Seizure 19 (2010) 165–172

Contents lists available at ScienceDirect

Seizure

journal homepage: www.elsevier.com/locate/yseiz

Oxidative stress mediates hippocampal neuron death in rats after lithium–pilocarpine-induced status epilepticus

Jinzhi Liu [a], Aihua Wang [a,*], Lili Li [b], Yanfei Huang [a], Ping Xue [a], Aijun Hao [b]

[a] Department of Neurology, Qianfoshan Hospital, Medical School of Shandong University, No. 66, Jingshi Road, Jinan, Shandong 250014, PR China
[b] Department of Histology and Embryology, Shandong University, Jinan, Shandong 250012, PR China

ARTICLE INFO

Article history:
Received 5 October 2009
Received in revised form 12 January 2010
Accepted 15 January 2010

Keywords:
Oxidative stress
Status epilepticus
Pilocarpine
Hippocampus
Caspase-3

ABSTRACT

Oxidative stress, which is defined as the over-production of free radicals, can dramatically alter neuronal function and has been linked to status epilepticus (SE). The pathological process and underlying mechanisms involved in the oxidative stress during SE are still not fully clear. In the current study, SE was induced in rats by lithium–pilocarpine administration. Our data show that hippocampal neuron death occurs at 6 h and is sustained for 7 days after SE. The production of nitric oxide (NO) started to increase at 30 min and was evident at 6 h and 7 days after SE, which coincided with increased expression of neuronal nitric oxide synthase (nNOS), inducible nitric oxide synthase (iNOS) and malondialdehyde (MDA) after SE, whereas, activated caspase-3 prominently appeared at 7 days after SE. Further, FK506, an immunosuppressant, partially rescued the neuron death and attenuated the expression of NO, nNOS, iNOS, MDA and activated caspase-3. Taken together, our study indicates that oxidative stress mediated hippocampal neuron death occurs prior to caspase-3 activation and that FK506 plays an important role in protecting hippocampal neurons during status epilepticus.

1. Introduction

Status epilepticus (SE) is a severe clinical manifestation of epilepsy and has functional and structural consequences resulting in brain damage.[1] There are a number of physiological and neurochemical changes that take place during the seizure activity.[2] Notably, oxidative stress can dramatically alter neuronal function and has been associated with neurochemical changes observed during SE and spontaneous recurrent seizure (SRS) induced by pilocarpine.[3,4]

Oxidative stress is defined as an imbalance between higher cellular levels and reactive oxygen species (ROS), such as superoxide radical, hydrogen peroxide and nitric oxide (NO),[5] and cellular antioxidant defense including superoxide dismutase (SOD).[6] Brain tissue is particularly vulnerable to oxidative damage because of its high consumption of oxygen and the consequent generation of high quantities of free radical. Growing data suggest that injury resulting from oxidative stress may play an important role in the pathophysiology following acute neurological insults such as stroke and seizures.[7]

NO is formed from arginine by the action of three kinds of nitric oxide synthase (NOS) isoforms, two calcium-dependent forms, i.e., neuronal (nNOS) and endothelial (eNOS), and one calcium-independent form, i.e., inducible nitric oxide synthase (iNOS). In the nervous system, nNOS is largely responsible for NO production at early stages after SE. iNOS has been implicated in some important central processes[8] and produces large amounts of NO continuously for long periods, a feature that is responsible for the cytotoxicity of NO. It may play an important role during chronic stress and apoptotic cell death in the hippocampus in SE rats.

As a free radical, NO is widely regarded as a messenger molecule that participates in diverse physiological processes in the central nervous system (CNS), including brain development, pain perception, neuronal plasticity, memory and behavior.[9] However, NO is also involved in a number of pathological conditions, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis, amyotrophic lateral sclerosis, cerebral ischemia, traumatic brain injury and epileptic seizure.[10] A strong NO activation was found in the hippocampus and other brain regions following KA-induced SE. On the basis of animal experiments, NO has been implicated in many of the molecular mechanisms of epileptic seizures, ranging from mediation of an excitotoxic cascade to modulation of the CNS blood flow during the episodes, and finally to participation in the subsequent neuronal injury.[11–13] When overproduced, much of the newly synthesized NO will be converted into peroxynitrite, which is an extremely potent free radical.[14,15] This substance subsequently interferes with

* Corresponding author. Tel.: +86 13791120819.
E-mail address: wangah_0052@sina.com (A. Wang).

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doi:10.1016/j.seizure.2010.01.010

mitochondrial energy metabolism, and may even cause death of neurons by necrosis or apoptosis.[16]

FK506, an immunosuppressive agent, commonly used in clinical settings for the prevention of allograft rejection[17,18] is also known for its strong neuroprotective effects following brain damages of different origins.[19,20] In a recent study, we have shown in our lab that FK506 could control chronic refractory seizure via inhibiting calcineurin (CaN) mediated $GABA_AR$ dephosphorylation.[21] Our current study was designed to investigate whether and how oxidative stress participates in this process using lithium chloride–pilocarpine-induced SE (LPCSE) rat model.

2. Experimental procedures

2.1. Experimental animals

8–12 weeks-old male Wistar rats weighing between 250 and 280 g were used in this study (Experimental Animal Center of Shandong University, China). The rats were housed under controlled temperature and light conditions (12 h light; 12 h dark cycle with lights on at 08:00 a.m.), with free access to food and water. The experimental procedures were approved by the Commission of Shandong University for ethics of experiments on animals in accordance to international standards.

