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Research report

Pre-treatment with nimodipine and 7.5% hypertonic saline protects aged rats against postoperative cognitive dysfunction via inhibiting hippocampal neuronal apoptosis

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ABSTRACT

Objective: This study aimed to investigate the effects of pre-treatment with nimodipine and 7.5% hypertonic saline (HS) on postoperative cognitive dysfunction (POCD) in aged rats.
Methods: Healthy Sprague-Dawley aged rats were randomly assigned into 4 groups: POCD group, nimodipine group, HS group, and nimodipine + HS group. Rats in POCD group received normal saline injection and then splenectomy 30 min later under 1.8% isoflurane inhalation for 2 h. In remaining groups, rats received injection of 1 mg/kg nimodipine (i.p) and/or 4 ml/kg 7.5% HS (i.v) and then splenectomy. Morris water maze test was performed before and after surgery. The hippocampus was harvested for the detection of neuronal apoptosis rate (AR), cytoplasmic calcium ($[Ca^{2+}]_i$), Bcl-2 and Bax mRNA expression and hippocampal neuronal ultrastructure.
Results: When compared with POCD group, the latency to escape, neuronal AR, $[Ca^{2+}]_i$, Bax mRNA expression and Bax/Bcl-2 ratio reduced dramatically, but the times of crossing the platform and Bcl-2 mRNA expression increased significantly ($P < 0.05$) in nimodipine group, NS group and nimodipine + HS group. In addition, the latency to escape, neuronal AR, $[Ca^{2+}]_i$, Bax mRNA expression and Bax/Bcl-2 ratio reduced markedly, but the times of crossing the platform and Bcl-2 mRNA expression increased significantly in nimodipine + HS group as compared to nimodipine group and NS group ($P < 0.05$). Hippocampal neuronal ultrastructure damage was observed in all 4 groups, but it was the mildest in nimodipine + HS group.
Conclusion: Pre-treatment with both nimodipine and 7.5% HS exerts better protective effects, which is related to the inhibition of hippocampal neuronal apoptosis.

1. Introduction

Postoperative cognitive dysfunction (POCD) is used to describe a deterioration of cognition that is temporally associated with surgery, and it has been a common post-operative complication in the elderly (≥65 years) [1,2]. POCD is usually self-limited, but occasionally can be long-lasting or even permanent [1]. Its incidence varies from 20 to 79% in cardiac surgery and 4.1–22.3% in non-cardiac surgery [1,3]. The advanced age is the most important risk factor for POCD although other risk factors (such as genetic polymorphism, idiosyncratic condition, metabolic syndrome and neurological diseases) are also found to increase the risk for POCD [4]. In recent years, increasing studies have been conducted to investigate the pathogenesis and prevention of POCD due to its association with higher mortality, longer hospitalization, greater use of medical resources and poorer quality of life [5,6].

POCD is characterized by post-operative memory impairment and intellectual reduction. The hippocampus is an important component of limbic system and involved in the regulation of learning, memory, emotion and behaviors [2]. Although the mechanism underlying the pathogenesis of POCD is still poorly understood, studies have revealed that reactive oxygen species (ROS) [7], hippocampal neuronal apoptosis [8], and neuroinflammation [9] are closely related to the pathogenesis of POCD, and thus several measures have been investigated in the prevention of POCD in animal models [10–12].

Perturbed neuronal Ca^{2+} homeostasis is implicated in age-related cognitive impairment [13,14]. It has been known for more than 30 years that Ca^{2+} levels are increased in aging neurons [15,16]. Ca^{2+} is a key regulator of synaptic plasticity, excitatory amino acid neurotransmission [17] and neuronal apoptosis [18],

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and therefore it is easy to see how dysregulation of Ca^{2+} leads to cognitive abnormalities.

Calcium channel blockers commonly used to treat hypertension might relieve Alzheimer disease –like symptoms in many models. Nimodipine is a 1,4-dihydropyridine-derivative Ca^{2+}-channel blocker developed approximately 30 years ago [19]. It is highly lipophilic, and can cross the blood-brain barrier and reach brain and cerebrospinal fluid (CSF). Nimodipine is a safe drug with an important place in pharmacotherapy and with the main documentation for reduction in the severity of neurological deficits resulting from vasospasm in subarachnoid hemorrhage patients. [20]. Nimodipine was found to prevent transient cognitive dysfunction after moderate hypoxia in adult mice, [21], and improve the disruption of spatial cognition induced by cerebral ischemia in rats [22]. Hypertonic saline (HS) solutions have received renewed attention as effective agents for the treatment of cerebral edema and the brain resuscitation in a variety of brain injury paradigms. [23]. However, no study has been conducted to investigate the cerebroprotection of HS solutions. Our previous studies showed pre-treatment with nimodipine or 7.5% HS alone was able to prevent POCD in old rats [24,25]. The present study was undertaken to investigate whether the neuroprotection of pre-treatment with nimodipine and HS solution (7.5% saline) on POCD is superior to that of nimodipine or HS alone in aged rats.

