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Motor and cognitive impairment in a mouse model of ischemic carotid artery disease

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HIGHLIGHTS

- This mouse model replicates outcomes following human carotid artery disease.
- Affected mice develop motor incoordination and weakness.
- Affected mice develop spatial working memory impairment.
- Affected mice develop spatial reference memory impairment.
- This mouse model is useful to explore new treatments for carotid artery disease.

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ABSTRACT

We have recently established a novel mouse model of bilateral common carotid artery gradual occlusion. This model serves as a mimic of severe carotid artery disease with multiple cerebral infarctions induced by cerebrovascular insufficiency. In this study, we examined whether locomotor and cognitive impairment was induced in these mice using a test battery for neurological and cognitive functions. Adult C57BL/6J male mice were subjected to either ameroid constrictor (AC) placement to gradually narrow the bilateral common carotid arteries or to sham surgery. At 28 days post-surgery, locomotor activity was assessed by rotarod and wire hang tests, and cognitive function was assessed using the Y-maze and Morris water maze tests. Rotarod and wire hang tests showed a significantly shorter latency to fall in mice subjected to the placement of ACs compared with sham surgery mice. AC-implanted mice showed significant impairments in working memory on the Y-maze test and in spatial learning and reference memory on the Morris water maze test. Therefore, the current mouse model with AC placement on the bilateral common carotid arteries showed locomotor disability, learning deficits, and memory impairment, which well-replicated the outcomes of patients with ischemic carotid artery disease. This model will be useful for investigating the mechanisms underlying the neurological and cognitive deficits following cerebrovascular insufficiency and for exploring pharmacological interventions for stroke patients with severe carotid artery disease.

1. Introduction

Carotid artery occlusive disease gradually develops over time, eventually leading to cerebral infarction [18] and cognitive impairment [2]. Cerebral infarction causes serious damage that requires rehabilitation and leaves many patients bedridden. The number of patients with carotid artery disease has increased globally because of excess caloric intake [5,9,12]. Among them, patients with bilateral carotid artery occlusion have a particularly poor prognosis

Abbreviations: AC, ameroid constrictor; CCA, common carotid artery; CBF, cerebral blood flow; TIA, transient ischemic attack.

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because of the high incidence of subsequent symptomatic stroke [16]. Therefore, developing novel treatments for carotid artery disease is an urgent task. We have recently established a novel mouse model of bilateral common carotid artery (CCA) gradual occlusion as a mimic of severe carotid artery disease in which multiple cerebral infarctions were induced as a result of cerebrovascular insufficiency [11].

The mouse model is generated by implanting ameroid constrictors (ACs) on the bilateral CCAs, gradually decreasing cerebral blood flow. Multiple cerebral infarctions subsequently develop in the gray and white matter in three-quarters of the animals. As in the case in humans, we hypothesized that cerebral infarctions distal to carotid artery disease could cause neurological and cognitive impairment. To address this question, we studied the behavior of affected mice using a test battery for neurological and cognitive functions. If behavioral phenotypes are recapitulated, this mouse model would further help to investigate the effects of cerebrovascular insufficiency and explore treatments for human carotid artery disease.

2. Materials and methods

2.1. Experimental protocol

Male C57BL/6J mice of 10–12 weeks of age (CLEA Japan, Tokyo) were assigned into the following two groups: (1) sham surgery group ($n = 15$), and (2) AC-implanted group ($n = 29$). Male C57BL/6J male mice aged 1 year old (CLEA Japan, Tokyo) were also used and assigned into the following two groups: (1) sham surgery group ($n = 10$), and (2) AC-implanted group ($n = 10$). After sham surgery or the implantation of AC to each group of mice, neurological and cognitive assessments were made 28 days after surgery in younger mice. Cerebral blood flow (CBF) was assessed by laser speckle flowmetry pre- and post-surgery in both younger and older mice. All mice were housed in a room with a 12-h light/dark cycle (lights on at 7 am) and were given access to food and water ad libitum. We monitored the condition of the animals daily until the 14th day after surgery, after which conditions were monitored twice weekly. All procedures in this study were conducted in strict accordance with the guidelines for animal experimentation from the Animal Research Committee of National Cerebral and Cardiovascular Center. Surgeries were performed under anesthesia, and all efforts were made to minimize suffering.