2.2. Pre-treatment of animals

The rats were randomly divided into four groups (n = 54 in each group, totaling 216): control group, pilocarpine group (pilo group), saline + pilo group and FK506 + pilo group (FK506 group). In FK506 group, the animals were further treated with FK506 (Fujisawa, Japan) (2 mg/kg, i.p.) 24 h and 1 h before pilocarpine administration. In addition, rats in saline + pilocarpine group were treated as FK506 group except that FK506 was replaced by the same dose of 0.9% saline. Pilocarpine group animals were treated with pilocarpine with no pre-treatment. Control group animals were treated as pilocarpine group with the same dose of 0.9% saline instead of pilocarpine. The animals of each group, which consisted of seven subgroups (six rats in each subgroups) with 0.5 h, 2 h, 6 h, 12 h, 1 day, 3 days, and 7 days after treatment, were sacrificed for the estimation of NO, NOS, MDA, SOD and immunoblotting. Six rats were killed at 6 h and six rats at 7 days for immunohistochemical techniques.

2.3. Induction of seizures

The rats were pretreated with lithium chloride (3 mequiv./kg, i.p.) 24 h before injection of scopolamine methylnitrate (1 mg/kg, s.c.) that does not cross the blood–brain barrier to prevent peripheral cholinergic effects without affecting the central nervous system. Pilocarpine was administered 30 min after scopolamine methylnitrate (s.c.). Every effort was made to reduce the number of animals used and minimize animal suffering. After 1 h when the rats developed convulsive seizures at stage IV or V according to Racine,[22] they were treated with diazepam (10 mg/kg, i.p.) to stop SE. Control group animals were treated with the same dose of 0.9% saline instead of pilocarpine.

2.4. Tissue sampling

Six animals from each group were sacrificed at 0.5 h, 2 h, 6 h, 12 h and 1 day, 3 days and 7 days after SE. Since numerous studies have identified that hippocampus is one of the most important brain regions involved in pathological process of epilepsy, the hippocampus was isolated, and stored at −80 °C until further processing.

2.5. Histology

Rats were perfused with 4% paraformaldehyde in PBS under anesthesia at different times after seizures. The brains were sectioned coronally in 10 μm thickness. H&E staining with hematoxylin and eosin was then performed. The surviving cells were defined as round-shaped, cytoplasmic membrane-intact cells, without any nuclear condensation or distorted aspect. The surviving pyramidal cells in the hippocampal CA3 region were seen at high magnification (400×).

2.6. Assay for NO and NOS activity

Left hippocampus of rats was weighed, and homogenized in saline (10%wt/vol) or HEPES buffer (20%wt/vol). Saline homogenate was used for the estimation of NO content, and NOS activity was measured in the HEPES-buffered homogenate.

It is reported that nNOS is the main isoform of cNOS (constitutive nitric oxide synthase, including the isoforms of nNOS and eNOS).[23] Since the activity of nNOS has not been measured directly, cNOS activity was measured to reflect the activity of nNOS indirectly. The activities of cNOS, iNOS as well as the concentrations of NO in the supernatant were determined using commercially available kits (Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer's instructions. NO is an extremely labile molecule and turns rapidly to NO_2^- and NO_3^- in vivo. The assay of NO level was based on nitrate reductase which specifically convert NO_3^- to NO_2^- that was measured at a wavelength of 550 nm. cNOS is calcium-dependent and iNOS is calcium-independent, the criteria on which the discrimination of two kinds of isoforms were based.

2.7. Assay for MDA and SOD

Saline homogenate was used for the estimation of MDA contents and superoxide dismutase (SOD) activities by using commercially available kits (Jiancheng Bioengineering, NanJing, China). All procedures completely complied with the manufacturer's instructions. Lipid peroxidation (LPO) was assessed by measuring the concentration of malondialdehyde in the form or a stable chromophoric reaction product with thiobarbituric acid (TBA) measured at a wavelength of 532 nm. The assay of SOD activity was based on its ability to inhibit the oxidation of oxymine by superoxide anion produced from the xanthine–xanthineoxidase system. One unit of SOD activity was defined as the amount that reduced the absorbance at 550 nm by 50%.

2.8. Immunohistochemistry

The rats were anesthetized with chloral hydrate (500 mg/kg IP) at various times after SE and perfused via the left cardiac ventricle with 4%paraformaldehyde in PBS. The brain was removed and placed in the same fixative at 4 °C for at least 24 h. The cerebellum and olfactory bulbs were removed. The first frontal 6 mm of the cerebral hemispheres were removed and the following 8 mm, which contained hippocampus, were collected and fixed with paraffin. The brains were placed in a 20% sucrose solution in phosphate buffer at room temperature for 48 h and in a 30% sucrose solution at least 48 h. The brains were positioned on the stage of a freezing microtome and were cut at a nominal thickness of 10 μm in the horizontal plane and stored at −20 °C. The sections were incubated with 3% H_2O_2 for 5 min and then with phosphate-buffered saline for 5 min. The sections were then treated with primary antibodies for 24 h at 4 °C and thereafter incubated with biotinylated goat antibodies against rabbit IgG as the secondary antibody for 10 min. Subsequently, the sections were incubated

Table 1
Behavioral episodes after LPCSE.

