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Neuronal damage, central cholinergic dysfunction and oxidative damage correlate with cognitive deficits in rats with chronic cerebral hypoperfusion

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ABSTRACT

Chronic cerebral hypoperfusion has been identified to be a risk factor for cognitive decline in aging, vascular dementia, and Alzheimer's disease. Substantial evidence has shown that chronic cerebral hypoperfusion may cause cognitive impairment, but the underlying neurobiological mechanism is poorly understood so far. In this study, we used a rat model of chronic cerebral hypoperfusion by permanent bilateral common carotid artery occlusion (BCCAO) to investigate the alterations of neuronal damage, glial activation oxidative stress and central cholinergic dysfunction, and their causal relationship with the cognitive deficits induced by chronic cerebral hypoperfusion. We found that BCCAO rats exhibited spatial learning and memory impairments and working memory dysfunction 12 weeks after BCCAO compared with sham-operated rats, simultaneously accompanied by significantly increased neuronal damage and glial cell activation in the cerebral cortex and hippocampus. Twelve weeks of BCCAO treatment in rats resulted in central cholinergic dysfunction and increased oxidative damage compared with sham-operated rats. Correlational analyses revealed that spatial learning and memory impairments and working memory dysfunction were significantly correlated with the measures of neuronal damage, central cholinergic dysfunction and oxidative damage in the cerebral cortex and hippocampus of rats with BCCAO. Moreover, the measures of neuronal damage and central cholinergic dysfunction were significantly correlated with the indexes of oxidative damage in rats with BCCAO. Collectively, this study provides novel evidence that neuronal damage and central cholinergic dysfunction is likely due to increased oxidative stress under the condition of chronic cerebral hypoperfusion. Furthermore, the results of the present study suggest that neuronal damage, central cholinergic dysfunction and oxidative damage in the brain following the reduction of cerebral blood flow could be involved in cognitive deficits induced by chronic cerebral hypoperfusion.

1. Introduction

Chronic cerebral hypoperfusion is a common event in elderly people by advanced age. Considerable evidence indicates that a persistent reduction in cerebral blood flow (CBF) may compromise the disorder of learning and memory processes, which contributes to the development and progression of dementia (Farkas, Luiten, & Bari, 2007; Liu & Zhang, 2012; Zlokovic, 2011). The epidemiologic studies have identified that cerebral hypoperfusion as a vascular risk factor links vascular dementia (VD) and Alzheimer's disease (AD), the major two forms of dementia prevalent in elderly population (Breteler, 2000; Cankurtaran et al., 2008; Jhoo et al., 2008; Sekita et al., 2010).

Increasing studies have shown that stroke increases the risk of cognitive impairment and dementia, and may contribute to the progression of VD and AD (Kalaria, Akinyemi, & Ihara, 2012; Kim et al., 2012). Apart from clinical stroke itself, cerebral hypoperfusion is associated with the development of cognitive impairment and dementia (Austin et al., 2011; Kalaria et al., 2012; Kim et al., 2012). The decreased CBF, particularly in the temporal and parietal cortices, is found in subjects with AD, and its association with the severity of AD has been firmly established (Alegret et al., 2010; de la Torre, 2002; Farkas & Luiten, 2001; Kume et al., 2011; Matsuda, 2001; Mazza, Marano, Traversi, Bria, & Mazza, 2011). Additionally,

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the CBF reduction according to the severity of dementia is noted in VD patients (Kato et al., 2008; Shim, Yang, Kim, Shon, & Chung, 2006; Yang et al., 2002). Cerebral hypoperfusion is also observed in subjects with mild cognitive impairment (MCI), and its degree has been suggested as a predictive marker for the progression to AD (Borroni et al., 2006; Hanyu et al., 2010; Hirao et al., 2005). Overall, emerging evidence has shown that chronic cerebral hypoperfusion during old age may lead to cognitive decline and contribute to the development of VD and AD (de la Torre, 2004; Kalaria et al., 2012; Humpel & Marksteiner, 2005). However, the neurobiological mechanisms underlying the cognitive impairments caused by chronic cerebral hypoperfusion have not been well understood so far. A further insight into the neurobiological basis of cognitive deficits induced by chronic cerebral hypoperfusion will help to identify potential targets for effective therapies.

