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P38 MAPK pathway mediates cognitive damage in pentylenetetrazole-induced epilepsy via apoptosis cascade

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ABSTRACT

Objective: Our group has previously reported the role of P38 mitogen-activated protein kinase (MAPK) pathway in the memory impairment of pentylenetetrazole (PTZ)-kindled rats. However, any contribution of p38 MAPK pathways to the cognitive dysfunction of PTZ-kindled rats remains unclear. The objective of this study is to verify the relationship between p38 MAPK pathway and cognitive function of epileptic rats, and discuss probable mechanisms.

Methods: Thirty male SD rats were divided into three groups, namely, PTZ, inhibitor, and sham groups. All rats except those from the sham group were treated with PTZ to establish temporal lobe epilepsy (TLE) models, whereas the P38 MAPK inhibitor SB 203580 was given to the inhibitor group. Morris water maze test was performed to assay their learning and memory abilities. The levels of phosphorylated p38 (p-p38) and caspase 3 were confirmed using Western blot.

Results: In the probe test of water maze, the PTZ group had the longest escape latency and least time to pass through the platform. Compared with the PTZ group, the inhibitor group had better performance in escape latency and spatial probe tests. Performance in the water maze test corresponded with the level of p-p38 and caspase 3 in hippocampus. We also found that the down-regulation of p-p38 in the inhibitor group led to down-regulated levels of caspase 3.

Conclusions: P38 MAPK pathway contributed to cognitive damage in PTZ-induced epilepsy via apoptosis cascade.

1. Introduction

Memory impairment is one of the most common comorbidities of epilepsy (Devinsky et al., 2016), especially in patients with temporal lobe epilepsy (TLE) (Leritz et al., 2006). It is believed that memory and learning are facilitated by the hippocampal cortex (Bohbot and Corkin, 2007). Using a TLE-related experimental animal model, previous studies reported that neuronal cell loss (Cendes et al., 2014) and abnormal expression of bioactive compounds might play crucial roles in cognitive deficits. Moreover, by miRNA microarray analysis, our previous study found four upregulated and seven down-regulated miRNAs related to memory impairment in TLE rats. Target gene prediction and gene enrichment analysis revealed that mitogen-activated protein kinase (MAPK) signaling pathway might be a key factor for memory impairment in TLE rats (Liu et al., 2015).

Apoptosis is a process of programmed cell death in multicellular organisms. It occurs widely in healthy tissues, and dysregulation of this process can result in devastating health outcomes. It has been shown that defective apoptotic processes are common in TLE (Liu et al., 2015; Waldbaum and Patel, 2010). Caspases serves as one of the mediators of apoptosis (Shalini et al., 2015) and can be regulated by multiple factors. P38 is a key factor in the MAPK pathway. A previous study showed that inhibition of the p38 MAPK pathway impeded GTP-mediated caspase activation (Moosavi et al., 2007), indicating that p38 may act upstream of caspases, mediating apoptosis in hippocampus, and contributing to cognitive deficits in TLE.

In this study, we sought to verify the relationship between the p38 MAPK pathway and cognitive function in TLE rats, and discuss the importance of apoptosis in this process.

2. Materials and methods

2.1. Grouping and kindling induction

Thirty adult male SD rats (6–7 weeks old; 200–250 g of body weight) were divided into a PTZ group, an inhibitor group, and a sham

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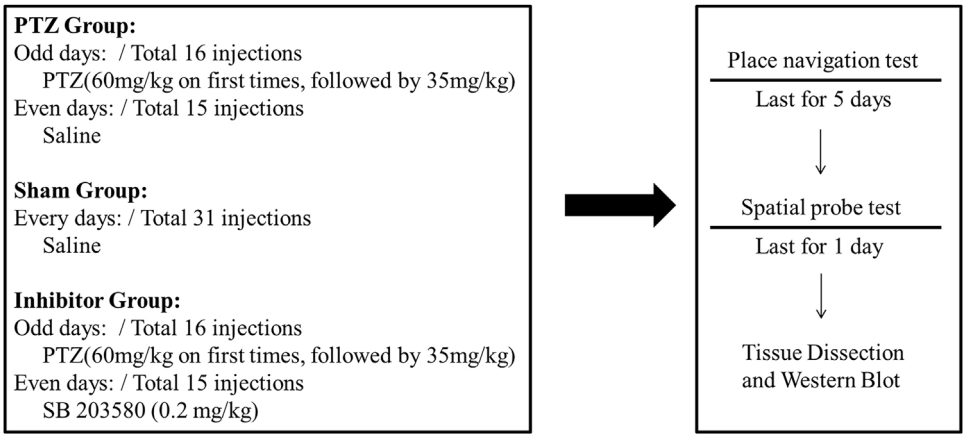

Fig. 1. Flowchart for this study.

group. Animals were housed in groups of five under the following conditions: 22–26 °C room temperature, 50–60% humidity, and a 12:12 light-dark cycle, with lights on at 8:00 AM The rats were given free access to food and water.

