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Research report

Cognitive impairment and persistent anxiety-related responses following bilateral common carotid artery occlusion in mice

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HIGHLIGHTS

- BCCAO induces memory impairments and anxiety-related behaviors in Swiss mice.
- BCCAO promotes CA1–CA4 hippocampal neurodegeneration and decreases hippocampal neurogenesis.
- The behavioral and neurohistological changes induced by BCCAO are time-dependent.

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ABSTRACT

The present study examined the behavioral and neurohistological changes induced by the bilateral common carotid artery occlusion (BCCAO) model of brain ischemia in Swiss mice. The post-ischemic behavioral effects of 17 min BCCAO were recorded 7, 14, and 28 days after reperfusion in the Morris water maze, open field, and elevated plus maze to assess spatial learning and memory, general locomotor activity, and levels of anxiety-like behavior, respectively. After behavioral testing, the brains were removed and processed to evaluate hippocampal neurodegeneration using Nissl staining and Fluoro-Jade C histochemistry and hippocampal neurogenesis using doublecortin immunohistochemistry. BCCAO induced memory impairment 7 and 14 days after reperfusion, with apparent functional recovery 28 days later. Anxiety-related behaviors remained elevated in ischemic compared to sham mice tested 28 days after reperfusion. Hippocampal neurodegeneration was detected in all hippocampal subfields (CA1–CA4) from day 7 to day 28. Decreased hippocampal neurogenesis was observed 14 and 28 days after BCCAO. The effects of BCCAO on spatial memory were transient, whereas anxiety-like behavior was persistent and might be related to CA3 hippocampal injury induced by BCCAO in mice.

1. Introduction

Transient global ischemia occurs in patients who suffer from cardiac arrest or shock or undergo complex cardiac surgery, resulting in a broad range of neurological and emotional dysfunction [1–3]. Cognitive impairments are among the more fully characterized neurobehavioral sequelae following brain ischemia, with memory being the most affected domain, followed by attention and executive function [3–7]. *Post mortem* studies have suggested that hippocampal damage may be a key factor associated with cognitive impairment after ischemic events [4,8]. Although relatively neglected, depressive symptoms [2], persistent anxiety [6,9,10], increased social isolation [1], and increased risk for posttraumatic stress disorder [11] have also been reported after transient brain ischemia. These sequelae affect perceived improvements in health and social functioning [6].

Bilateral occlusion of the common carotid arteries (BCCAO) has been used as a model of transient global brain ischemia in mice [12–17]. The main findings of this model include CA1 hippocampal damage associated with impaired memory function [13,14,18,19]. The magnitude and type of cognitive deficits, however, vary considerably, depending on the behavioral test, duration of occlusion, and survival time following reperfusion [20,21]. Differences have also been found in the patterns of brain damage following BCCAO, which has been associated with individual differences in collateral blood flow through the circle of Willis among different strains or even in the same strain [12,22–24]. All of these differences represent an important source of variability and may influence the outcome of BCCAO in mice.

Functional outcome represents an essential component of the preclinical testing of drugs that target the acute (neuroprotection)

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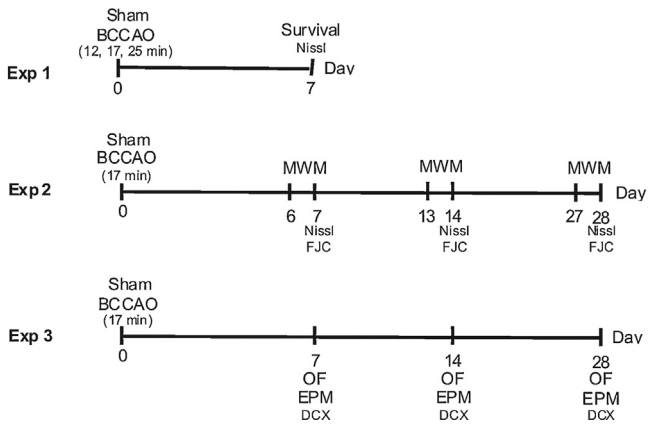

Fig. 1. Experimental design. BCCAO = bilateral common carotid arteries occlusion, Morris water maze = MWM, open field = OF, elevated plus maze = EPM, DCX = doublecortin.

and chronic (repair and recovery) stages of brain ischemia [25]. With regard to emotional behavior, only few and inconclusive studies have addressed the effects of BCCAO on anxiety-related behavior in mice, and such studies have also been performed during the early phase of brain ischemia and reperfusion. For example, BCCAO mice presented anxiogenic-like behavior in the elevated plus maze (EPM) and social interaction test 2 and 7 days after reperfusion [26,27]. Anxiolytic-like effects were detected in the light-dark box 3 and 4 days after BCCAO [18].