Group	*n*	Latency period to stage IV (mean ± SD)	Proportion of stage V	Proportion of stage V
Pilo	54	47.4 ± 9.1	42.6%	57.4%
Pilo + saline	54	46.9 ± 9.6	50%	50%
Pilo + FK506	54	67.5 ± 9.7*	74.1%*	25.9%*

The data for behavioral episode after LPCSE or LPCSE with FK506. When FK506 was administered prior to pilocarpine injection, the latency period was significantly longer and the percent of stage V was considerably lower. Data are mean ± SD.
* $P < 0.05$ vs. pilo or saline + pilo.

with peroxidase-labelled streptoavidin for 10 min and then the colour was developed with diaminobenzene. Finally, the sections were counterstained with hematoxylin. The following primary antibodies were used: rabbit polyclonal antibodies against rat brain nNOS (1:1000; Chemicon), iNOS (1:5000; Chemicon) and active caspase-3 (1:10; Chemicon).

2.9. Western blot analyses

For Western blot analyses of nNOS, iNOS and active caspase-3, the right hippocampi were homogenized (1/10, w/v) in ice-cold lysis buffer (Beyotime, China) with 1% PMSF (phenylmethylsulfonyl fluoride). Samples were centrifuged at 12,000 rpm for 10 min at 4 °C and the protein concentration of the resulting supernatant was determined by the BCA (bicinchoninic acid) protein assay kit (Beyotime, China) and stored at −80 °C.

Thirty micrograms of protein were separated by sodium dodecyl sulfate polyacrylamide gel electropheresis (SDS-PAGE) and transferred to nitrocellulose membranes. After blocking in 5% fat-free milk for 1.5 h, the membranes were incubated with primary antibodies including anti-nNOS (1:1500; Chemicon), and anti-iNOS (1:5000; Chemicon), anti-active caspase-3 (1:100; Chemicon), at 4 °C overnight. Then the membranes were washed and incubated with horseradish peroxidase (HRP)-conjugated second antibody (1:3000; Santa Cruz Biotechnology, CA, USA) for 1 h. Immunoreactivity was developed by chemiluminescence kit (Pierce, Rockford, IL, USA) and exposed to film. The bands on the film were scanned and analyzed with an image analyzer (Alpha Innotech, San Leandro, CA, USA).

2.10. Statistical analysis

Values are expressed as mean ± standard deviation (S.D.). Differences in experimental groups were determined by one-way analysis of the variance followed by Newman–Keuls test, and groups of animals with stage 4 and 5 seizures were analyzed by Chi-square test. Significance was accepted at $P < 0.05$.

3. Results

3.1. Behavioral episodes

Behavioral episodes induced by pilocarpine injections showed typical increases in their intensity and duration, gradually progressing towards status epilepticus. The rats treated with

