample. Equal amounts of protein were separated on 10% sodium dodecyl sulfate-polyacrylamide gels and transferred to immobile polyvinylidene difluoride membranes (Millipore, CA, USA) for approximately 90 min. The PVDF membrane was then placed in 5% nonfat milk powder and sealed at room temperature for 1 h. Antibodies were diluted with PBS solution at the appropriate concentration according to the specification of each antibody, anti-IL-1β (1: 800 Bioworld USA), anti-TLR4 (1: 500 Boster China), anti-NF-κB (1: 1000 Bioworld USA), and anti-β-actin (1: 1000 Santa Cruz, CA, USA). The PVDF membranes were incubated in the diluted antibody at 4°C overnight and washed with 0.1% TBST 3 times for 5 min each time. The goat anti-rabbit fluorescent secondary antibody (1: 10000 Rockland Immunochemicals, Limerick, PA, USA) was added to the solution, and the membranes were incubated at room temperature on a shaking table for 1 h and washed with TBST 3 times for 5 min each time. Odyssey imaging system scan film and ImageJ software were used to determine the density of the target band. The density of the IL-1β, TLR4, and NF-κB bands was measured relative to that of the internal control (β-actin).

Data analysis

All values are displayed as the mean ± standard error (SEM). SPSS 22.0 was used for statistical analyses. SRS frequency and duration were compared between the EP and EP+EGCG groups using the unpaired *t* test. Analysis of variance (ANOVA) for repeated measures was used to analyze the escape latencies in the Morris water maze test among the groups over a period of 5 days. One-way ANOVA was used to compare the other data among the 3 groups. The statistical significance level was set at $p<0.05$.

Results

EGCG treatment after SE reduces SRS frequency and duration in pilocarpine-treated rats

We observed the effect of EGCG after treatment on SE at the chronic stage. We found that SRSs appeared in the EP group at approximately 18±2 days, which was earlier than that in the EGCG post-treatment EP group, but the difference was not significant. However, there were significant differences in behavior between the EP and EP+EGCG groups. The rats in the EP group showed irritability and aggression, as well as untidy fur. In the EEG recordings during class IV/V seizures, epileptic discharges were characterized by high amplitude (>2×baseline), high frequency (>5 Hz), and long duration (>3 s). In the EP group, the epileptic discharges were longer than those in the EP+EGCG group, whereas no epileptic discharge was observed in the control group (Figure 1A). A combination of behavioral and EEG analyses revealed that post-SE EGCG treatment appeared to reduce seizure severity. SRS frequency was higher in the EP group than in the EP+EGCG group, and the average seizure duration was longer in the EP group than in the EP+EGCG group ($P<0.001$). Taken together, these findings suggest that EGCG treatment tended to reduce SRS frequency and seizure duration (Figure 1B, 1C).

EGCG treatment post SE improves cognitive dysfunction

In the MWM test, the navigation experiment revealed that the escape latency of rats in each group was shortened with training (Figure 2A). The escape latency of the 3 groups was not significantly different on the first 2 days ($P>0.05$), On days 3–5, the escape latency in each group was shorter than that on the previous 2 days. Compared with the control group, the escape latencies in the EP group was longer ($P<0.05$). However, the poor performance was improved by treatment with EGCG ($p<0.01$). The spatial exploration test revealed that rats in the EP group exhibited significantly fewer platform crossings than those in the control group ($P<0.05$ Figure 2B). However, treatment with EGCG significantly increased the number of crossing ($p<0.01$). The duration of time spent in the target quadrant of the EP group was significantly shorter than that of the control group ($P<0.01$), while treatment with EGCG significantly improved the performance ($P<0.05$).

EGCG treatment after SE partly reverses epilepsy-induced synaptic dysfunction in L-LTP

As shown in Figure 3, in the control group, the average fEPSP amplitude rapidly increased to 241.46±7.18% of baseline and remained at 186.16±5.93%, 172.89±5.34%, and 160.11±4.60% at 60 min, 120 min, and 180 min after HFS, respectively (Figure 3B). In contrast, the average fEPSP amplitude in the EP group was 175.07±9.02% immediately after HFS and 137.13±5.10%, 119.61±5.42%, and 107.96±5.66% at 60 min, 120 min, and 180 min after HFS, respectively, all of which were significantly lower than the values in the control group ($P<0.01$) (Figure 3B). However, the average fEPSP amplitude in the EP+EGCG group was 209.25±9.92% immediately after HFS and 159.21±4.68%, 147.88±4.12%, and 138.51±5.45% at 60 min, 120 min, and 180 min after HFS, respectively, suggesting that EGCG partly reversed epilepsy-induced synaptic dysfunction ($P<0.05$ when compared to the EP group). Therefore, long-term EGCG treatment partially attenuated the epilepsy-induced impairments in L-LTP.