2.2. Ameroid constrictor (AC)

AC consists of a titanium casing surrounding a hygroscopic casein material with an internal lumen (Tokyo Instruments, Tokyo). The casein component gradually absorbs water and consequently swells, leading to predictable narrowing and occlusion of the arterial lumen it encases. The inner and outer diameters were 0.5 and 3.25 mm, respectively, and the length was 1.28 mm [15].

2.3. Surgical procedure

Under anesthesia with 1.5% isoflurane, the surgery was conducted after confirming the mice were completely static and unresponsive to a toe pinch. Both CCAs were exposed through midline cervical incision and freed from their sheaths. For mice in the AC-implanted group, ACs were applied to the bilateral CCAs. The rectal temperature was maintained between 36.5 °C and 37.5 °C using a self-regulating heating pad.

2.4. Rotarod test

The rotarod test was performed at 28 days after surgery by placing the mouse on a rotating drum (Muromachi Kikai, Tokyo) and measuring the time the mouse maintained its balance on the rod. The speed of the rotarod was accelerated from 4 to 40 rpm over a 5-min period. This test was repeated five times with an interval of 5 min between attempts [20].

2.5. Wire hang test

The wire hang test was performed at 28 days after surgery. A metallic wire (2 mm × 60 cm) was secured to the top of a transparent, rectangular, open-topped plastic box (30 cm height × 60 cm width × 40 cm length). The wire was tightly fixed to the top of the tank to avoid vibration, which could interfere with the performance of mouse. The mice were placed on the metallic wire and latency to fall was recorded. This was repeated five times with an interval of 5 min between attempts. The cutoff time was set at 60 s. Mean latency to fall was determined [20].

2.6. Y-maze test

The Y-maze test was conducted during the dark period (7–11 pm) at 28 days after surgery. This task is based on spontaneous alternation behavior and is used to measure spatial working memory. The maze consists of 3 arms (each 40 cm long, 9.5 cm high, and 4 cm wide), labeled A, B, or C, diverging at 120° angles from a central point. The experiments were performed in a dimly illuminated room. After each mouse was tested, the floor of the maze was cleaned using super hypochlorous water-soaked paper for the discrimination of smell to avoid olfactory cues. Each mouse was placed at the end of the start arm and allowed to move freely through the maze during an 8-min session without reinforcers such as food, water, or electric foot shock. The sequence of arm entries was manually recorded. A mouse was considered to have entered an arm when all 4 paws were positioned in the arm runway. An alternation was defined as entry into all 3 arms on consecutive occasions (e.g., the sequence, ABCBCBCA was counted as 2 alternations, with the first consecutive ABC and the last consecutive BCA out of 6 consecutive occasions; 33% alternations). The maximum alternation was calculated as the total number of arm entries minus 2, and the percentage of alternation was calculated as (actual alternation/maximum alternation) × 100. The total number of arms entered during the sessions, which reflects spontaneous activity, was also recorded. Mice that entered arms less than eight times during the test were eliminated because their data were not considered to reflect precise alternation [21].

2.7. Morris water maze test

The Morris water maze test was performed 28 days after the implantation of AC or sham surgery as previously reported with some modifications [10]. The Morris water maze test consists of a circular pool (diameter, 120 cm; depth, 40 cm) and a set of video analysis systems (EthoVision XT5; Noldus, Wageningen, Netherlands). The pool was filled with water containing non-toxic white paint to a depth of 30 cm. A clear, circular platform (diameter, 8 cm) was submerged 1 cm below the water surface in the center of one quadrant of the pool (target quadrant). Red cross sign and blue upward arrow at the opposite side of red cross sign were used as the cues for the mice to orient for swimming in the pool. On the first 4 days, we performed four trials per day with a 5-min interval between attempts (acquisition phase). The platform was kept in the same position during the acquisition phase. The mice were placed at the starting position (the quadrant adjacent to the