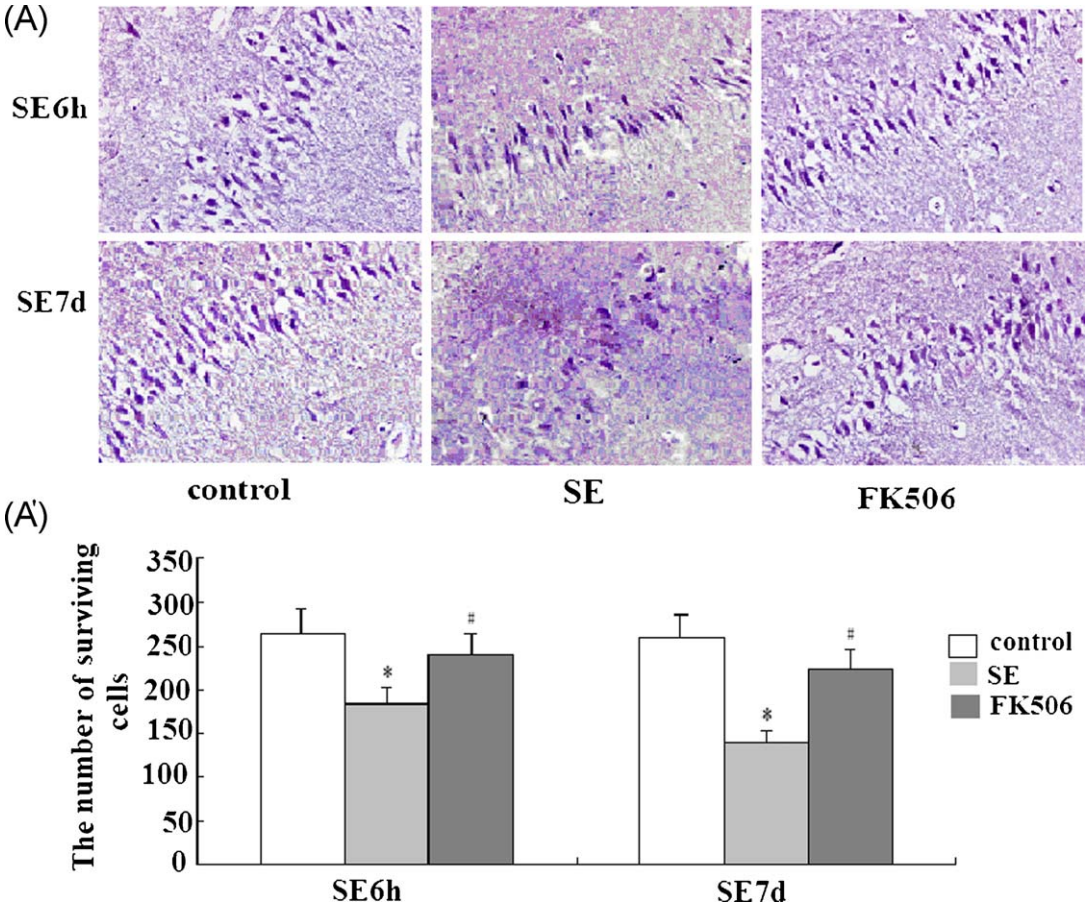

Fig. 1. High power (40×) photomicrographs showing H&E staining with hematoxylin and eosin of the hippocampal CA3 pyramidal neurons at 6 h and 7 days rats after LPCSE. (A) The changes of the hippocampal CA3 pyramidal neurons at 6 h and 7 days in rats after SE with or without FK506. Bar = 20 μm. (A′) The number of surviving cells after SE. Compared with control rats, the surviving cells of rats of SE group showed decrease significantly. FK506 could keep from cells damage. Bars indicate mean ± SD. *$P < 0.05$ vs. control. #$P < 0.05$ vs. pilo

FK506 showed highly significant differences in their latency period reaching to stage IV–V and the profile of proportions of rats suffering from seizures of different intensities. The latency period of the group with FK506 was significantly longer and the percentage of animals reaching stage V was considerably lower compared with pilo or saline + pilo group ($P < 0.05$) (Table 1). Control animals did not exhibit any behavioral seizure activity.

3.2. Neuron loss in hippocampus after seizure and effect of FK506 against neuron loss

H&E staining showed that dead neurons in hippocampal CA3 region with pyknotic nuclei are clearly distinguishable from surviving cells that show round and palely stained nuclei. Continuous seizures led to cell death from 6 h to 7 days. As shown in Fig. 1A and A′, the surviving neuron numbers after SE were significantly decreased compared with that of control ($P < 0.05$). Moreover, FK506, a CaN (calcineurin) inhibitor, significantly attenuated the neuron loss induced by seizures ($P < 0.05$) (Fig. 1A and A′).

3.3. Alteration of NO level and NOS activation after LPCSE

NO level started to increase at 30 min after SE and remained significantly elevated until 7 days with two peaks at 6 h and 7 days ($P < 0.01$, $P < 0.01$, respectively, Fig. 2A). cNOS activity started to increase at 30 min after SE, reached a maximum level at 6 h ($P < 0.05$, $P < 0.01$, respectively, Fig. 2B). In contrast, the iNOS activity did not increase until 12 h, and attained the peak at 7 days after SE ($P < 0.05$, $P < 0.01$, respectively, Fig. 2C). After pre-treatment with FK506, NO level at 6 h and 7 days, cNOS activation at 6 h and iNOS activation at 7 days induced by seizures decreased markedly (all $P < 0.05$, Fig. 2A′, A″, B′ and C′). The results suggested that a time-dependent increase in NO level after SE coincided with an increase in cNOS and iNOS activation, and that FK506 could inhibit cNOS and iNOS, activation and hence, total NO level.

3.4. Alteration in MDA level and SOD activation after LPCSE

The MDA level started to increase at 30 min after SE and reached a maximum level at 7 days ($P < 0.05$, $P < 0.01$, respectively, Fig. 3A). SOD activation started to increase at early stage ($P < 0.05$, Fig. 3B) with a peak at 2 h after SE ($P < 0.01$, Fig. 3B), and returned to basal level at 24 h after SE ($P > 0.05$, Fig. 3B). However, SOD activation started to increase again at 7 days after SE ($P < 0.05$, Fig. 3B). Pre-treatment with FK506 could remarkably decrease MDA level at 7 days after SE ($P < 0.05$, Fig. 3A′) and increase SOD activation at 6 h and 7 days after SE ($P < 0.05$, Fig. 3B′ and B″). The early increase in SOD activation following seizures maybe an early compensatory reaction in response to increased MDA levels, which is in agreement with earlier reports.[24]

3.5. The immunohistochemical analysis of nNOS, iNOS and caspase-3 expression after LPCSE

In order to determine if hippocampal neuron death observed at early stage following SE is attributable to oxidative stress and/or

