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RESEARCH ARTICLE

Comparison of cognitive performance between two rat models of vascular dementia

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Background and Purpose: An ideal animal model to explore that pathogenesis and prevention of dementia is essential. The present study was designed to compare the difference of behavior and cerebral blood flow of the two vascular dementia rat models at different time intervals. *Methods*: The rats were randomly allocated to three groups: bilateral common carotid artery occlusion (BCCAO) group, thromboembolism (TE) group and sham-operated (SHAM) group. The performance in the Morris water maze (MWM) was analyzed at 7, 14 and 28 d after operation and cerebral blood flow (CBF) was analyzed at 28 days after operation. *Result*: The results showed that the two models exhibited longer latency, less times to crossing platform in MWM and lower CBF than the SHAM rats. Compared with the TE rats, the BCCAO rats have a significant prolongation of escape latency at 7 days and 28 days. In the probe trial, the BCCAO rats showed less number of times across the platform. *Conclusion*: The BCCAO rats maybe provide a more useful model to study the physiopathological mechanisms of cognitive impairment related to chronic cerebral ischemia.

KEYWORDS: bilateral common carotid artery occlusion, thromboembolism, Morris water maze, learning and memory

Introduction

In the last decades, the incidence of dementia is increasing. Vascular dementia (VD) is defined as loss of the cognitive function resulting from ischemic, hypoperfusive, or hemorrhagic brain lesions due to cerebrovascular disease or cardiovascular pathology [1]. It is the second most common cause of dementia. VD involves the impairment of memory and cognitive function following cerebrovascular disease [2]. It is not a single disease, but a group of conditions with different pathological correlates and physiopathological mechanisms [3].

To mimic such a pathological condition and explore the underlying mechanisms, several animal models of chronic cerebral hypoperfusion have been developed [4]. In these animal models, the bilateral carotid artery occlusion and the embolus injection in rat are two commonly used methods. Which model is more reliable? There is little reported in the previous literature. Therefore, we designed this experiment to compare these two models in behavior and cerebral blood flow (CBF), in order to evaluate their characteristics of cognitive impairment after ischemia.

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Materials and methods

Subjects

All procedures in the present experiment were performed in accordance with the requirements of the Provisions and General Recommendations of Chinese Experimental Animal. Adult male Wister rats (8 weeks old) weighing 280–300 g were obtained from Vital River Laboratories (Beijing, China) and maintained on a 12-h light/dark cycle with free access to food and water. Animal room was maintained at 23 ± 1°C. Rats were divided at random into the following three groups: bilateral carotid artery occlusion group (BCCAO, $n = 30$), thromboembolism group (TE, $n = 30$) and sham-operated group (SHAM, $n = 30$). After surgery, all rats were allowed to recover for 7 days.

Surgery

Bilateral carotid artery occlusion group

Rats were induced by bilateral occlusion of the common carotid arteries using the method described previously [5]. Briefly, anesthesia was induced with 3.5% chloral hydrate, 1 mL/100 g. Through a midline incision, the left and right common carotid arteries were carefully exposed. The arteries were gently isolated from the cervical sympathetic and vagal nerves. The arteries were then doubly ligated with 5–0 silk sutures. During the procedure, particular care was taken to avoid damage to the vagus nerve in any way. Forty rats were operated by BCCAO and 10 rats did not survive in the recovery period.

Thromboembolism group

Rats were induced by injecting clot described previously [6]. Thirty-six hours before the surgery, 10 mL of blood was obtained from femoral artery in one male Wistar rat and stored at 37°C incubator for clot formation. The clot was fragmented into 100–200 μm, as measured by micrometer. The rats were anesthetized with 3.5% chloral hydrate, 1 mL/100g. Through a midline incision, the bifurcation of the right common carotid and external carotid artery was exposed. A temporary clip was applied to the external carotid artery away from the bifurcation. Then, 0.3 mL of 3% clot suspension diluted with saline was injected into the internal carotid artery. In the TE group, 35 rats were operated and five rats did not survive the recovery period.

Sham-operated group

The same surgical procedure was conducted in SHAM rats in which the bilateral carotid artery was not occluded and the internal carotid artery was not injected.

Morris water maze

The timeline used in this study is presented (Figure 1). The Morris water maze (MWM) was used to test spatial memory [7], using the method described by Gerlai [8]. In a 160 cm diameter circular pool filled with 40 cm deep water (23 ± 2°C), the pool was divided into four virtual quadrants (northeast, northwest, southeast and southwest). A circular plastic platform was placed into the middle of the northeast quadrant. The top surface of the platform was 2 cm below the water. A video camera which was mounted above the center of the tank was used to record all trials.

