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Oxiracetam can improve cognitive impairment after chronic cerebral hypoperfusion in rats

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ABSTRACT

Chronic cerebral hypoperfusion (CCH) induces cognitive deficits. Although CCH can be improved, cognitive impairment is not improved accordingly. To date, many studies have focused on investigating the pathophysiological mechanisms of CCH; however, the treatment of the induced cognitive impairment remains ineffective. Thus, the mechanisms underlying cognitive impairment after CCH and potential agents for treating this impairment need to be explored further. Oxiracetam is a nootropic drug that improves clinical outcomes for some central nervous system (CNS) disorders. Whether it can improve cognitive impairment after CCH is unknown. In this study, we used behavioural methods, electrophysiology, biochemistry, histopathological staining and transmission electron microscope to investigate rat's cognitive impairment by CCH, and found that Oxiracetam could improve CCH-induced cognitive impairment and prevent deficits of neural plasticity, white matter lesions, and synaptic ultrastructure. These results suggest that Oxiracetam may be effective as a potential agent against CCH-induced cognitive impairment.

1. Introduction

Chronic cerebral hypoperfusion (CCH) appears in many diseases with cognitive deficits, such as mild cognitive impairment, Alzheimer's disease (AD), and vascular dementia (VaD). The initiators of CCH include hypertension, diabetes mellitus, smoking, hypercholesterolemia, and atherosclerosis, as well as other genetic and environmental factors (Dong et al., 2011; Pimentel-Coelho et al., 2013; Roman et al., 2002). However, preventing or treating these risk factors and comorbidities has not been convincingly shown to reduce cognitive decline or dementia in the elderly (Debette, 2013; Godinho et al., 2015). Thus, once these risk factors and comorbidities trigger CCH, intervention does not effectively mitigate CCH-induced cognitive impairment or repair the pathological damage, despite CCH is a chronic but reversible process. Evidences show that cerebral hypoperfusion can lead to chronic hypoxia, focal lesion formation, diffuse axonal degeneration (D'Haeseleer et al., 2015), impairment of the blood brain barrier (BBB) integrity, white matter dysfunction (Choi et al., 2015), cerebral amyloid angiopathy, cholinergic dysfunction, and oxidative stress, because CCH can bring the inadequate blood supply and energy matter to brain areas associated with cognition (Gupta et al., 2015). Since the neurons and their fibres white matters are vulnerable to various risk factors related to lack of blood-oxygen, decreasing energy metabolism may cause deterioration in neuronal structures and functions, leading to cognitive deficits. Not only are resources for neuronal metabolism reduced, but also metabolic capability is decreased. The impaired metabolic capability triggered by CCH may not be completely restored, even though the blood-oxygen supply recovers. So the impairment of neural plasticity (Hai et al., 2010; Jing et al., 2015), synaptic microstructure (Bayat et al., 2015), and white matter lesions (Ueno et al., 2002) do not recover to the normal level because of lack of the energy and other resources, thereby leading to cognitive deficits. But whether the recovery of the impaired metabolic capability may improve the above pathological alterations to ultimately ameliorate the cognitive impairment remains unreported.

Oxiracetam is a nootropic drug of the racetam family that can penetrate the blood-brain barrier, and reach the hippocampus and cerebral cortex with high concentration (Gouliaev and Senning, 1994).

Abbreviations: CCH, Chronic cerebral hypoperfusion; AD, Alzheimer's disease; VaD, vascular dementia; BBB, blood brain barrier; CNS, central nervous system; PKC, protein kinase C; mAb, Mouse monoclonal antibody; CREB, cAMP-response element binding protein; BDNF, brain derivative growth factor; fEPSPs, field excitatory postsynaptic potential; PP, perforant path; LTP, long-term potentiation; HFS, high frequency stimulation; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; LTD, long-term depression; TEM, transmission electron microscopy; PSD, postsynaptic density; NMDA receptor, N-methyl-d-aspartic acid receptor

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Oxiracetam has a very mild stimulant and notably improves the clinical outcomes of several CNS disorders, such as cognition and memory disorders, epilepsy and seizure, neurodegenerative diseases, stroke and ischemia, and stress and anxiety, etc (Malykh and Sadaie, 2010). Many studies had shown that Oxiracetam improved the synthesis of phosphatidylcholine and phosphatidylethanolamine, proteins, and nucleic acids. Additionally, Oxiracetam can increase the content of high-energy phosphates (Gabryel et al., 1999), the level of membrane-bound protein kinase C (PKC), and specifically hippocampal PKC activity, which is important for cognitive function (Fordyce et al., 1995). Moreover, Oxiracetam potentiates neurotransmission (Gouliaev and Senning, 1994). So Oxiracetam can improve the metabolism level of brain. Although substantial evidences suggested that Oxiracetam could promote metabolism and play the neuroprotective roles in several cognitive impairment (Hlinak and Krejci, 2000, 2002, 2005; Mondadori et al., 1996), the effect of Oxiracetam on CCH-induced cognitive impairment has not been reported so far and the underlying mechanisms are unknown. Normal neural plasticity (Hai et al., 2010; Jing et al., 2015), synaptic microstructure (Bayat et al., 2015), and white matter structure (Ueno et al., 2002) play the critical roles in preserving normal cognitive function, and the cognitive function was impaired in CCH (Sarkaki et al., 2014; Sekhon et al., 1997; Ueno et al., 2002), but whether Oxiracetam can regulate the neural plasticity, synaptic microstructure, and white matter structure in CCH model remains unknown.

