Neuroscience 325 (2016) 111–123

THE INHIBITION OF TRANSFORMING GROWTH FACTOR BETA-ACTIVATED KINASE 1 CONTRIBUTED TO NEUROPROTECTION VIA INFLAMMATORY REACTION IN PILOCARPINE-INDUCED RATS WITH EPILEPSY

Q. TIAN,[a] Q. XIAO,[a] W. YU,[b] M. GU,[a] N. ZHAO[b] AND Y. LÜ[a]*

[a] *Department of Geriatrics, The First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, China*

[b] *Institute of Neuroscience, Chongqing Medical University, Chongqing 400016, China*

Abstract—Recently, more and more studies support that inflammation is involved in the pathogenesis of epilepsy. Although TGFβ signaling is involved in epileptogenesis, whether TGFβ-associated neuroinflammation is sufficient to regulate epilepsy remains unknown to date. Furthermore, tumor necrosis factor-α receptor-associated factor-6 (TRAF6), transforming growth factor beta-activated kinase 1 (TAK1), which are the key elements of TGFβ-associated inflammation, is still unclear in epilepsy. Therefore, the present study aimed to explore the role of TRAF6 and TAK1 in pilocarpine-induced epileptic rat model. Firstly, the gene levels and protein expression of TRAF6 and TAK1 were detected in different time points after pilocarpine-induced status epilepticus (SE). 5z-7-oxozeaenol treatment (TAK1 antagonist) was then performed; the changes in TRAF6, TAK1, phosphorylated-TAK1 (P-TAK1), interleukin-1β (IL-1β) levels, neuronal survival and apoptosis, and seizure activity were detected. Our results showed that expressions of TRAF6 were increased after SE, reached the peak in 7 day, maintained at the high level to 30 days, and the TAK1, P-TAK1 levels were increased after SE following time. After 5z-7-oxozeaenol treatment in epileptic rats, TRAF6–TAK1–P-TAK1 signaling protein expressions were reduced, inflammatory cytokine IL-1β expression was decreased, neuron survival index was improved, the neuron apoptosis index was decreased and seizure durations were alleviated. In conclusion, the expression of TRAF6 and TAK1 are related to the progression of epilepsy. TAK1 might be a potential intervention target for the treatment of epilepsy via neuroprotection.

Key words: epilepsy, seizure, TRAF6, TAK1, neuroprotection.

INTRODUCTION

Epilepsy is a chronic disease characterized by the hyperexcitatory of the brain neurons. Approximately 1% of people suffer from epilepsy around the world, and about 30% of people with epilepsy become drug-refractory epilepsy (Sisodiya, 2005). Previous studies of epileptogenesis have mainly focused on neurotransmitters and ion channels (De Sarro et al., 1992; Russo et al., 2012; Miceli et al., 2015). However, recently studies have shown that inflammation is closely related to the disruption of the blood–brain barrier (BBB), neurotoxicity, functional change of neurotransmitters and abnormality of ion channels (Samland et al., 2003; Viviani et al., 2003; Kim et al., 2013; Ye et al., 2013). Moreover, the numbers of inflammatory cytokines increased in epilepsy, including interleukin-1β (IL-1β), cicloossigenasi-2 (COX-2), tumor necrosis factor-α (TNF-α), transforming growth factor beta (TGF-β) (Ravizza et al., 2008; Sharma et al., 2009; Mercado-Gómez et al., 2014; Arisi et al., 2015); and the inhibitions of proinflammatory cytokines, such as IL-1β, TNF-α, significantly reduce the frequency and severity of the seizures (Maroso et al., 2011). Therefore, the inflammatory mechanism of epilepsy is worth further studying.

Growing evidence suggests that TGF-β signal facilitates epileptogenesis (Ivens et al., 2007; Cacheaux et al., 2009). Our previous studies have shown that several TGF-β pathway-associated proteins are abnormally changed in the epileptic patients and pilocarpine-induced rats, including the TGF-β receptor type I, sorting nexin 25, serine-threonine kinase receptor-associated protein and smad-7 (Lu et al., 2009; Du et al., 2013; Liu et al., 2014), indicating TGF-β signaling involves in the pathogenesis of epilepsy. However, these studies focus on the classical pathway of TGF-β signaling. Therefore, we turn our attention to the other side of TGF signaling pathway, in the hope to find better targets of epilepsy.

*Corresponding author. Tel: +86-23-89011622; fax: +86-23-68811487.
E-mail address: lyu_yang@126.com (Y. Lü).

Abbreviations: ANOVA, analysis of variance; AP-1, activator protein-1; BBB, blood–brain barrier; COX-2, cicloossigenasi-2; DMSO, dimethyl sulfoxide; ELISA, enzyme-linked immunosorbent assay; IL-1β, interleukin-1β; IL-6, interleukin-6; MAPK, mitogen-activated protein kinase; NF-kB, nuclear factor-kappa B; NMDA, N-methyl-D-aspartate; P-TAK1, phosphorylated TAK1; RT-PCR, real-time polymerase chain reaction; SE, status epilepticus; SRS, spontaneous recurrent seizures; TAK1, transforming growth factor beta-activated kinase 1; TBST, Tris-Buffered Saline and Tween; TGF-β, transforming growth factor beta; TLR, toll-like receptor; TNF-α, tumor necrosis factor-α; TRAF6, tumor necrosis factor-α receptor-associated factor-6; TUNEL, terminal deoxynucleotidyl transferase mediated dUTP nick end-labeling.

