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Chronic oleoylethanolamide treatment improves spatial cognitive deficits through enhancing hippocampal neurogenesis after transient focal cerebral ischemia

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ABSTRACT

Oleoylethanolamide (OEA) has been shown to have neuroprotective effects after acute cerebral ischemic injury. The aim of this study was to investigate the effects of chronic OEA treatment on ischemia-induced spatial cognitive impairments, electrophysiology behavior and hippocampal neurogenesis. Daily treatments of 30 mg/kg OEA significantly ameliorated spatial cognitive deficits and attenuated the inhibition of long-term potentiation (LTP) in the middle cerebral artery occlusion (MCAO) rat model. Moreover, OEA administration improved cognitive function in a manner associated with enhanced neurogenesis in the hippocampus. Further study demonstrated that treatment with OEA markedly increased the expressions of brain-derived neurotrophic factor (BDNF) and peroxisome proliferator-activated receptors α (PPARα). Our data suggest that chronic OEA treatment can exert functional recovery of cognitive impairments and neuroprotective effects against cerebral ischemic insult in rats via triggering of neurogenesis in the hippocampus, which supports the therapeutic use of OEA for cerebral ischemia.

1. Introduction

The prevalence of cognitive impairments after stroke is high [1]. Spatial memory is closely dependent on hippocampal formation [2]. Neurogenesis in the hippocampus has been correlated with learning and memory [3]. Focal cerebral ischemia provokes newly born neurons from the subgranular zone (SGZ) to migrate into the granule cell layer of the dentate gyrus (DG) [4]. They subsequently incorporate into the synaptic circuitry, which contributes to the formation of hippocampal-dependent memories [5]. These studies raise the possibility that a compensatory increase in hippocampal neurogenesis may mitigate spatial cognitive deficits after stroke. However, the majority of ischemia-induced newly born cells undergo death within several weeks, and the survival rate of newly generated cells is low. The therapeutic potential of neurogenesis induced by cerebral ischemia itself is insufficient [6]. Therefore, finding new compounds directed at increasing endogenous hippocampal neurogenesis may be a new strategy for restoring cognitive function impaired after stroke.

Oleoylethanolamide (OEA) is a potent endogenous ligand for Peroxisome proliferator-activated receptors α (PPARα), nuclear receptors involved in the transcriptional regulation of lipid metabolism, inflammation, and neuroplasticity [7,8]. The synthesis of endogenous PPARα ligand, OEA, is induced on demand after local accumulation of stimuli in the brain following an injury, such as cellular stress and cerebral ischemia [9,10]. The

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neuroprotective effects of OEA have been described in animal models of neurological disorders [11–13]. A recent study also indicates that OEA protects the nigrostriatal circuit from 6-OHDA-induced neurotoxicity through a PPAR α-dependent mechanism [14]. Moreover, our previous study showed that OEA had the ability to improve neurological dysfunction, reduce infarct volume, and alleviate brain edema 24 h after focal cerebral ischemia [15]. Thus, although the positive effects of OEA on acute cerebral ischemia has been verified, little is known about the long-term positive effects of chronic OEA administration on ischemic stroke, especially the progress of spatial cognitive function recovery, electrophysiological behavior, and endogenous hippocampal neurogenesis. Therefore, we set out to examine the functions of OEA as well as to investigate whether OEA has the ability to protect a rat brain during the chronic phase of ischemic stroke.

To test this hypothesis, a middle cerebral artery occlusion (MCAO) rat model was established. The best-studied cellular model for hippocampal learning and memory is the detection of long-term potentiation (LTP), which is a long-lasting increase in the synaptic transmission efficiency induced by the high frequency stimulation [16]. The Morris water maze (MWM) test is a memory test based on the capacity of animals to rescue themselves by reaching a hidden goal platform in a pool of water. Animals with a damaged hippocampus display spatial navigation impairments and perform poorly in the MWM test [17]. In the present study, we investigated the effects of OEA on improving cognitive deficits in a focal cerebral ischemia model induced by MCAO. In brief, MWM, LTP and endogenous hippocampal neurogenesis and neuroplasticity were used to evaluate the effects of OEA on rat brain function at the later stages of ischemic stroke, which may provide an interesting view of the potential application of OEA for ischemic stroke.

2. Materials and methods

2.1. Experimental animals

All experimental procedures were performed in accordance with the Provisions and General Recommendation of Chinese Experimental Animal Administration Legislation, and all efforts were made to minimize pain and suffering in the animals. Male Sprague-Dawley (SD) rats weighing 260–280 g were purchased from Beijing Vitalriver Experimental Animal Co. (Beijing, China) and housed under a 12/12 h dark/light cycle and specific pathogen-free (SPF) conditions. The animals were fasted without food deprivation for 12 h before the MCAO procedure was performed.