The rat model of chronic cerebral hypoperfusion by permanent bilateral common carotid artery occlusion (BCCAO) is a well-established model of human vascular disease-related dementia (Farkas et al., 2007; Jiwa, Garrard, & Hainsworth, 2010). The temporal evolution of regional cerebral blood flow (CBF) reduction in selected brain structures has been measured at a number of time points after BCCAO in rats, ranging between 2.5 h and 6 months (Choy et al., 2006; Farkas et al., 2007). In the acute phase after the start of BCCAO in rats, the CBF drops sharply and reduces to 35–45% of the control level in the cerebral cortex and to 60% of the control level in the hippocampus (Otori et al., 2003; Ulrich, Kroppenstedt, Heimann, & Kempski, 1998). From week 1 after BCCAO onset, the CBF values start to gradually increase in the cerebral cortex and hippocampus, but remain significantly lower levels of the control and reach the baseline at 6 months (Choy et al., 2006; Otori et al., 2003; Tomimoto et al., 2003). Accordingly, previous studies using this model have reported that BCCAO in rats produces long-lasting CBF reduction, which can result in cognitive impairments using eight-arm radial maze task (Sopala & Danysz, 2001), Morris water maze task (Cechetti, Pagnussat et al., 2012; Cechetti, Worm et al., 2012; Peng et al., 2007) and object recognition task (Cechetti, Pagnussat et al., 2012; Tanaka et al., 1998), as well as neuronal damage (Cechetti, Pagnussat et al., 2012; Peng et al., 2007; Sayan-Ozacmak, Ozacmak, Barut, & Jakubowska-Dogru, 2012), white matter lesions (Farkas et al., 2004; Peng et al., 2007; Tomimoto et al., 2003; Wakita, Tomimoto, Akiguchi, & Kimura, 1994), axonal damage (Tomimoto et al., 2003; Wakita et al., 1994, 2002), glial activation (Cechetti, Pagnussat et al., 2012; Farkas et al., 2004; Peng et al., 2007; Wakita et al., 1994; Zhang, Deng et al., 2011), oxidative damage (Kasparová et al., 2005; Peng et al., 2007; Sayan-Ozacmak et al., 2012; Zhang, Deng et al., 2011), and cholinergic dysfunction (Peng et al., 2007; Tanaka, Ogawa, Asanuma, Kondo, & Nomura, 1996; Xiong, Zhang, Sun, & Liu, 2011). The pathological changes in this model imitate many features of human VD (Du et al., 2013; Farkas et al., 2007; Jiwa et al., 2010). In addition, several recent studies have shown that chronic cerebral hypoperfusion may accelerate cerebral amyloid pathology in mice (Garcia-Alloza et al., 2011; Okamoto et al., 2012; Pimentel-Coelho, Michaud, & Rivest, 2013) and in rats (Choi et al., 2011; Liu, Xing, Wang, Liu, & Li, 2012; Wang, Xing et al., 2010) as well as enhance tau hyperphosphorylation in rats (Yao, Zhang, & Xie, 2012). Therefore, the BCCAO rats are widely accepted as a well-characterized experimental model of cerebral hypoperfusion to explore the cerebral hypoperfusion-induced cognitive dysfunction, metabolic changes, neuropathological changes, and the causal relationships between these factors (Farkas et al., 2007; Jiwa et al., 2010; Wang, Zhang et al., 2010; Wang, Xing et al., 2010).

In the present study, we employed BCCAO rats to examine the cognitive function, neuronal damage, glial cell activation, oxidative stress and central cholinergic function, and sought to determine the correlates of cognitive deficits induced by chronic cerebral hypoperfusion.

2. Material and methods

2.1. Animals

Adult male Sprague–Dawley rats (aged 6 months; weighing 380 ± 20 g) used in this study were obtained from Laboratory Animal Center of Fourth Military Medical University (Xi'an, China). The rats were housed in individual cages and maintained in temperature- and humidity-controlled rooms with *ad libitum* access to food and water throughout the experimental period. All procedures were in accordance with the University Policies on the Use and Care of Animals and were approved by the Institutional Animal Experiment Committee of Fourth Military Medical University, China.

2.2. Surgery

The surgical procedure was performed as described previously (Cechetti, Pagnussat et al., 2012; de la Torre & Aliev, 2005; Du et al., 2013; Liu et al., 2012; Wang, Zhang et al., 2010; Wang, Xing et al., 2010). In brief, after rats were anesthetized with intraperitoneal administration of sodium pentobarbital (100 mg/kg), a midline cervical incision was made and then both common carotid arteries were exposed and gently separated from carotid sheath and vagus nerve. In rats chosen randomly for cerebral hypoperfusion group ($n = 10$), each common carotid artery was ligated with a 4-0 type surgical silk suture. As sham-operated control group ($n = 10$), the animals received the same operation without artery ligation. During the surgical procedure, the rats were put on a heating pad to maintain body temperature at 37.5 ± 0.5 °C and kept on it until recovery from anesthesia.

2.3. Morris water maze test

Twelve weeks after the surgery, the hippocampus-dependent spatial learning and memory of all rats were evaluated by the Morris water maze test as described previously (Cechetti, Pagnussat et al., 2012; Du et al., 2013; Wang, Zhang et al., 2010; Wang, Xing et al., 2010). The apparatus consisted of a circular water tank (120 cm in diameter and 50 cm high) and filled to a depth of 30 cm with water at 23 ± 1 °C. Water was made opaque by the addition of skimmed milk. The pool was divided into four equal quadrants: northeast (NE), northwest (NW), southeast (SE), and southwest (SW).

During the acquisition training period of the task, the translucent acrylic platform (10 cm in diameter) was located in the center of the northeast quadrant and submerged 1.5 cm below the water surface throughout training. The rats were subjected to four trials per day for 5 consecutive days. In each of the four trials, the rats were gently released into the water by facing the tank wall at four different starting positions equally spaced around the perimeter of the pool. The rats were given a maximum of 120 s to find the hidden platform. On reaching the platform, the rats were allowed to stay on it for 20 s. If the rat failed to find the platform within 120 s, the training was terminated and a maximum score of 120 s was assigned. The rat was then guided to the hidden platform by hand and allowed to stay on the platform for 20 s. The latency to escape onto the hidden platform was recorded as the performance of spatial learning.

To assess the spatial memory, a probe trial was performed 24 h after the last training trial. In this trial, the platform was removed from the tank, and the rats were allowed to swim freely for 60 s in the pool before they were removed from water by hand. The time that an individual rat spent in the target quadrant previously containing the platform was recorded as a measure of spatial memory.