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The homeostasis of iron and suppression of HO-1 involved in the protective effects of nimodipine on neurodegeneration induced by aluminum overloading in mice

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Abstract

Aluminum intoxication can cause damage to the cognitive function and neurodegenerative diseases. In the present study, we investigated the role of iron homeostasis and heme oxygenase-1 (HO-1) expression in the protective effects of nimodipine on the neurodegeneration induced by aluminum overloading in mice. 2 μl of 0.25% aluminum chloride solution was intracerebroventricularly injected once a day for five days to induce the neurodegeneration of mice. Nimodipine was administered by intragastric gavage (80 mg/kg per day) for 30 days. We observed that nimodipine could improve the performance of behavior test related to the learning and memory function and ameliorate pathological changes of hippocampi caused by aluminum. Results of western blot, immunohistochemistry study, biochemical test and inductively coupled plasma-atomic emission spectrometry showed that nimodipine could suppress the increased expression of HO-1 protein, and decrease the elevation of both HO activity and iron level in hippocampi, induced by aluminum overloading. These results indicate that nimodipine can suppress the neurodegenerative development induced by aluminum overloading and the mechanism of its action is at least partly related to keeping the homeostasis of iron through blunting the expression of HO-1 in hippocampus.

Keywords: Aluminum; Iron; Heme oxygenase; Neurodegeneration; Nimodipine; Hippocampus

1. Introduction

Neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, etc., will increase with the increasingly ageing population in the next decades. It is believed that many factors, including genetics, cerebral vessel diseases, excessive reactive oxygen species, and abnormalities of aluminum, iron and other metal ions contribute to the onset of these diseases. Among them, aluminum for Alzheimer's disease and iron for Parkinson's disease have been well recognized (Borie et al., 2002; Feigin and Zgaljardic, 2002; Pratico et al., 2002).

Heme oxygenase (HO) functions as the rate-limiting enzyme in heme degradation (Abraham et al., 1998; Maines, 1988). There are three isoforms of HO: HO-1, HO-2 and HO-3 (Maines and Kappas, 1974; Yoshida et al., 1974; Maines et al., 1986; Trakshel et al., 1986; McCoubrey et al., 1997). HO-1 is an inducible form that can be induced by many factors (Wu, 2005) and expressed in different regions of brain, especially the hippocampus (Scapagnini et al., 2002). HO-1 is thought to be heavily involved in the catabolism of heme which is degraded into CO, biliverdin and iron. HO-2 that is a non-inducible isoenzyme of HO is thought to be particularly involved in signaling pathways. The physiological role of HO-3 is uncertain.

Nimodipine as a calcium channel blocker belongs to dihydropyridine compounds, which has a high selectivity to the cerebral vessel and shows an admiringly protective effect on the brain injury from cerebral ischemia and its complications (Ma and Zhang, 2006). It was reported that nimodipine could

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ameliorate the age-related working memory deficits in aged animals (Devo et al., 1989; Veng et al., 2003) and improve memory ability as well as attention ability of the patients with mild cognitive impairment (Wang et al., 2006). However, the mechanism is still unclear. It was reported that high concentration of glucose that affected the survival of pancreatic islet cells, elevated the expression of HO-1 in pancreatic beta cells through increasing calcium influx, which could be reversed by nimodipine (Jonas et al., 2003). Heme degraded by HO-1 is the main source of endogeneous iron. Our research has demonstrated that aluminum overloading caused damage to the brain of mice with significant elevation of iron (He et al., 2006). Documents also showed that the abnormal increase of iron involved the development of neurodegeneration (Ke and Qian, 2007). So far, the effects of nimodipine on neurodegeneration induced by aluminum overloading, hippocampal HO-1 expression and homeostasis of iron have not been elucidated.

