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A modified bilateral carotid artery stenosis procedure to develop a chronic cerebral hypoperfusion rat model with an increased survival rate

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HIGHLIGHTS

- Bilateral carotid artery occlusion results in substantial mortality in rats.
- Modifications prolong the experimental period or require additional materials.
- Approximately 89 ± 8% stenosis of both carotid arteries was established with a 32G needle.
- This method produced a chronic cerebral hypoperfusion model with an increased survival rate.

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ABSTRACT

Background: Bilateral carotid artery occlusion (2-vessel occlusion, 2VO) in rats is a classic and frequently used approach to develop an animal model of chronic cerebral hypoperfusion. However, this method results in substantial mortality in rats.
New method: We investigated whether a modified 2VO procedure, which induces bilateral carotid artery stenosis via ligation of each bilateral common carotid artery (CCA) with a 32 gauge (G) needle followed by needle removal, could produce a chronic cerebral hypoperfusion rat model with an increased survival rate. Sprague-Dawley (SD) rats were treated with the standard or modified 2VO procedure, and changes in cerebral blood flow (CBF) and survival rates were determined. On day 28, cognitive function was assessed with the Morris Water Maze (MWM) test, and neuronal survival and degeneration within the hippocampal CA1 area were measured. Damage to the white matter (WM) within the corpus striatum was assessed via Luxol fast blue (LFB) staining and analyses analyzing the levels of the myelin basic protein (MBP) protein levels.
Results: The modified 2VO procedure induced similar cognitive impairments, hippocampal lesions and WM damage compared with the standard 2VO procedure in rats; however, it had an increased survival rate.
Comparison with existing methods This novel method can be used to quickly and effectively establish a chronic cerebral hypoperfusion rat model with common materials and an improved survival rate.
Conclusion: Bilateral carotid artery stenosis using a 32G needle is a useful and reliable method to develop a rat model of chronic cerebral hypoperfusion with increased survival.

1. Introduction

Chronic cerebral hypoperfusion contributes to the cognitive Q3 deficiency present in patients with vascular dementia, Alzheimer's disease and aging (Iadecola, 2013; Baskys and Cheng, 2012; Zhao and Gong, 2015). Bilateral carotid artery occlusion (2-vessel occlusion, 2VO) in Sprague-Dawley (SD) rats is a classic and frequently used approach to establish an animal model of chronic cerebral

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hypoperfusion (Jiwa et al., 2010). The well-developed circle of Willis in SD rats provides compensatory blood flow from the vertebral arteries to the regions that would typically be supplied by the occluded carotid arteries; thus, the 2VO procedure causes global cerebral hypoperfusion rather than serious stroke (Jiwa et al., 2010; Farkas et al., 2007). There are three phases that follow the onset of 2VO with regard to changes in cerebral blood flow (CBF) and the metabolic or homeostatic state of the brain tissue. The CBF dramatically decreases in the acute phase, which lasts for 2–3 days; the chronic hypoperfusion phase subsequently follows for 8–12 weeks (Parra et al., 2005). The CBF of 2VO rats is very low in the chronic phase, especially in the hippocampus (~60% of the control level) and white matter (WM) areas (35–45% of the control level), which creates moderate hypoxic–ischemic conditions that closely resemble the reduced CBF in human dementia (Farkas et al., 2007).

However, the abrupt decrease in CBF during the acute phase after the 2VO procedure causes substantial mortality rates in rats (50–60%) (Wang et al., 2014; Shibata et al., 2004). Many modified 2VO protocols have been explored to minimize the number of animals used in research by alleviating the sudden reduction of CBF. Cechetti et al. (2010, 2012) demonstrated that rats that had two common carotid arteries (CCAs) occluded separately with a 1-week interval exhibited similar behavioral and morphological outcomes but an increased survival rate (90%) compared with rats treated with a standard 2VO procedure. Shibata et al. demonstrated that the bilateral placement of microcoils with an inner diameter of 0.18 mm on the CCAs in mice was an effective method to establish a chronic cerebral hypoperfusion model (Shibata et al., 2004). This novel method caused appropriate WM lesions and a significantly lower mortality rate (15%) compared with the 0.16 mm group (75%) (Shibata et al., 2004). However, these modified procedures prolong the experimental period or require additional materials.

Artery stenosis via arterial ligation with a syringe needle followed by needle removal is widely used in the establishment of heart failure models (Hara et al., 2002; Yaoita et al., 2002). This method preserves portions of the blood flow and causes different degrees of artery stenosis using different gauge (G) needles. A 32G needle, which has an outer diameter of 0.23 mm, is a common fine needle widely used in insulin injection. To identify a quick and effective method to establish a chronic cerebral hypoperfusion rat model with improved survival rates, we hypothesized that bilateral carotid artery stenosis using a 32G needle could induce chronic cerebral hypoperfusion in SD rats with increased survival.