Hidden platform trial

In order to adapt to the environment, the rats were allowed to swim a 90 s in the pool without any platform before the day of start training. During the time of the training, milk was dissolved into the water every day to make it opaque. Rats were randomly placed in four different positions (never in the northeast quadrant) and released facing the wall. Once the rat had found the platform, it was left for 10 s to orient and then was removed, dried in a towel and putted back in cages. If the platform was not found after 90 s, the rat was gently guided to it for 10 s and the escape latency (the time taken to find the hidden platform) as 90 s to record. Animals were tested in the water maze at 7 days post-surgery using a paradigm consisting of 4 trials per day over 3 consecutive days. They were also tested at 14 days post-surgery and 28 days post-surgery using the same paradigm. Escape latency and swimming speed were collected.

Probe trial

A probe test was conducted immediately after the three-day period at 28 days after surgery. The animals were allowed to swim freely for 90 s in the swimming pool which the platform was removed. The number of times across the platform was recorded.

Measurement of cerebral blood flow

A laser-Doppler flowmeter (LDF; Moor Instruments, Axminster, UK) was used for continuous CBF monitoring of the right area of the cerebral cortex supplied [9]. The rats were placed and the head fixed in a stereotactic frame. Then, an LDF probe was positioned on the position (5 mm lateral and 1 mm posterior to the bregma). In this position, a burr hole was drilled without injury to the dura mater. The CBF was continuously recorded 5 min. Measurements were made at 28 days after surgery.

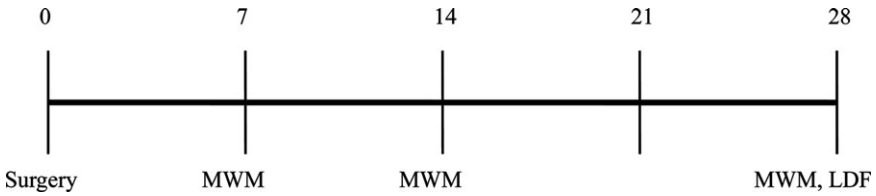

Figure 1. Timeline for testing. Numbers indicate days post-surgery. Morris water maze (MWM): the animals received 4 trials per day for 3 consecutive days at 7, 14 and 28 days after surgery. Measurement of cerebral blood flow by LDF at 28 days post-surgery.