EGCG protects hippocampal pyramidal neurons from damage

To observe the effect of post-SE EGCG treatment on the impaired hippocampal pathology in post-SE rats, hippocampal

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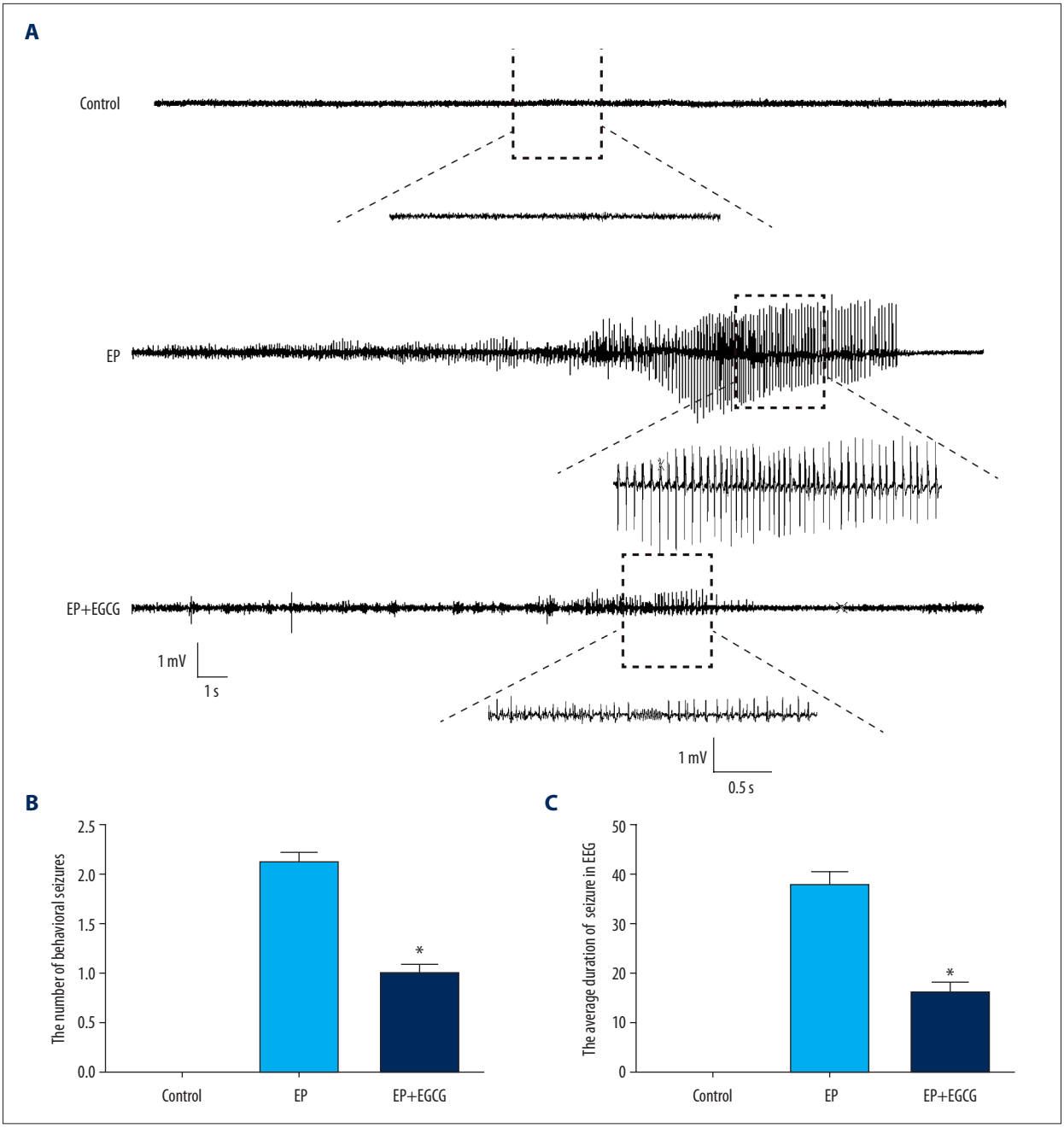