Therefore, the present study is performed to determine whether Oxiracetam can improve the CCH-induced cognitive impairment in rat, and explore whether neural plasticity, white matter lesions, and synaptic microstructure are implicated in the cognitive decline caused by CCH. At same time, we would study whether Oxiracetam could regulate these structural and functional changes to improve in the cognitive decline caused by CCH.

2. Material and methods

2.1. Antibodies and chemicals

Mouse monoclonal antibody (mAb) against total β-actin, rabbit pAb CREB against cAMP-response element binding protein (CREB), and rabbit pAb p-CREB-Thr133 against phosphor-CREB at Thr133 were purchased from Abcam (Cambridge, CB, UK). Rabbit pAb BDNF against brain derivative growth factor (BDNF) was from Millipore (Billerica, Massachusetts, MA, USA). Goat anti-rabbit or anti-mouse IgG conjugated to IRDye™ (800CW) was from Licor biosciences (Lincoln, NE, USA). The BCA protein assay kit was from Pierce Chemical Company (Rockford, IL, USA). TRIzol was purchased from Invitrogen, (Singapore). The first-strand cDNA synthesis kit was from Thermo Fisher Scientific (Waltham, MA, USA). Oxiracetam was from Sencee Pharmaceutical Co. Ltd. (Guangzhou, China) and was diluted to a final working concentration of 3.75 mg/ml. Luxol fast blue dye was purchased from Sigma (St Louis, MO, USA).

2.2. Animals and Chronic cerebral hypoperfusion (CCH) Model

Adult Sprague-Dawley rats (male, 220–240 g) were from the Experimental Animal Center of Tongji Medical College, Huazhong University of Science and Technology, and were housed with accessible food and water ad libitum. Rats were kept on a 12-h light/dark cycle with the light on from 7:00 am to 7:00 pm. All animal experiments were performed according to the University's Policies on the Use of Animals and Humans in Neuroscience Research. After a 1-d period for acclimation, all of the rats were put into the water maze and their memory was tested.

The rats were administered with chloral hydrate (0.4 g/kg) intraperitoneally for anesthetization. During surgery, temperature was maintained at 37 °C with a heating pad. After a ventral midline incision, both common carotid arteries were gently separated from the carotid sheath and vagus nerve (Briones et al., 2000). Bilateral common carotid arteries were doubly ligated with 4–0 silk suture just below the carotid bifurcation, respectively. In control rats, similar surgery was made but no vessel ligation. After the surgery was finished, the rats stayed under 37 °C until recovery. Through the surgery, the brain of rats didnot become softening wholly or partly.

2.3. Treatment, Morris water maze, and pole climbing test

In our study, the rats were divided into the following four groups: 1) the group receiving CCH using 2-vessel occlusion (2VO) (n=11); 2) the sham group (Con) (n=10); 3) the group receiving 2VO and Oxiracetam treatment intraperitoneally (30 mg/kg) (2VO+OR) (n=11); and 4) the sham group receiving Oxiracetam treatment (OR) (n=11). In the 2VO group, bilateral common carotid arteries had been ligated for 4 weeks and the rats were receiving saline daily. In the sham group, the rats had no occlusions but received saline. In 2VO+OR group, the rats received 4 weeks of occlusion and Oxiracetam treatment daily. The OR group received 4-weeks treatment of Oxiracetam daily but no occlusions. A criterion of the 2VO model was that blood flow was reduced to 70% below normal. To verify the 2VO model, we used a laser Doppler system to detect the level of blood flow.

After 30 days of cerebral hypoperfusion or Oxiracetam treatment, all rats received spatial memory training in the Morris water maze. For spatial memory acquisition, rats were trained in the water maze to find a hidden platform for 7 consecutive days, 3 trials per day, with a 30-s interval from 2:00 to 8:00 pm. Each trial started with the rat in the middle of the outer round edge facing the wall of the pool and ended when the animal climbed on the platform. The rats that could not find the platform in 60 s were guided to the platform. The rats' swimming pathways and latencies to find the hidden platform were recorded as previously reported using a video camera fixed to the ceiling of the room, 1.5 m from the water surface (Morris, 1984). The scores of first trials to arrive the platform during 7 days were recorded to evaluate the learning ability. For the memory retention test, the platform was removed and the place where the platform originally existed was recorded as the cued area in the software. Rats were put into the 1st quadrant of the maze, and the swimming pathway and latency to the first time to reach the cued area, the number of times crossing the cued area, the total swimming distance and the total time in the 3rd quadrant were recorded.

Basing on a negative passive avoidance response to conditioned stimuli, pole climbing apparatus was used to examine cognitive function. The apparatus consisted of a chamber (25×25×40 cm) with the stainless-steel grid floor, a 2.5 cm-in-diameter pole in the center of the grid floor, and a loudspeaker on top of chamber. Electric shock (foot shock: 50 Hz 6 mA) is delivered to the grid floor. The rats were placed in the chamber to be allowed to acquaint the chamber for 60 s. Buzzer signal, as conditioned stimulus (CS), was turned on and electric shock, as unconditioned stimulus (US), delivered through grid floor for 60 s. Animal learned to build the associate between the buzzer and impending foot shock and to avoid the foot shock through climbing the pole after buzzer signal. Every rat was trained 5 trails for 5 days and transfer latency time (TLT) was recorded to check the retention of conditioned avoidance response (CAR) and escape response (ER). Avoidance response was defined as climbing reaction time during the 10-second buzzer signal. Escape response was defined as climbing reaction time during the 60-second foot shock.

2.4. Electrophysiology

After the spatial memory retention test, rats were anaesthetized with urethane (1.6 g/kg, i.p.). Electrodes were implanted in the following coordinates: 4.0 mm posterior to the bregma and 2.0 mm lateral to the midline for the recording electrode, and 7.0 mm posterior