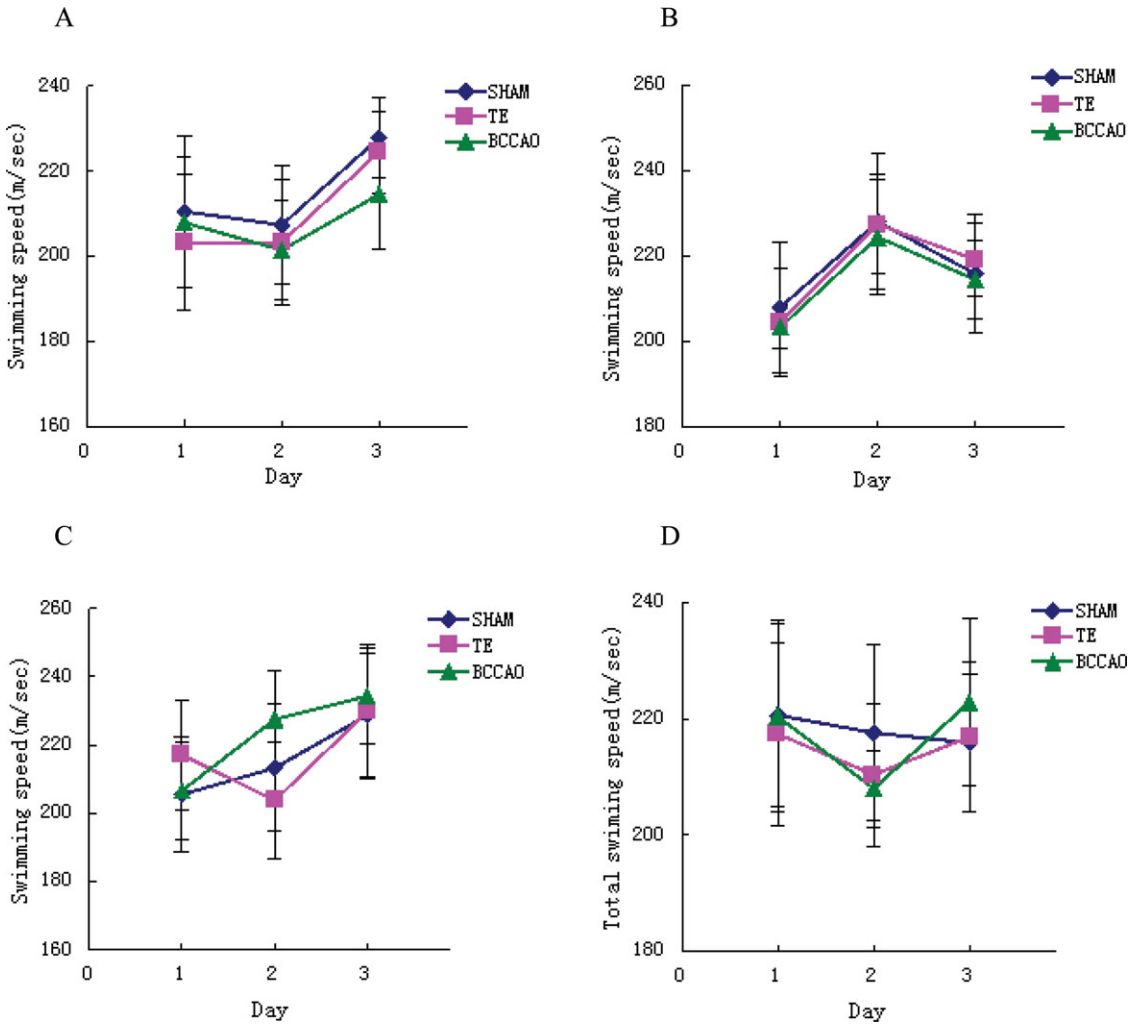

Figure 2. Swimming speed of the three groups in the Morris water maze. The values are presented as means ± SEM. (A) Swimming speed of the three groups at 7 days after surgery. (B) Swimming speed of the three groups at 14 days after surgery. (C) The swimming speed of the three groups at 28 days after surgery. (D) Total swimming speed of the three groups at 7, 14 and 28 days after surgery.

Data analysis

All statistical analysis was performed with SPSS software (version 16.0). Escape latency was analyzed by two-way analysis of variance with repeated measures. Other data were analyzed by one-way analysis of variance. All results were shown as means ± SEM. In all statistical comparison, $p < 0.05$ was used as significant.

Results

Morris water maze

Hidden platform trial

In MWM test, with the prolongation of training, the escape latency became shortened in each group. The swimming speed was not significantly different in any group of animals (Figure 2). Compared with the SHAM rats, there is a significant prolongation of escape latency in BCCAO rats and TE rats ($p < 0.05$). The rats from the BCCAO group showed longer escape latency than did the TE rats ($p < 0.05$) at 7 days (Figure 3A) and 28 days (Figure 3C) post-surgery. The BCCAO rats and the TE rats presented a longer latency to find the platform than the SHAM rats ($p < 0.05$). However, there is no significant difference in the escape latency time between the BCCAO rats and TE rats ($p > 0.05$) at 14 days after surgery (Figure 3B). During all testing period, BCCAO rats and TE rats took a longer time to find the platform than did the SHAM rats ($p < 0.05$). The total escape latency of the BCCAO rats increased compared with the TE rats ($p < 0.05$) (Figure 3D).