Figure 1. Effects of long-term EGCG treatment on SRS and seizure duration in pilocarpine-induced epilepsy rats. SRS was compared between the EP and EP+EGCG groups (n=12/group). (**A**) Control, EP, and EP+EGCG EEG recordings. In the EP group, the epileptic discharges were longer than those in the EP+EGCG group. No epileptic discharge was observed in the control group. SRS frequency was 2.1±0.9 per day in the EP group and 1.0±0.08 per day in the EP+EGCG group, and the average seizure duration was longer in the EP group (37.57±0.89) than in the EP+EGCG group (16.08±0.6). (**B, C**) Show that EGCG treatment tended to reduce SRS frequency and seizure duration. Values are expressed as the means ±SEM (n=6/group). * $P<0.001$ compared to the EP group.

pyramidal neurons were labeled using Nissl staining, which shows surviving pyramidal neurons. The hippocampal CA1 and CA3 pyramidal neurons in the control group were intact, and almost no pyramidal neurons were lost (Figure 4A, 4D, 4G). Compared with the control group, the EP group lost significantly more pyramidal neurons (Figure 4B, 4E, 4H), and the CA1 subfield in the EP group was more severely damaged than the CA3 subfield. In the EP+EGCG group, the structure of pyramidal

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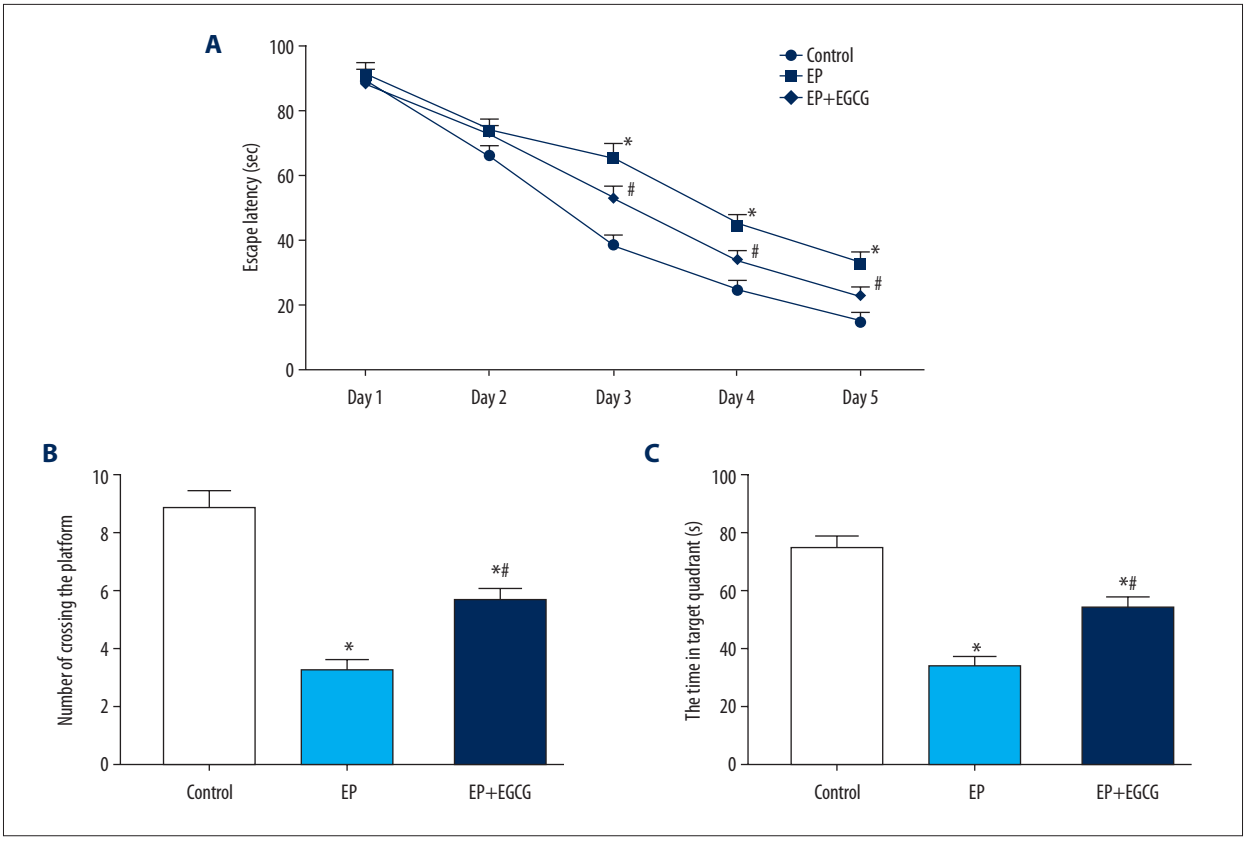