http://dx.doi.org/10.1016/j.neuroscience.2016.03.045
0306-4522/

Transforming growth factor beta-activated kinase 1 (TAK1) is a serine/threonine kinase and is a member of the mitogen-activated protein kinase (MAPK) kinase kinase family. TAK1 is activated by several of stimuli, including bone morphogenetic protein, oxidative stress, TNF-α, TGF-β, IL-1β, toll-like receptor (TLR) (Yamaguchi et al., 1995; Cheng et al., 2010; Gu et al., 2014; Morioka et al., 2014; Yu et al., 2014a; Onodera et al., 2015). The activated TAK1 participates in cell growth, differentiation, apoptosis, tumor metastasis and inflammation signaling (Sakurai et al., 1998; Kang et al., 2013). The phosphorylation of Thr-187 and Ser-192 is crucial for TAK1 kinase activity, and such phosphorylation is caused by an E3-ligase TNF receptor-associated factor-6 (TRAF6). Furthermore, TAK1 involves in pro-inflammation and anti-apoptosis functions (Gingery et al., 2008; Sorrentino et al., 2008) and the inhibition of TAK1 confers neuroprotection after brain injury in rats (Zhang et al., 2013, 2015). However, the functions of TAK1 in epilepsy have not been reported to our knowledge.

As the key component of inflammation signaling, the role of TAK1 and TRAF6 in epileptogenesis remains unknown. In the present study, we investigated the relationship among TAK1, TRAF6 and epilepsy and the potential role of the TAK1 inhibition in epilepsy by 5Z-7-oxozeaenol, the TAK1 antagonist, to identify whether the TAK1 pathway is a potential treatment target for epilepsy.

EXPERIMENTAL PROCEDURES

Animals

Adult male Sprague–Dawley rats were housed in specific pathogen-free environment with a controlled light (12-h reversed light–dark cycle), constant temperature (25 °C) and rats could freely access water and food. The given and consumed food and water for every rat were almost the same during the experiments. All procedures were approved by the Ethics Committee of Chongqing Medical University on animal experiments and performed according to international standards.

Rats weighing 200–250 g were given the intraperitoneal injection of lithium chloride (127 mg/kg, Sigma, USA) 20 h before the pilocarpine injection. In order to reduce the side effects of peripheral cholinergic of pilocarpine, atropine (1 mg/kg) was intraperitoneally injected 30 min before pilocarpine injection. Then the rats were treated with the intraperitoneal injection of pilocarpine (50 mg/kg, Sigma, USA) and the behavioral changes were observed. When the seizure level reached Racine scale (Racine, 1972) stage four or five for 1 h, rats were injected with diazepam (10 mg/kg) to terminate status epilepticus (SE).

Experiment design

In the first phase of the experiment, adult male Sprague–Dawley rats were randomly assigned to control group and epilepsy group. The control group rats underwent the same procedure of the epilepsy model, except that all drugs were replaced by 0.9% saline. The epilepsy group rats were randomly divided into three groups (1 day, 7 days and 30 days, $n = 7$ each group) after SE. Those three groups represented the three phases of the epilepsy: acute phase, latent phase and chronic phase. The expression levels of TRAF6, TAK1, and phosphorylated TAK1 (P-TAK1, the activated form of TAK1) were detected in the cortex and hippocampus in pilocarpine-induced the epileptic rats.

In the second phase of the experiment, 5Z-7-oxozeaenol (Sigma, USA) was used to inhibit TAK1 in lithium-pilocarpine-induced rats. Dimethyl sulfoxide (DMSO) was used as solvent of 5Z-7-oxozeaenol. A solution of 5z-7-oxozeaenol mixed DMSO in 4 μg/μl concentration was prepared before using. 5 μl of solutions with 5z-7-oxozeaenol (20 μg) was injected into the rat right intracerebroventricle at 3 h after termination of SE (1.0 mm posterior to the bregma, 2 mm lateral to midline, and 4.5 mm below the skull surface). Five microliter of DMSO injections was considered as vehicle. Then, the epileptic rats were observed 30 days; and the rats were randomly assigned to 6 groups ($n = 7$ each group): DMSO-treated or 5z-7-oxozeaenol-treated group in 1 day, 7 days and 30 days, respectively. The role of 5z-7-oxozeaenol treatment was investigated in the brains of epileptic rats.

In order to investigate the behavioral changes among the epileptic group, vehicle-treated group and 5z-7-oxozeaenol treated group, the spontaneous recurrent seizures (SRS) of all surviving rats were continuously video monitored after termination of SE for a period of 15–30 days. Seizure score, seizure frequency and seizure duration were observed by an observer blinded to the experimental conditions. The severity of the epilepticus was assessed according to the Racine score: (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; (5) generalized clonic convulsions associated with loss of balance (Racine, 1972).

All the rats were sacrificed at time points. Some of rats were perfused transcardially with precooled 0.9% saline and the brain were moved out quickly and the cortex and hippocampus was dissected out on ice, then stored in −80 °C for western blots, enzyme-linked immunosorbent assay (ELISA) and real-time polymerase chain reaction (RT-PCR). Some of the rats were perfused transcardially and fixed with 4% paraformaldehyde. The brains were moved out and immersed in 4% paraformaldehyde for 24 h, then processed, and embedded in paraffin. The coronal plane sections (4 μm) were prepared for the terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) and Nissl staining.

RT-PCR

The total RNAs of the cortex and hippocampus in rats' brain were obtained using RNAiso Plus (Takara, Japan), cDNA was synthesized using PrimeScript®RT kit (Taraka, Japan) in accordance with the manufacturer's