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Deferoxamine pre-treatment protects against postoperative cognitive dysfunction of aged rats by depressing microglial activation via ameliorating iron accumulation in hippocampus

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ABSTRACT

Postoperative cognitive dysfunction (POCD) is a common complication of elderly patients after surgery. The mechanisms of POCD have not been clarified. Iron accumulation is a feature of neurodegeneration. Recent reports showed that iron content was increased with impaired cognition induced by surgery. We sought to investigate whether iron chelation would attenuate POCD. In this study, male aged (18 months) Sprague-Dawley rats received 100 mg/kg deferoxamine or saline solution (0.9%) for 6 days before exploratory laparotomy. Cognition was evaluated by Morris water maze before and after surgery. Additional rats received deferoxamine or saline were used to determine hippocampal iron content, iron transport-related proteins (transferrin receptor, divalent metal transporter 1, ferroportin 1 and hepcidin), oxidative stress, microglial activation and brain cell apoptosis. It was found that deferoxamine improved postoperative spatial memory in aged rats. Deferoxamine significantly reduced hippocampal iron concentration and ferritin. Surgery increased divalent metal transporter 1 and hepcidin, decreased transferrin receptor and ferroportin 1, and enhanced ferroportin 1 mRNA. However, deferoxamine reversed the changes of these proteins. Furthermore, deferoxamine sharply reduced the hippocampal reactive oxygen species, malondialdehyde concentration and OX-42 that is a marker of microglia, which might reduce postoperative brain cell apoptosis. This study showed that deferoxamine may improve postoperative cognition of aged rats by ameliorating oxidative stress induced by hippocampal iron accumulation, microglial activation and brain cell apoptosis. This study suggests a potential therapeutic method for reducing POCD.

1. Introduction

Postoperative cognitive dysfunction (POCD) is a common postsurgical syndrome and primarily affects aged patients. The incidence of POCD varies from 20 to 79% in cardiac surgery (Newman et al., 2006; Selnes et al., 2012; Trubnikova et al., 2014) and 4.1–22.3% in non-cardiac surgery (Fang et al., 2014; Krenk et al., 2014; Silbert et al., 2014). A latest investigation in Chinese patients who had non-coronary bypass cardiovascular surgery showed that the incidence of early POCD was 33.0% (Xu et al., 2013). Researches have shown that POCD is associated with higher mortality, longer hospitalization, greater use of medical resources and poorer quality of life (Newman et al., 2001; Roach et al., 1996). It is generally accepted that aging is the most crucial risk factor for the development of POCD (Caza et al., 2008; Moller et al., 1998; Monk et al., 2008). Since anesthesia and surgery are coexisted, which one of the two leads to POCD is still controversial. Investigators of International Study of Postoperative Cognitive Dysfunction have reported that general anesthesia is not associated with long-term POCD (Rasmussen et al., 2003). A recent clinical trial (Silbert et al., 2014) reported that there was no significant difference in the incidence of POCD in patients undergoing different types of anesthesia. Although these studies did not demonstrate a direct relationship between surgery and the occurrence of POCD, surgical

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Abbreviations

POCD	postoperative cognitive dysfunction
DFO	deferoxamine
MWM	Morris water maze
ICP-MS	inductively coupled plasma mass spectrometer
Bcl-2	B cell lymphoma 2
Bax	Bcl-2-associated X protein
Fpn1	ferroportin 1
TfR	transferrin receptor
DMT1	divalent metal transporter 1
ROS	reactive oxygen species
MDA	malondialdehyde
SOD	superoxide dismutase
TUNEL	Terminal deoxynucleotidyl transferase mediated dUTP nick end labeling
IRE	iron responsive element
IRP	iron regulatory protein
LPS	lipopolysaccharide
IL-1β	interleukin-1β

stress on the development of POCD may be an important stimulus. In addition, evidence from animal experiments supported cognition decline induced by surgery (An et al., 2013; Hovens et al., 2013; Zhang et al., 2013).