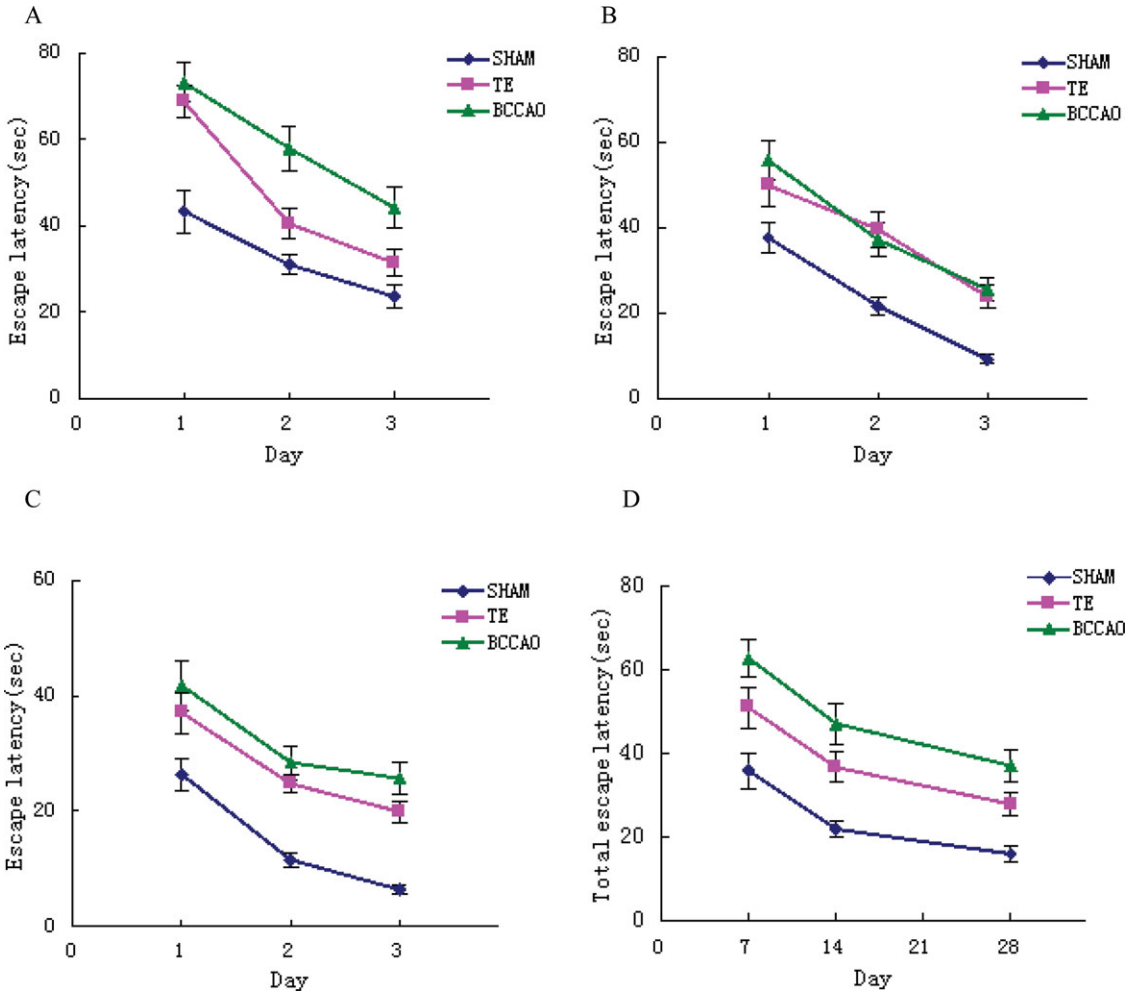

Figure 3. Escape latency of the three groups in the Morris water maze. The values are presented as means ± SEM. (A) Escape latency of the three groups at 7 days after surgery. (B) Escape latency of the three groups at 14 days after surgery. (C) The escape latency of the three groups at 28 days after surgery. (D) Total escape latency of the three groups at 7, 14 and 28 days after surgery.

Probe trial

In the probe test, the results are shown at 28 days (Figure 4): the number of times across platforms of the BCCAO rats and TE rats at 28 days were significantly lower than that of the SHAM rats ($p < 0.05$). The BCCAO rats showed less number of times across the platform than did the TE rats ($p < 0.05$).

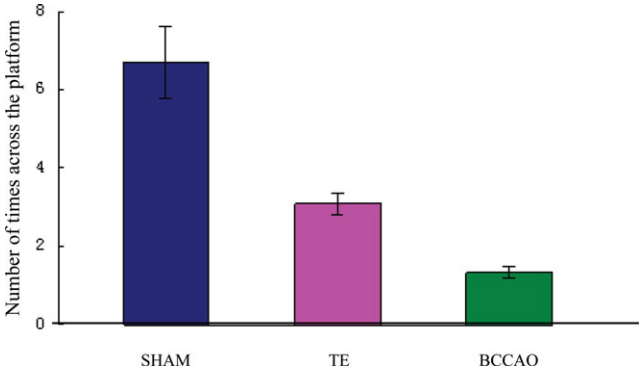

Figure 4. The number of times across the platform in the probe test at 28 days after surgery. The data are means ± SEM.

Measurement of cerebral blood flow

Compared with the SHAM rats, there is a significant decrease in CBF of the BCCAO rats and the TE rats ($p < 0.05$). The BCCAO rats showed significant decrease in CBF compared to the TE rats ($p < 0.05$; Figure 5).