Figure 2. Effects of long-term EGCG treatment on cognition in the MWM in pilocarpine-induced epilepsy rats. (**A**) Results of the navigation experiment, escape latencies in the MWM. (**B**) Number of platform crossings during the spatial exploration experiment. (**C**) The time spent in the target quadrant during the spatial exploration experiment. Values are expressed as the means ±SEM (n=12/group). * $P<0.001$ compared to the control group, # $P<0.05$ compared to the EP group.

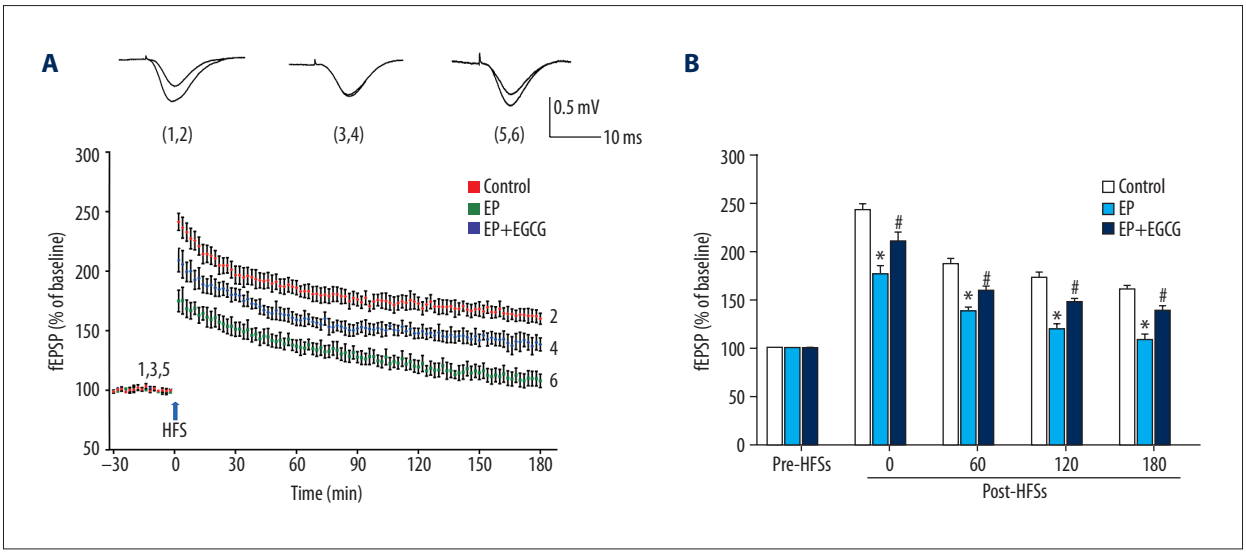

Figure 3. Effects of long-term EGCG treatment on *in vivo* L-LTP in the hippocampal CA1 region. (**A**) Scatter plots showing that L-LTP in the EP group was significantly suppressed in the hippocampal CA1 region. Each point represents the mean ±SEM of the fEPSP amplitude. Insets: typical fEPSP traces recorded 15 min before and 3 h after HFS in the 3 groups. (**B**) Histograms displaying the average fEPSP amplitude in 3 groups at different time points before and after HFS. Each column represents the mean ±SEM (n=6/group). * $P<0.01$ compared with the control group at the same time; # $P<0.05$ compared with the EP group.