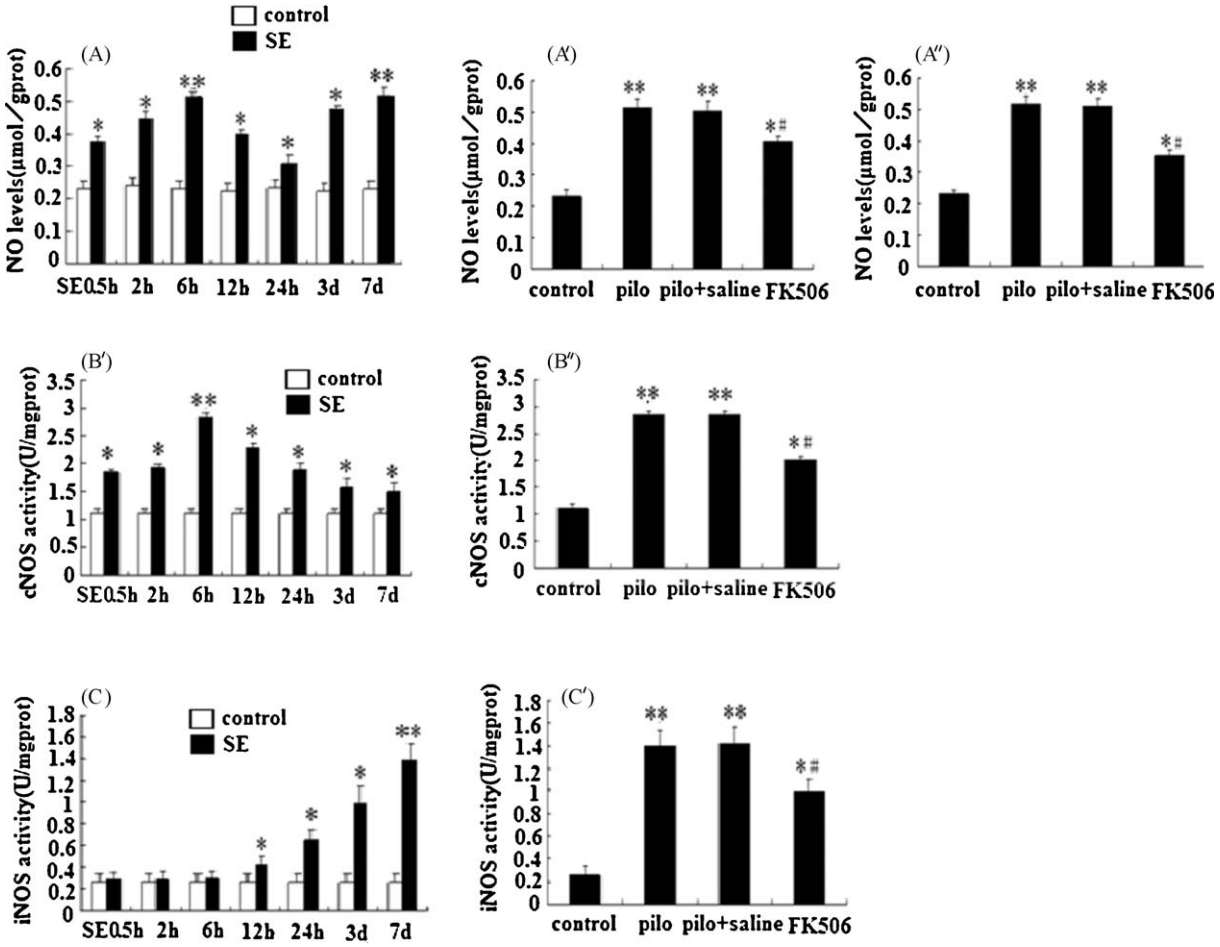

Fig. 2. Changes of NO level, cNOS and iNOS activity after LPCSE. (A) Changes of NO level after SE; (A′ and A″) the effects of FK506 on NO level at 6 h and 7 days after SE. (B) Changes of cNOS activity after SE; (B′) the effects of FK506 on cNOS activity at 6 h after SE. (C) Changes of iNOS activity after SE; (C′) the effects of FK506 on iNOS activity at 7 days after SE. Bars indicate mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control. $^{\#}P < 0.05$ vs. pilo and or saline + pilo.