Although the exact mechanism of POCD is largely unknown, preclinical and clinical studies have shed light on the relationship between iron accumulation and neurodegenerative disorders (Bartzokis et al., 2000; House et al., 2006; Zecca et al., 2004). For example, high-iron diet may lead to iron overload in rat brain, which subsequently impairs learning and memory, and inhibits long-term potentiation in hippocampus (Sripetchwandee et al., 2014). An et al. (An et al., 2013) reported that increased brain iron content was associated with cognitive decline after surgery in a POCD rat model, suggesting a potential relationship between iron overload and POCD. A research based on magnetic resonance imaging also showed that R_2, a parameter strongly correlating to iron concentration, was increased in some brain regions of patients with memory complaints (House et al., 2006). Furthermore, Schröder et al. (Schroder et al., 2013) had summarized a series of studies involving iron-induced memory deficits in human and animal models. As a transition metal, iron has the chemical property of electron transfer by switching between ferrous state and ferric state. Iron is able to react with intracellular hydrogen peroxide or lipid peroxides. As a results, ferrous iron is oxidized to ferric iron; meanwhile hydroxyl radical or lipid radicals are generated. These molecules are highly reactive and destructive to cellular macromolecules and structures. Thus, excessive iron is toxic to cells. Alleviating brain iron overload may be an available therapeutic strategy to improve cognitive function and slow down neurodegenerative progress. Deferoxamine (DFO), an iron chelator, can penetrate blood-brain barrier after peripheral administration (Palmer et al., 1994) and exhibit reliable efficacy on animal model of cognition decline or neurodegenerative diseases(Chen et al., 2013; de Lima et al., 2008; Guo et al., 2013). Our previous study had observed that peripheral DFO administration may improve spatial memory of aged rat and reduce hippocampal iron deposition, without any obvious adverse effect. However, the effect and mechanism of iron chelator on POCD have not been evaluated. We hypothesize that pretreatment with DFO may effectively alleviate iron accumulation in the brain, leading to reduced oxidative stress and microglial activation, and, thus, protection against neuronal apoptosis and improved postoperative cognitive function after surgery.

2. Materials and methods

2.1. Animals

All procedures were carried out in accordance with the guidelines from the National Institutes of Health. Protocols of this study were approved by the committee Animal Research of Southwest Hospital. Eighteen-month-old male Sprague-Dawley rats, weighing 525 ± 43 g, were purchased from Chongqing Academy of Chinese Materia Medica. All animals were housed two rats per cage in a controlled environment at 24 °C and a humidity of 60%, under 12 h light-dark cycle with *ad libitum* access to water and rodent chow.

2.2. Drug administration and surgery

Rats were randomly allocated to Group NS and Group DFO, and received intraperitoneally saline solution (0.9%) or DFO (100 mg/kg daily for up to 6 days), respectively. On the next day after drug injection finished, rats were anesthetized with 5% chloral hydrate and underwent exploratory laparotomy, which was according to a previous description, but with some modification (Barrientos et al., 2012). The surgical field and instruments were maintained sterile throughout the procedure. Briefly, a midline abdominal incision (~4 cm) was made. The abdominal organs were explored in the following order: liver, spleen, left kidney, right kidney and bowel. Exploration lasted around 20 min. The incision was infiltrated with 0.2% lidocaine for postoperative analgesia and then closed with suture. Animals were recovered and returned to their cages and kept individually.

2.3. Tissue preparation

Rats were sacrificed by an overdose of 5% chloral hydrate. Brains were obtained at these four time points: before surgery (Pre), 1 day after surgery (Post 1), 3 days after surgery (Post 3) and 7 days after surgery (Post 7). Rats received no drug administration and surgical procedure were killed as normal control (Ctrl). Different batches of rats were used for immunohistochemical studies and other analyses, respectively. For immunohistochemistry, six rats in each group were killed at every time-point. Animals were transcardially perfused with saline followed by 2.5% paraformaldehyde. The brains were dissected and post-fixed in 2.5% paraformaldehyde overnight at 4 °C, and then placed in 30% phosphate-buffered sucrose solution. Coronal frozen sections (30 μm) and paraffin sections (5 μm) were cut through hippocampus. For other determinations except for immunohistochemistry, additional six rats in each group were killed and perfused with heparinized saline to remove blood. Hippocampus were isolated and stored at −80 °C.

2.4. Morris water maze

A new batch of rats (n = 12 for each group) were used for Morris water maze (MWM) test. This experiment was conducted in a closed, quiet, light and temperature-controlled room during the light phase of light-dark cycle of the rats. Rats were transferred to this room to acclimate the conditions the day before test. The device was a 200 cm diameter black pool with 30 cm depth water at 24 ± 1 °C, in which water was premixed with black non-toxic dye. Pool was divided into four quadrants, one of which had a 12-cm diameter transparent platform that was submerged 2 cm below the surface of the water. This quadrant was the target quadrant.