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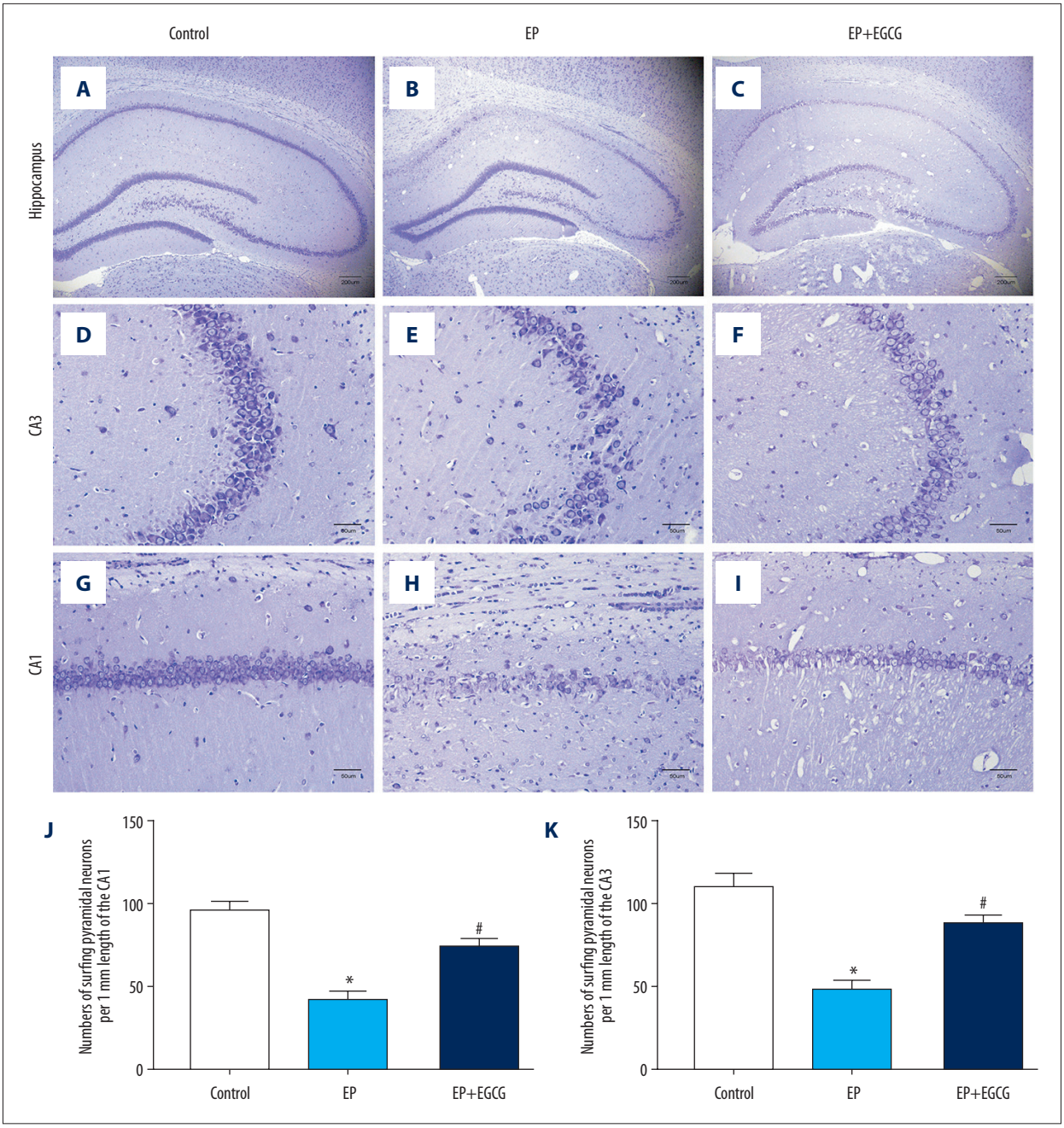

Figure 4. Effect of long-term EGCG treatment on the number of pyramidal neurons in the hippocampal CA1 and CA3. Photomicrographs demonstrating the whole hippocampus (**A–C**), CA1 subfield (**D–F**), and CA3 subfield (**G–I**). The hippocampal pyramidal neurons in the control group: (CA1: 96.17±5.77, CA3: 109.67±9.810), in the EP group: (CA1: 41.5±5.858, CA3: 48.17±4.929), and in the EP+EGCG group (CA1: 74.67±3.739, CA3: 87.00±6.137). Quantitative representation of the remaining pyramidal neurons in CA1 (**J**). Quantitative representation of the remaining pyramidal neurons in CA3 (**K**). The results are expressed as the means ± SEM (n=6 per group). * $P<0.001$ compared with the control group, # $P<0.05$ compared with the EP group.

neurons was partially intact, and significantly more Nissl bodies were present (Figure 4C, 4F, 4I) than in the EP group. Analysis of hippocampal pyramidal neuron survival revealed that there were significantly fewer neurons in the CA1 and CA3 in the EP group than in the corresponding regions in the control group (P<0.05). After EGCG treatment, the number of surviving pyramidal neurons was significantly increased compared to that in the EP group (P<0.05).