Temporal lobe epilepsy preconditioning was performed using intraperitoneal injection of PTZ (Sigma–Aldrich, USA) at a dose of 60 mg/kg on the first day, followed by repeated injections of PTZ of 35 mg/kg every other day, starting on the third day. Rats were considered epileptic if their behavioral changes reached level IV–V according to the Racine standard (Racine et al., 1972). Rats in the sham group were injected with the same dose of 0.9% physiological saline instead of PTZ. Rats in the inhibitor group were given SB 203580 (Selleck, USA) intraperitoneally at a dose of 0.2 mg/kg on the days between PTZ injections. Morris water maze test was conducted starting on the 31st day after kindling induction (Fig. 1).

2.2. Morris water maze test

Dysfunction of learning and memory in rats were analyzed using the Morris water maze (MWM). The MWM apparatus (Panlab, Spain) consisted of a circular pool, a round colorless platform, and a camera installed above the center of the pool. The data were acquired using SLY-WMS MWM system v.2.1 software.

The place navigation test lasted 5 days. Rats were placed in water in a clockwise sequence by the first, second, third, and fourth quadrants, recording the time that the rats took to find the platform within 60 s. The escaping latency was recorded as 60 s if the rat failed to find the platform within stipulated time.

Spatial probe test was conducted on the sixth day. Each rat was released one by one on four quadrants. Each attempt lasted 100 s, and the total number of times the rats passed through the platform was calculated.

2.3. Tissue dissection and western blot analysis

Hippocampal tissue was harvested and stored as previously described (Liu et al., 2015). Expressions of p-p38 MAPK and caspase-3 were investigated by Western blot. Hippocampal tissues were homogenized in an ice-cold mixture of RIPA buffer (Beyotime, China) and protease inhibitors, and then incubated over ice for 30 min. After centrifugation, the supernatant of the homogenates was collected, and total protein concentrate was detected by the BCA Protein Assay Kit (Beyotime, China). Samples containing an equal quantity of denatured protein (50 μg) were loaded, and were electrophoresed in 10% SDS-PAGE (Beyotime, China) followed by electrophoretic transference onto PVDF membranes (Millipore Corp, Massachusetts). Membranes were blocked with 5% skimmed milk and then probed overnight at 4 °C with rabbit anti-phosphorylated p38 (p-p38) and caspase-3 primary antibodies (Cell Signaling, USA). After incubating with fluorescent goat anti-rabbit IgG (Abcam, USA) at 25 °C for 90 min, the PVDF membranes were scanned using a Licor Odyssey Infrared Imaging System.

2.4. Statistical analysis

All results are presented as mean ± standard deviation. Data in this study, including escape latencies in the first 5 days, the times the rats passed through the platform on day 6 in the MWM test, and relative densities of immunoreactive bands in Western blots were analyzed by one-way ANOVA using the SPSS 17.0 package. Values were considered statistically significant when $p < 0.05$.

3. Results

3.1. PTZ kindling

Rats were observed for 30 min immediately after each injection. After PTZ administration, all rats in the PTZ group and inhibitor group reached levels IV–V of Racine standard. The duration that rats remained in stage IV/V seizure varied across individuals, ranging from 4 min to 10 min.

3.2. Morris water maze test

The escape latency of all three groups decreased progressively over the five training days. Statistically significant differences were observed on the third, fourth and fifth days. In comparison to the sham group, the latency of the PTZ group was higher. Latency of the inhibitor group was shorter than that of the PTZ group. There were no significant differences between the inhibitor and sham groups (Fig. 2**A**).

In the spatial probe test on the sixth day, the PTZ group passed less frequently through the platform compared to the sham group. The number of times the inhibitor group passed through the platform was higher than that for the PTZ group. There was no significant difference between the PTZ and inhibitor groups (Fig. 2**B**).

3.3. Western blot analysis

Western blot was performed to determine the expression of p-p38 and caspase 3 in the hippocampus and to assess differences among the three groups. Expression levels of p-p38 and caspase 3 were upregulated in the PTZ group. In comparison, the level of p-p38 was lower in the inhibitor group compared to the PTZ group. No significant difference was detected in the level of caspase 3 across groups (Fig. 3).