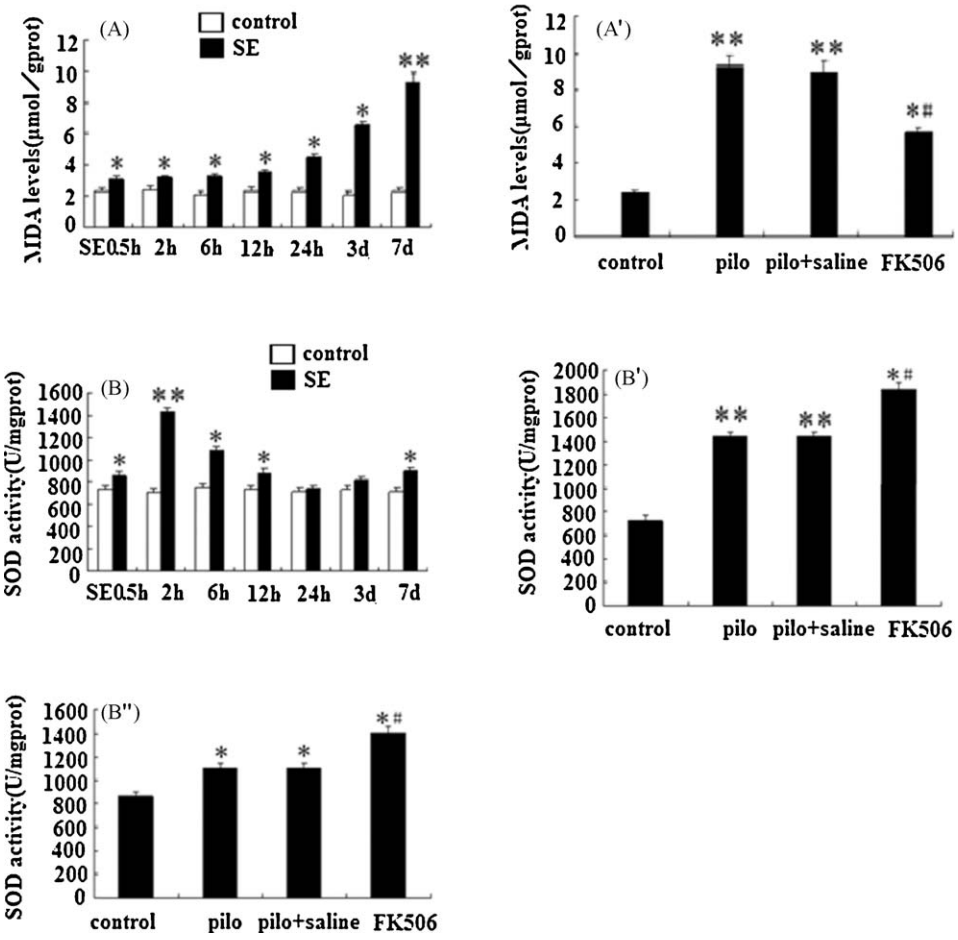

Fig. 3. Changes of MDA level and SOD activities after SE induced by LPCSE. (A) Changes of MDA level after SE; (A′) the effects of FK506 on MDA level at 7 days after SE. (B) Changes of SOD activity after SE; (B′ and B″) the effects of FK506 on SOD activity at 2 h and 7 days after SE. Bars indicate mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control. $^{\#}P < 0.05$ vs. pilo or saline + pilo.

caspase-3 cascade, we measured nNOS, iNOS and caspase-3 positive cells by immunohistochemistry. The results showed that nNOS positive cells appeared at 6 h (Fig. 4A′) and iNOS and caspase-3 positive cells appeared significantly at 7 days after SE (Fig. 4B′ and C′), respectively coincident with hippocampal neuron death at 6 h and 7 days after SE (Fig. 1A and A′). FK506 was found to inhibit the appearance of cells positive for nNOS, iNOS and caspase-3 (Fig. 4A″, B″ and C″). These results suggested that oxidative stress mediated hippocampal neuron death was prior to caspase-3 activation, and that FK506 could protect hippocampal neurons from damage by suppressing oxidative stress at early stage after SE and by inhibiting oxidative stress and caspase-3 cascade at later stage after SE.

3.6. The protein expression of nNOS, iNOS and caspase-3

To further determine if increased activation of nNOS, iNOS and caspase-3 is related to an increase in their protein expression after SE, Western blot analysis was performed at 2, 6, 72 h and 7 days. As shown in Fig. 5A and A′, protein expression of nNOS increased at 2 h and reached a peak at 6 h after SE ($P < 0.05$, $P < 0.01$, respectively), but protein expression of iNOS started to increase at 6 h and reached maximum at 7 days after SE ($P < 0.05$, $P < 0.01$, respectively). Meanwhile, protein expression of caspase-3 gradually increased from 72 h to 7 days after SE ($P < 0.05$, $P < 0.01$, respectively). FK506 substantially inhibited protein expression of nNOS at 6 h after SE ($P < 0.05$, Fig. 5B and B′), and protein expression of iNOS ($P < 0.05$, Fig. 5C and C′) and caspase-3 at 7 days after SE ($P < 0.05$, Fig. 5D and D′). The above results suggested that increased activation of nNOS, iNOS and caspase-3 was related to an increase of protein expression of nNOS, iNOS and caspase-3 after SE. FK506 plays an important neuroprotection role by inhibiting activation and protein expression of nNOS, iNOS and caspase-3.

4. Discussion

In our current model, NO overactivation and neuron death in hippocampus was clearly observed after LPCSE. Administration of FK506, an imunosupressive agent, could significantly rescue neurons from death induced by SE. We found that NO was activated at 30 min to 7 days with peak at 6 h and 7 days after SE, which coincided with activation of cNOS and iNOS. Meanwhile, MDA and SOD emerged in this period of LPCSE, but activated caspase-3 protein was predominantly found at 3–7 days. These results suggested that oxidative stress could be partially responsible for SE-induced neuron degeneration in the early stage. It is known that after SE, the function of Mg^{2+}/Ca^{2+}ATPase that regulates intracellular Ca^{2+} concentrations is compromised,[25,4] and that ATPase mediated uptake of Ca^{2+} into the endoplasmic reticulum is also less efficient, which could potentially result in higher than normal resting intracellular Ca^{2+} concentrations.[4] The increased intracellular Ca^{2+} could potentially contribute towards oxidative stress associated with SE.[26,27] Oxidative stress is one of the important mechanisms that play a role in the etiology of seizure-induced neuronal death after first hour of acute phase of seizures.[7] The most important effect of free radicals is lipid