2. Materials and methods

2.1. Animals and ethics statement

Male adult SD rats aged 11–12 weeks (260–300 g) were purchased from the Animal Experimental Center of Zhengzhou University. All rats were maintained on a 12-h light/dark cycle at a constant temperature of 22 ± 1 °C; the rats were housed in plastic cages (6 rats per cage) with free access to food and water. All protocols were approved by the Animal Care and Use Committee of Zhengzhou University. All efforts were made to minimize the number of animals used and their suffering.

2.2. Modified 2VO procedure and groups

The rats were anesthetized with chloral hydrate (400 mg/kg) via an intraperitoneal injection. A neck ventral midline incision was performed, and the CCAs were carefully separated from their sheaths. The rats assigned to the standard 2VO procedure (2VO group, $n = 30$) (Jia et al., 2012) had both CCAs permanently occluded with 5–0 silk suture. In the modified protocol (modified 2VO group, $n = 33$), both CCAs were gently banded with a 5–0 silk suture tied around a blunt 32G needle; the needle was then quickly withdrawn. The sham-operated controls (sham group, $n = 21$) received the same surgical procedures without carotid artery ligation. Three rats each from the sham and modified 2VO groups were used to determine the severity of CCA stenosis induced by the modified 2VO procedure one hour after surgery. The survival rates of each group over the course of the experiment were recorded. Body weights were measured weekly after surgery and expressed as a percentage change as follows: (body weight at each time point-body weight before surgery)/body weight before surgery × 100%.

2.3. CBF measurement

Six rats per group were used to determine the CBF of the frontal cortices after surgery using laser-Doppler flowmetry as previously described (Shibata et al., 2004; Sutherland et al., 2014). The rats were anesthetized with chloral hydrate (400 mg/kg) via an intraperitoneal injection, and the skin overlying the right skull was reflected. The skull was thinned at a point 2.5 mm posterior and 2.5 mm lateral to the bregma using a microdrill until only a small translucent sheet of bone remained. A plastic guide cannula for laser-Doppler flowmetry was subsequently fixed perpendicular to the thinned skull using dental resin. The CBF before and 1, 3, 7, 14, and 28 days after the surgery were recorded for each rat by placing a probe through the guide cannula. The mean CBF values were expressed as a percentage of the baseline value.

2.4. Morris water maze (MWM) test

Ten rats per group were assessed for cognitive impairment via the MWM test on day 28 as previously described (Cechetti et al., 2012). A Water Maze automatic control recorder (using Shuo Ling Yuan-Water Maze System, SLY-WMS) with a black circular pool (150 cm in diameter, 50 cm in height) was filled with water (23–24 °C, 28 cm in depth; Beijing Sunny Instruments Co. Ltd, Beijing, China). An escape platform 12 cm in diameter was fixed in the middle of the northeast quadrant and placed 2 cm below the water surface. In the reference memory protocol, the rats received five training sessions (one session per day), which each consisted of three trials in 15-min intervals. Each rat was placed in the pool and released facing the side wall at one of three randomly chosen starting positions, which were not repeated. The rat was allowed to swim until it found the hidden platform. If it did not succeed within 120 s, the rat was guided to the platform and was allowed to remain for 10 s; its escape latency was recorded as 120 s. The mean escape latency and swimming speed of the daily trials were recorded. On the sixth day, a probe trial, which consisted of one 2-min trial with the platform removed, was conducted. The mean time that each rat spent swimming in the northeast quadrant and the number of times the animal crossed the annulus were recorded.

2.5. Histological evaluation of hippocampal damage

Six rats per group were used to identify the histological changes in the hippocampus on the day after the MWM test (day 34). The rats were deeply anesthetized with an overdose of 10% chloral hydrate and transcardially perfused with 0.01 mol/L phosphate buffered saline (PBS) followed by 4% paraformaldehyde in 0.01 mol/L PBS (pH 7.4). The brains were removed, post-fixed and stored in 30% sucrose/0.01 mol/L PBS until the tissues sank and were subsequently cut into 15-μm sections with a cryostat microtome (CM1100, Leica Biosystems, Germany). Sections of the dorsal hippocampal area (bregma, −2.8 to −3.2 mm; internal sections, 50 μm) were used for cresyl violet staining, Fluoro-Jade B (FJB) staining and the assessment of WM damage.

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