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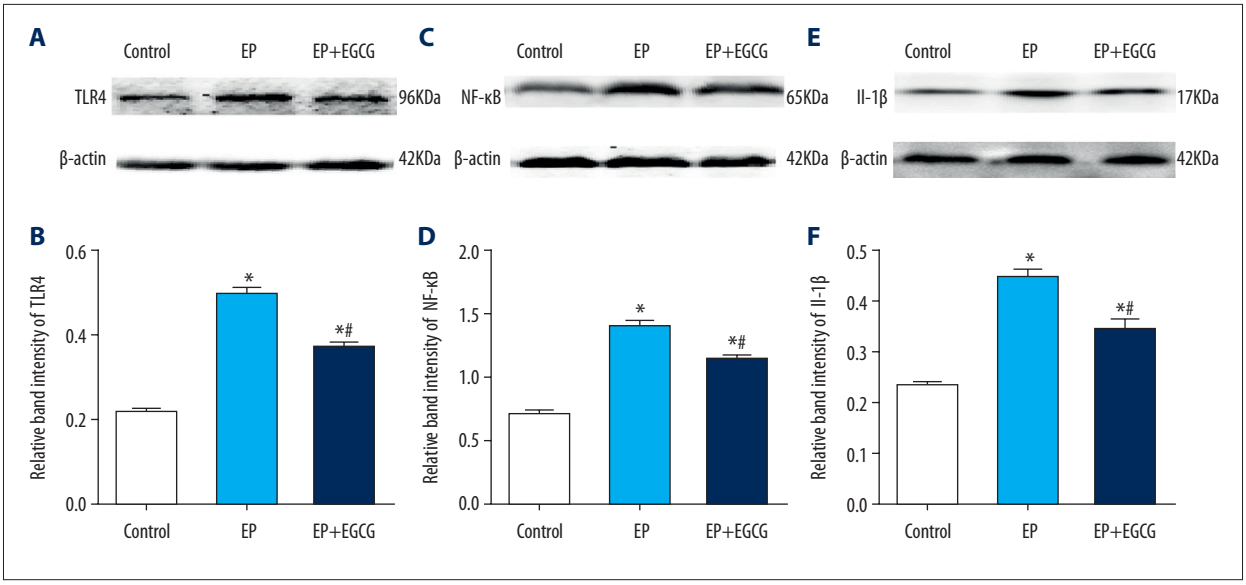

Figure 5. Effects of long-term EGCG treatment on hippocampal expression of TLR4, NF-κB, and IL-1β. (**A, C, E**) Representative Western blot images of TLR 4, NF-κB, and IL-1β in the control, EP, and EP+EGCG groups. (**B, D, F**) Histograms showing the quantitative analyses of TLR 4, NF-κB, and IL-1β. Data are expressed as the means ±SEM. (n=6/group). * $P<0.001$ compared with the control group; # $P<0.05$ compared with the EP group.

EGCG treatment after SE decreases inflammatory protein expression

TLR4, NF-κB, and IL-1β were normally expressed in the hippocampal tissues of the control group. Compared with the expression in the control group, the expression of TLR4, NF-κB, and IL-1β in the EP group was significantly increased ($P<0.05$), further suggesting that inflammatory pathways play an important role in the development of epilepsy (Figure 5). Compared with the expression in the EP group, the expression of TLR4, NF-κB, and IL-1β in the EP+EGCG group was significantly decreased ($P<0.05$), suggesting that EGCG downregulated the expression of TLR4, NF-κB and IL-1β in the process of epileptic formation.

Discussion

Based on the present results, we reached the following conclusions: EGCG treatment after SE (1) markedly reduced SRS frequency in pilocarpine-treated rats, (2) improved epilepsy-induced cognitive impairment and reversed epilepsy-induced synaptic dysfunction in L-LTP *in vivo*, (3) protected hippocampal neurons from damage after SRS, and (4) significantly attenuated the increase in TLR-4/NF-κB and IL-1β hippocampal levels. The above findings clearly show that EGCG exerts antiepileptogenesis and neuroprotective effects on pilocarpine-induced epilepsy.