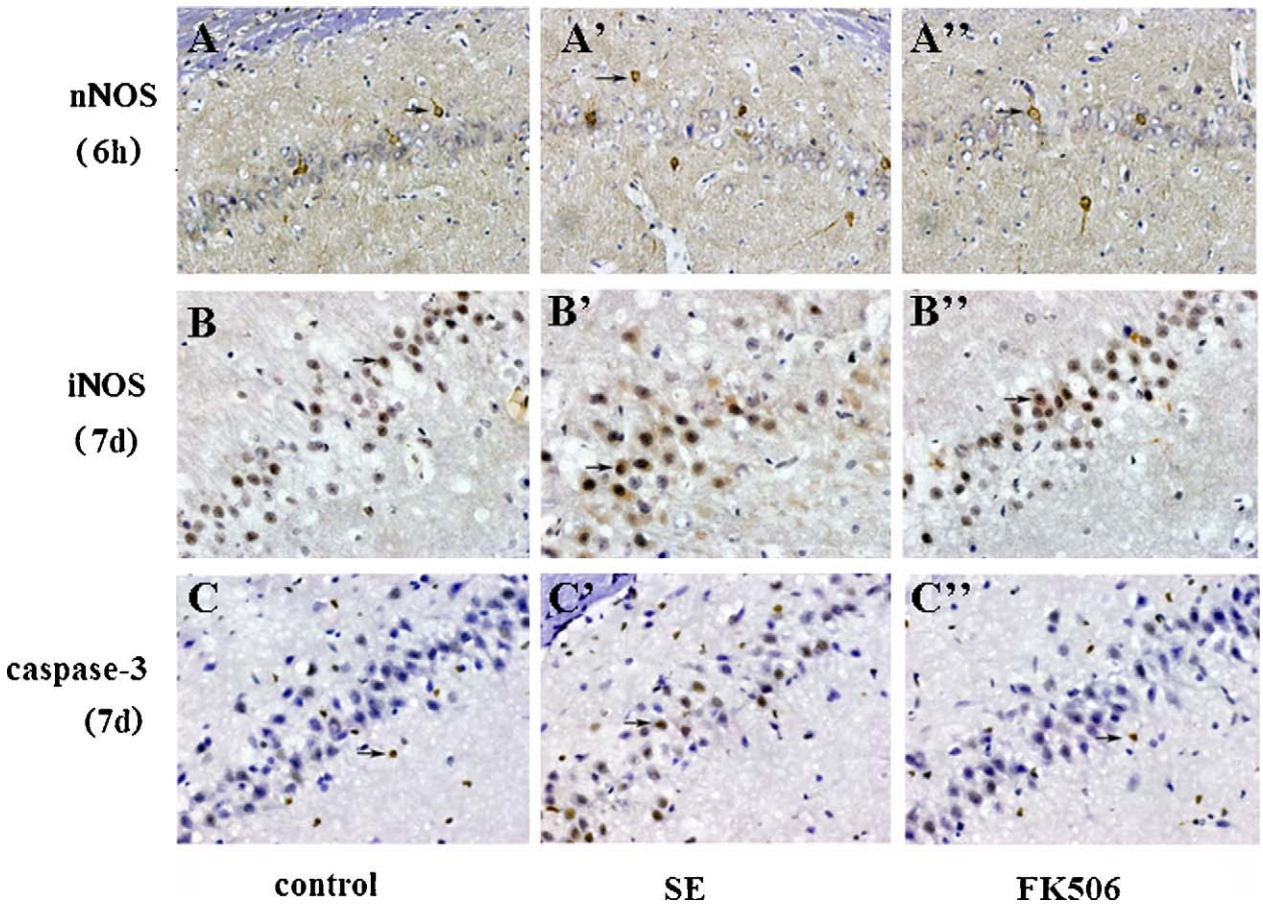

Fig. 4. The expression positive cells of nNOS, iNOS and caspase-3. (A, A′ and A″, 400×) Immunohistochemistry showed the effects of FK506 on positive cells of nNOS at 6 h after SE. (B, B′ and B″, 600×) Immunohistochemistry showed the effects of FK506 on positive cells of iNOS at 7 days after SE. (C, C′ and C″, 600×) Immunohistochemistry showed the effects of FK506 on positive cells of caspase-3 at 7 days after SE. Bars indicate averages ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control. $^{\#}P < 0.05$ vs. pilo or saline + pilo.

peroxidation. There is a growing body of evidence suggesting that elevated lipid peroxidation levels and/or its metabolites are potentially neurotoxic.[28,29] Seizures alter membrane lipid composition that can affect membrane fluidity permeability and consequently the function of membrane-bound enzymes, which in turn, may have serious consequences on neuronal functioning.[30] A previous study has also reported little evidence of caspase-3 activation, even with DNA laddering occurring at 24 h after SE in the brain regions.[31] Consistent with this, our data suggest that oxidative stress mediated hippocampal neuron death occurs prior to caspase-3 activation after LPCSE. The finding potentially explains the mechanism of early cell death after SE and suggests antioxidant-based neuroprotective strategies for SE.

NO alone is a poorly reactive species, however, it is able to react rapidly with superoxide anion and produces highly oxidizing and nitrating species, NO_2^-, NO_3^- and peroxynitrite that cause oxidative damage via lipid peroxidation.[32] Peroxynitrite, which is a highly reactive free radical, has been shown to mediate much of the toxicity of NO.[33] These secondary reactive nitrogen species are also capable of modifying a diversity of biomolecular structures in the cell,[8] including antioxidant enzymes leading to oxidative damage.

In our study, we found the activity of cNOS started to increase at 30 min and reached peak at 6 h after SE, while the activity of iNOS increased at 12 h and reached peak at 7 days after SE, accounting for much of the increase in NO activity. However, the acute increase in NO activity is mainly mediated by cNOS, especially nNOS.[23] These results provide support for the participation of nNOS contributing to oxidative damage immediately after SE, which may be one of the potential mechanisms of seizures-induced neurotoxicity. It is interesting to note that although labile nNOS activity is mainly regulated by Ca^{2+}, our results show another mechanism of its regulation following SE, i.e., its increased expression.