2.2. Preparation of the focal cerebral ischemia model

Focal cerebral ischemia was induced by middle cerebral artery occlusion (MCAO) using the intraluminal filament technique, as previously described [18]. In brief, after anesthesia was induced with chloral hydrate (400 mg/kg, *i.p.*), 4-0 nylon monofilament suture, with rounded tips, was introduced into the right internal carotid artery (ICA) through the external carotid stump, advanced approximately 20 mm past the ECA/ICA bifurcation to occlude the origin of the middle cerebral artery (MCA) at the junction of the circle of Willis. The suture was withdrawn 120 min after to allow reperfusion. Sham-operated rats were treated with an identical surgery except that the intraluminal filament was not inserted. Throughout the procedure, body temperature was maintained at 37 ± 0.5 °C. Rats were excluded if hemorrhage was found in the brain slices or at the base of the circle of Willis during postmortem examination.

2.3. Experimental protocol and drug treatment

Fig. 1 shows the chemical structure of OEA (Sigma, St. Louis, MO, USA) and the design of experiments. On day 0, rats were subjected to MCAO and then returned after 24 h of recovery to standard, individual cages. Behavioral assessments were made on day 7 after surgery to give the animals sufficient time to recover from surgery. Our previous study showed that both 20 and 40 mg/kg OEA had protective effects against focal cerebral ischemic injury, while the effects of OEA at dose of 40 mg/kg were more potent than those of 20 mg/kg [15]. In the present study, rats received *i.p.* injection of OEA (dissolved in PEG/tween 80/saline, 5:5:90, 30 mg/kg) daily starting on day 8 after ischemia induction and continuing to 14 or 35 days after MCAO. To determine whether OEA treatment improves spatial cognitive impairment, the MWM test was tested during days 43–48 after MCAO. The design of this experiment included a 7-day withdrawal (from day 36 to 42 after MCAO) following 28 days of chronic OEA treatment before we performed the MWM test. The purpose of selecting this paradigm is to exclude the possibility of acute responses of OEA treatment on cognitive performance and to reflect the effects of hippocampal neurogenesis on cognitive performance. To examine whether OEA increases neurogenesis, rats were injected *i.p.* with 5-bromo-2′-deoxyuridine (BrdU; Sigma) 50 mg/kg twice daily, at 8-h intervals. For the cellular proliferation study, animals were sacrificed 12 h after the last BrdU injection (from post-ischemic day 12 to 13 after MCAO) to examine the number of newly formed cells in the DG of injured hippocampus. To determine the survival and cell phenotype of the newly born cells, BrdU was administered to rats for 3 days (from post-ischemic day 11 to 13), animals were sacrificed 28 days after the last BrdU injection (at post-ischemic day 42). To determine whether hippocampal neurogenesis is necessary for the effects of OEA on spatial cognitive performance after ischemic stroke, we used a telomerase inhibitor, 3′-Azido-deoxythymidine (AZT; Sigma), to ablate neurogenesis. Rats were treated with 100 mg/kg AZT per day *i.p.* during the period of OEA treatment (from day 8 to 35 after MCAO). The dose of AZT used was based on a previous study [19]. The MWM test and neurogenesis performed 42 days after MCAO (28 days after the last BrdU administration) by the same approach mentioned previously.

The inhibition of PARP-1 by OEA (0.032–10,000 μM) was measured by universal PARP colorimetric assay kit (Trevigen, Gaithersburg, MD, USA) according to the manufacturer's instructions. The 3-aminobenzamide (3-AB) (Trevigen, USA) is provided at 200 mM in Ethanol as a control inhibitor. The experiment was repeated three times. IC50 values were calculated using GraphPad Prism 5 software.

2.4. Morris water maze test

The Morris water maze was used to test spatial learning and memory [17], and was performed during days 43–48 after MCAO. Briefly, the maze consisted of a circular water tank (120 cm in diameter and 50 cm high). The water temperature was kept at 25 ± 1 °C. A black circular platform (10 cm in diameter) was 2.0 cm below the surface of the water in the center of the quadrant and remained in the same position. There were prominent visible cues around the room. The experiment included two phases:

Phase I. *Acquisition*: Acquisition training consisted of 5 days of conditioning with four trails per day from day 43 to 47 after ischemia. For each trail, the rat was placed in the water facing the wall of the pool at one of the four starting points (north, south, east or west) and allowed to swim for a maximum of 90 s. If the rat found the platform, it was allowed to remain on it for 15 s. If it did not find the platform, it was guided to it and allowed to remain there for 15 s.