EGCG has superb antioxidant, antiinflammatory, radical-scavenging, and antiapoptotic properties. Therefore, in neurodegenerative disease [23], inflammatory diseases [24], and cognitive dysfunction [25], EGCG plays a potential protective role. EGCG can be detected in the brain 5 days after oral administration, indicating that EGCG can pass through the blood-brain barrier and localize in the brain. To better simulate the treatment of clinical patients with diagnosed epilepsy, we gave EGCG after the occurrence of SE. In our study, we found that EGCG reduced SRS. However, SRS was still observed, showing that EGCG was unable to completely inhibit disease progression. This partial effect may be attributed to its weak availability, stability, and solubility, and nanotechnology might help to enhance the antiepileptic effect of EGCG [26].

Some studies have reported that 30% to 40% of epilepsy patients have various levels of cognitive impairment, mainly due to decreased attention, memory, and judgment [27]. Among all comorbidities, cognitive impairment is the most closely associated with epilepsy, according to a study that included older adults [28]. Hermann et al. suggested that cognitive function in patients with TLE was significantly reduced [29]. Synaptic plasticity is an ubiquitous phenomenon in the central nervous system and plays a key role in learning and memory [30]. N-methyl-D-aspartate receptor-mediated LTP is a widely studied synaptic plasticity that is closely related to memory behavior [31]. Kirill et al. showed that LTP induction was decreased 7 days after SE in hippocampal neurons [32]. In our research, post-SE EGCG treatment partly reversed epilepsy-induced synaptic dysfunction in L-LTP *in vivo*. EGCG repaired the impairment in synaptic plasticity induced by epilepsy. EGCG has been shown to ameliorate nerve damage caused

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by LPS by suppressing inflammatory pathways and restoring neurogenesis [17], as well as improving cognition in Alzheimer transgenic mice [33]. EGCG has been used in phase 2 clinical trials in patients with Down's syndrome, showing that participants treated with EGCG had higher scores in visual recognition than did the control participants [34].These conclusions further confirm that EGCG can partly improve cognitive abilities.

Histopathological staining of rat brains showed that EGCG could also improve the damage of pyramidal neurons in hippocampal CA1 and CA3 regions and reduce the loss of neurons, indicating that EGCG has a neuroprotective effect in pilocarpine-induced epilepsy rats.

Increasing evidence suggests that inflammation plays a crucial role in epileptogenesis [9,35]. Some scholars suggest that antiinflammatory treatment will become a new therapy for epilepsy [36]. Kleen has suggested that inhibition of TLR4 can reduce seizures [12]. In an animal model, activation of the TLR4 pathway increases seizure susceptibility [37]. In our study, the expression of TLR4, NF-κB, and IL-1β was significantly higher in the EP group than in the control group. Treatment with ILR-1/TLR4 antagonists has been shown to modify the epileptogenic network to prevent further progression of epilepsy [13]. Our data indicate that EGCG reduced spontaneous chronic seizures, accompanied by a reduction in TLR4, NF-κB, and IL-1β expression. Rahimifard et al. reported that EGCG targets the TLR4 signaling pathway and is a novel therapeutic strategy for neuroinflammation [16]. These findings support the hypothesis that EGCG can prevent epilepsy progression through its anti-inflammatory effects. Activation of the TLR4 pathway leads to the disappearance of hippocampal neurons [38], which is consistent with the observed results. Our results showed a significant loss of hippocampal neurons in the EP group and that EGCG treatment protected hippocampal neurons from damage after seizure. These results further confirmed the neuroprotective effect of EGCG in the pilocarpine epilepsy model.

Conclusions

Our study shows that EGCG can suppress seizure frequency and time shorten the duration of seizures. EGCG treatment after SE improves cognitive dysfunction in MWM testing, and partly reverses epilepsy-induced synaptic dysfunction in L-LTP. EGCG protects hippocampal pyramidal neurons from damage. In our study, we found the inflammatory protein expression increases in SE rats, and after treatment with EGCG, the inflammatory protein expression decreases. These findings show that EGCG can protect against lithium-pilocarpine-induced epilepsy by inhibiting the TLR4/NF-kB signaling pathway. Our findings suggest that EGCG may be a novel adjuvant therapeutic approach in epilepsy by improving epileptic behavior and cognitive dysfunction.

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