In the nervous system, nNOS is largely responsible for NO production in neurons, while iNOS has been implicated in an inflammation response of glial cells and produces large amounts of NO continuously for long periods. An induction of iNOS may therefore play an important role during chronic stress and apoptotic cell death in the hippocampus in SE rats.[8]

We found that the level of MDA, a measure of lipid peroxidation, increased significantly after SE from 30 min with peak value on day 7. The results of increased MDA level following SE are consistent with other reports.[33] The increase of MDA level observed at 30 min corresponds to an increase in NO level. These results suggest that NO toxicity may be mediated by a direct effect of peroxynitrite during the development of SE induced by pilocarpine.

SOD, which is considered to be an important antioxidant enzyme, is capable of removing superoxide anion from the cell. The increased SOD activity following SE at the early stage is in agreement with earlier reports,[24] and may represent an early compensatory reaction in response to increased MDA levels found to be increased at the same time. The decrease in SOD activity at 24 h to 3 days after SE that we discovered may be due to the inactivating action of the secondary reactive nitrating species on this antioxidant enzyme.

Being an immunosuppressive agent, FK506, has been used in clinical settings for the prevention of allograft rejection.[18,19] It also has strong neuroprotective effect following brain damages of different origin[20,21] and antiepileptic influences.[34]

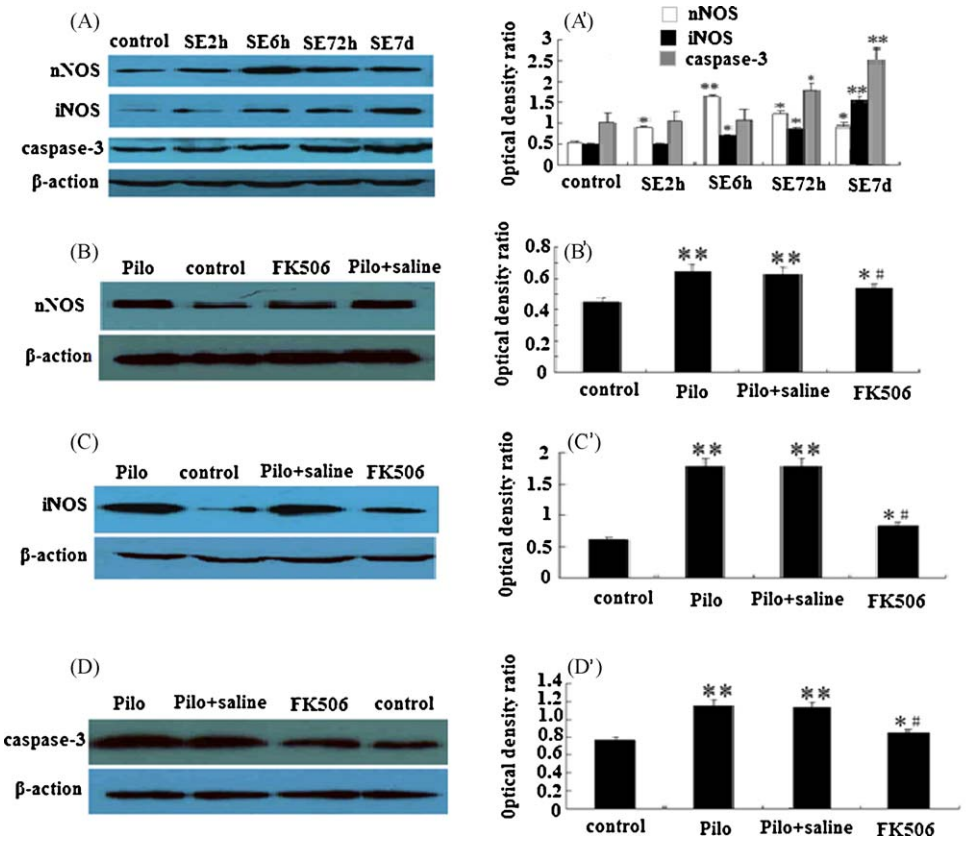

Fig. 5. Protein expression of nNOS, iNOS and caspase-3 after SE. (A and A′) The changes of protein expression of nNOS, iNOS and caspase-3 after SE in different times. (B and B′) Protein expression of nNOS and the effect of FK506 on nNOS at 6 h after SE. (C and C′) Protein expression of iNOS and the effect of FK506 on iNOS at 7 days after SE. (D and D′) Protein expression of caspase-3 and the effect of FK506 on caspase-3 at 7 days after SE. Bars indicate averages ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control. $^{\#}P < 0.05$ vs. pilo or saline + pilo.

KF506 binds to FK-binding proteins (FKBPs), to inhibit the activity of calcineurin, which is an enzymatic protein with phosphatase activity.[35] It is reported that FK506 can inhibit NMDA-induced death of neurons by increasing nNOS phosphorylation, which in turn results in the inhibition of the enzyme activity and hence decreased levels of reactive nitric oxide species.[36]

In our study, we found that FK506 not only inhibits NO and MDA level and the expression of nNOS, iNOS and caspase-3 but also increases SOD activity, suggesting that FK506 protects hippocampal neurons from damage via suppressing oxidative stress at early stage after SE and by inhibiting oxidative stress and caspase-3 cascade at later stages after SE.

In conclusion, our results suggest that NO-mediated oxidative stress may have dual neurotoxic effects following SE: An early damage likely resulting from mitochondrial dysfunction and a prolonged apoptotic neuronal cell death involving in the activation of caspase cascade. The findings have implications for potential clinical use of FK506 against SE.

Acknowledgment

This study was supported by a grant from the Planned Science and Technology Project of Shandong Province, China (No. Y2007C168).

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