Thus, our working hypothesis is that nimodipine may prevent the neurodegeneration induced by aluminum overloading through suppressing the expression of HO-1 and reducing the production of iron. Our analyses included behavior experiment and histological examination to evaluate the effect of nimodipine on neurodegeneration induced by aluminum overloading. Then, to clarify whether nimodipine affected HO, we examined the activity and expression of HO. Finally, the free iron was detected by inductively coupled plasma-atomic emission spectrometry to determine the effect of nimodipine on homeostasis of iron.

2. Materials and methods

2.1. Reagents

Glucose 6-phosphate, NADPH, Hemin and Glucose 6-Phosphate Dehydrogenase were purchased from Sigma Co. Rabbit anti rat HO-1 polyclonal antibody and Rabbit anti rat HO-2 polyclonal antibody were purchased from Oncogene Co. Nimodipine were purchased from Bayer Co.

2.2. Animals

The 55 days old male mice of the NIH strain (supplied by Laboratory Center, Chong Qing Medical University, Certificate No: scxk20020001) were fed in the long-day light cycle condition (16 h light/8 h dark) at the surrounding temperature of 20±4 °C. All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by Chong Qing medical university.

2.3. Apparatus

The step down test apparatus came from Institute of Materia Medica, Chinese Academy of Medical Sciences. The apparatus was a rectangular box (10×15×40-cm) with copper grid floor electrified with 36 V alternating current when test was carried out and a rubber columnar platform (diameter: 3.0 cm, height: 3.5 cm) in one corner. Inductively coupled plasma-atomic emission spectrometry meter was purchased from PE Co, U.S.A.

2.4. Surgery

The surgery was performed according to the procedures of Toyoda's report (Toyoda et al., 1996). Briefly, mice were anesthetized with 4% (wt./vol) chloral hydrate solution (10 ml/kg, ip) and a stainless steel cylindrical cannula (outer diameter 0.6 mm; inner diameter 0.4 mm) with stopper was implanted into the left lateral ventricle of mice (1.3 mm lateral to the midline, 0.3 mm posterior to the bregma, 2.0 mm in depth). During the experiment, body temperature was monitored and maintained at 37±0.5 °C. The operated mice were allowed to rest for 7 days to recover from the surgery and then received intracerebroventricular injection.

2.5. Step down test

Mouse was placed on the platform. When it stepped down the floor and received a 36 V alternating current footshock, the mouse quickly jumped onto the platform to avoid the electric stimulation, which was counted as one error. The error number and electric shock time the mouse underwent during the experimental period of 10 min was recorded as learning score. 24 h later, the mouse was placed on the platform again without electricity on grid. Time the mouse took to put its two forefeet on the grid and time the mouse stayed on the platform in 5 min were recorded as step down latency and remaining time, respectively. The longer the step down latency and remaining time were, the better the memory ability of mouse was.

2.6. Histology

After being anesthetized with 4% (wt./vol) chloral hydrate solution (10 ml/kg, ip), mice were perfused transcardially with 100 ml of 0.9% (wt./vol) saline containing heparin (250 U) followed by 100 ml solution containing 3.5% (wt./vol) formaldehyde and 0.9% (wt./vol) saline in phosphate buffer (0.1 mol/L, pH 7.2). The brains were removed and kept in the same fixing solution for 5 days. Coronal sections of 4 μm in thickness from brain tissue were done with HE staining.

2.7. Immunohistochemistry study

According to the procedures of Maines's report (Maines et al., 1996), the immunohistochemistry of HO-1 was performed, except for antibody. Briefly, mice were anesthetized with 4% (wt./vol) chloral hydrate solution (10 ml/kg, ip) and perfused through the heart with 0.9% (wt./vol) saline, followed by 0.1 mol/L phosphate buffer containing 4% (wt./vol) paraformaldehyde and 1.5% (wt./vol) sucrose. Brains were fixed for 16 h (at 4 °C) prior to sequential equilibration in 10%, 20%, and 30% graded sucrose solutions (wt./vol, in 0.1 mol/L phosphate buffer pH 7.2). Brains were subsequently frozen, and 40 μm thick coronal sections were obtained using a sliding microtome and then cryopreserved in 0.1 mol/L phosphate buffer