Discussion

In VD, cerebrovascular insufficiency and ischemic injury are believed to cause the brain dysfunction that underlies the dementia [10]. Chronic ischemia is a

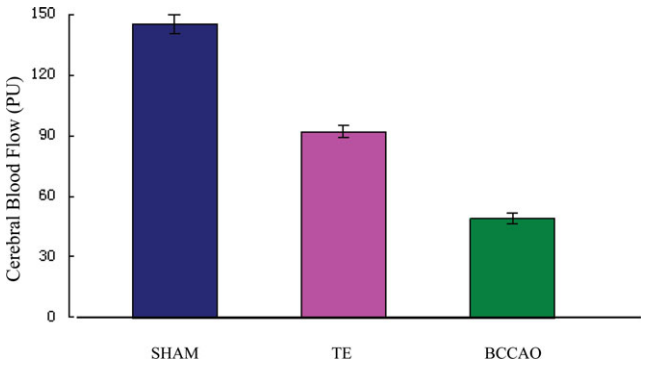

Figure 5. Measurement of cerebral blood flow at 28 days after surgery. Results are expressed as mean ± SEM.

progressive, dynamic process caused by cerebral hypoperfusion (CH) that may manifest with cognitive dysfunction asischemic conditions persist and ultimately leads to neuronal death [11]. VD and Alzheimer's disease are in fact frequently associated with a common pathophysiological state of chronic CH [12]. CH induces microvascular changes that could contribute to the progression of vascular cognitive impairment and dementia in the aging brain [13]. Animal models may therefore provide a better medium for examining the effects of vascular damage on cognition [14]. The use of appropriate animal models is essential to investigate mechanisms, prevention, and treatment of VD. Currently, many animal models of VD have been established. But most of those models produced acute single or multiple infarctions. They may be suitable to be used for studying transient ischemic.

The BCCAO model gives rise to ischemic cell change within selectively vulnerable structures, including the CA1 pyramidal neurons of the hippocampus, caudoputamen, and neocortex [15]. Cognitive deficits have been found to be associated with damage in the CA1 region of the hippocampus in studies using the BCCAO model [16]. Impaired learning and memory were apparent in MWM tasks at 7 days post-surgery [17]. Some hippocampal changes appeared, with increased astrocyte density and cell loss in the CA1 area [18]. According to previous studies, the changes caused by BCCAO are stable only at certain times. The percent reduction of local cerebral blood flow was between 25% and 94% of the control at 2.5 h after BCCAO [19]. The CBF values had already started to gradually recover at 1 week, but were still significantly lower than the control values 4 weeks after BCCAO. Between 8 weeks and 3 months, CBF returned to near to control levels [20–21]. In this experiment, we found that both the BCCAO model rats and the TE model rats showed the damage of learning and memory 4 weeks. BCCAO rats have a more stable and sustainable impairment than TE rats in learning and memory. We observed these changes only 4 weeks. Although, compared with the TE rats, the level of CBF was significantly reduced in BCCAO rats at 4 weeks, we were not sure these changes between 4 weeks and 3 months. Moreover, the reduction in blood flow is very similar to what is seen in patients with early-stage VD [22,23]. To summarize, the BCCAO model requires a simple surgical preparation and can be readily accomplished. It produces a chronic, global hypoperfusion state. Therefore, this model is easily suitable for chronic survival studies.

Embolic insults can produce multiple, ischemic foci [24]. Small infarcts [25] or a more-extensive territorial infarct [26] were seen. Animals with microinfarcts showed modest impairment in Barnes maze learning [27]. MWM learning was impaired acutely (2 days post-injury) but recovered by 5 weeks [28]. In this study, compared with the SHAM rats, the TE rats were demonstrated the cognitive impairment in MWM at 7 days, 14 days and 28 days after surgery and the decline in CBF at 28 days post-surgery. In addition, the location of the embolic clots was variable [29]. These scattered, focal lesions result in impaired learning and memory, with damage in corpus callosum, striatum, and hippocampus. The ischemic insult may be more severe in the basal ganglia [30]. Depending on the size of individual lesions, and the vascular territory affected (cortical, subcortical, or both) the resulting injury resembles lacunar state or multi-infarct vascular cognitive impairment. More severe embolization leads to a fused, ischemic lesion, resembling large artery stroke [31].

The two common VD models were studies in the previous papers. However, the first time we made a comparison of changes in CBF and memory impairment by the two models in the same period of time. In conclusion, our study shows that the BCCAO model rats and the TE model rats showed the damage of learning and memory. Furthermore, the BCCAO rats maybe provide a more stable and reliable model to study the physiopathological mechanisms of cognitive impairment related to chronic cerebral ischemia.

Declaration of Interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of this paper.

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