2. Materials and methods

2.1. Animals and grouping

Healthy male Sprague-Dawley rats (n = 120) weighing 450–500 g and aged 18 months were purchased from the Experimental Animal Center of Hebei Medical University. Animals were housed at a constant temperature and given *ad libitum* access to water and food. Rats were randomly assigned into 4 groups (n = 30 per group): POCD group, nimodipine group, HS group, and nimodipine + HS group. In POCD group, rats received splenectomy under inhalation anesthesia with 1.8% isoflurane (2 l/min; Sigma, USA), and intraperitoneal and intravenous injection of normal saline 30 min before surgery. In nimodipine group, rats were intraperitoneally injected with 1 mg/kg nimodipine (Bayer Schering Pharma AG, Germany) and intravenous injection of normal saline via the tail vein 30 min before surgery. In HS group, rats received intravenous injection of 4 ml/kg 7.5% HS solution (Shijiazhuang No.4 Pharmaceutical Co., Ltd.) via the tail vein and intraperitoneal injection of normal saline 30 min before surgery. In nimodipine + HS group, rats received injection of both nimodipine and 7.5% HS 30 min before surgery as abovementioned.

All experiments were performed in accordance with the Chinese Guidance for the Care and Use of Laboratory Animals, and the whole study was reviewed and approved by the Ethics Committee of Hebei Medical University.

2.2. Splenectomy

Splenectomy was performed at 30 min after pretreatment. Briefly, rats were anesthetized, the surgical site was shaved and sterilized, and a vertical incision of about 3 cm was made at the level of the left costal margin. Then, spleen was pulled out, the splenic vein and artery were ligated by using 4/0 polyglactin (Vicryl, Ethicon Endo-Surgery, Cincinnati, USA) suture. The spleen was resected after ligation. Once no bleeding was confirmed, sterile sutures were used to close the wound, and animals were allowed to stay at 37 °C until recovery of consciousness.

2.3. Morris water maze test

Morris water maze (MWM) test was performed 1 day before surgery and 1, 3 and 7 days after surgery. MWM was used to test spatial learning and memory based on reported methods [26] and performed in a 180-cm diameter water pool which was virtually divided into four quadrants. The pool was filled with water (22 ± 1 °C). A colorless escape platform (12 cm in diameter) was submerged 2 cm beneath the water surface, located in a designated target quadrant. Rats received 4-day training before the test. In each day, rats were placed in the pool facing the wall at each of the four possible start locations (north, east, south, and west) in a quasi-randomized manner. Each trial lasted until the rat climbed onto the platform or until 60 s had elapsed, whichever occurred first. The rats that failed to locate the escape platform within the allotted time were manually guided to it and allowed to stay on the platform for 15 s. The training consisted of four sessions per day and the times of the 4 daily trials for each rat were averaged. On the 4th day, the rats were placed in the water at the entry site where the last training was performed. The latency to escape (with a maximum of 180 s) was recorded at the time the animal got into the water and ending at the time the animal reached the submerged platform. In the probe trials without platform, the times of crossing the target quadrant where the platform had been located were recorded within 60 s. MWM test was conducted in JLBehv-MWM Morris water maze system (Shanghai Ji'liang Software Technology Co., Ltd).

2.4. Detection of hippocampal neuronal apoptosis

1 day before surgery and 1 and 7 days after surgery, 10 rats in each group were sacrificed after intraperitoneal anesthesia with 250 mg/kg chloral hydrate. The brain was collected, and hippocampus was separated and cut into blocks. The hippocampal tissues (100 mg) were filtered through a 200-mesh net, and the filtrate was centrifuged at 1000 rpm/min for 5 min. The supernatant was removed, and 500 μl of buffer was added to prepare single cell suspension (1×10^6/l). After addition of Annexin V, incubation was done for 5 min in dark. Then, the mixture was incubated with propidium iodide (PI) in dark for 5 min. Flow cytometry was performed to detect the apoptotic cells (Beckman Coulter) and the apoptosis rate was calculated.

2.5. Detection of intracellular calcium

The hippocampal neurons were separated as abovementioned. Cells were then washed twice with HBSS and incubated with 5 μmol/l Fura-3,AM at 37 °C for 30 min. After washing in HBSS twice, cells were re-suspended in HBSS, followed by incubation at 37 °C for 15 min. Flow cytometry was performed to detect the fluorescence intensity at excitation wavelength of 480 nm and emission wavelength of 525 nm. The fluorescence intensity reflects the concentration of intracellular calcium ($[Ca^{2+}]_i$).

2.6. Transmission electron microscopy

At 1 day before surgery and 1 and 7 days after surgery, rats were sacrificed, and hippocampus (about 1 mm × 1 mm × 3 mm) was collected, fixed in 4% glutaraldehyde and 1% osmium tetroxide. After dehydration in a series of ethanol, tissues were embedded in epoxy, followed by double staining with uranyl acetate – lead citrate. Transmission electron microscopy (TEM) was performed to observe the microstructure of hippocampal neurons (H-7500; Hitachi, Japan).