Hence the need to characterize functional and cellular outcome of brain ischemia in mice, the present study examined the neurohistological and behavioral changes induced by BCCAO in Swiss mice to determine whether it is a suitable model for the investigation of the neuroprotective effects of drugs that act on cognitive and emotional sequelae. Different post-ischemic survival intervals were used in our experimental protocol to increase the predictive validity of the model. Neurodegeneration and neurogenesis are parallel processes that are dynamically regulated by brain ischemia, and we also evaluated hippocampal cell death and hippocampal neurogenesis using Fluoro-Jade C (FJC) histochemistry and doublecortin (DCX) immunohistochemistry, respectively.

2. Material and methods

2.1. Animals

Outbred Swiss Webster albino male mice (30–40 g) were obtained from the central *vivarium* of the State University of Maringá, Maringá, Paraná, Brazil. We chose Swiss mice because the strain is less sensitive to BCCAO with an acceptable survival rate [13,16]. The animals were allowed to acclimate to a controlled temperature (22 ± 1 °C) with a 12 h/12 h light/dark cycle (lights on at 7:00 AM) room, the local *vivarium*, for 1 week prior to the experiments. The animals were housed in groups (n = 3–5) and were given a standard commercial show and tap water ad libitum. The experimental procedures were approved by the Ethics Committee on Animal Experimentation of the State University of Maringá (CEEA 004/2011). All efforts were made to minimize the number of animals used and their suffering.

2.2. Experimental design

A schematic of the experimental protocols is depicted in Fig. 1. Experiment 1 was designed to detect the duration of BCCAO that combines an acceptable survival rate and consistent neurodegeneration in the CA1 hippocampal subfield. Forty mice were randomly divided into four groups: sham-operated (n = 7), subjected to 12 min BCCAO (n = 8), subjected to 17 min BCCAO (n = 13), and subjected to 25 min BCCAO (n = 12). Seven days after surgery, the animals' brains were removed and processed for histological analysis to detect CA1 hippocampal neurodegeneration. Animal death was recorded, and the survival rate was calculated as a percentage, considering 100% the number of animals that entered the experiment. CA1 hippocampal neurodegeneration was qualitatively evaluated as present or absent, despite neuronal damage in other hippocampal subfields or brain areas. The results are expressed as the percentage of animals that presented CA1 hippocampal neurodegeneration compared with the sham-operated group.

Based on these results, in Experiments 2 and 3, 17-min BCCAO was chosen to evaluate cognitive and emotional behavior. The behavioral and quantitative histological analyzes were performed at different post-ischemic survival intervals, i.e. 7, 14 or 28 days following BCCAO. All mice entered Experiment II were subjected to MWM and had their brains assayed for quantitative analysis of neurodegeneration using Nissl staining; five animals of each experimental groups had their brains assayed for FJC staining. All animals from Experiment III were tested in the OF and subsequently in the EPM; five to seven animals of each experimental group had their brains assayed to DCX immunohistochemistry in order to evaluate neurogenesis.

2.3. Surgery

Transient global cerebral ischemia was induced using the BCCAO technique as previously described [18]. The mice were anesthetized with halothane (Tanohalo; Cristália, SP, Brazil), and an incision was made in the ventral neck to expose the common carotid arteries. Brain ischemia was induced by bilateral occlusion of the common carotid arteries using aneurysm clips. At the end of each occlusion, the aneurism clips were removed, and the arteries were visually inspected for reperfusion. The incision was then closed with sutures. Rectal temperature was carefully monitored during surgery and maintained at approximately 37.5 °C using a heating blanket. Throughout occlusion and for 3 h after reperfusion, the mice were maintained in a warming box at 30 °C. Sham-operated animals were subjected to the same anesthetic and surgical interventions, with the exception that the carotid arteries remained intact.

2.4. Behavioral testing

The animals were randomly allocated in different experimental groups as shown in Fig. 1. All of the experimental manipulations were performed during the light phase, between 8:00 AM and 2:00 PM, under identical conditions. The experiments were video-recorded, and the behavioral scores were later analyzed using ANYmaze image analyzer software (Stoelting, Wood Dale, IL, USA).

2.4.1. Morris water maze

Overall, 50 mice entered the experiment II. Seven BCCAO mice died after complete recovery from anesthesia and the remainder ischemic animals (n = 26) were randomly distributed into independent experimental groups: 7 day BCCAO (n = 8), 14 day BCCAO (n = 9) and 28 day BCCAO (n = 9). The sham group was composed of 17 animals.

The Morris water maze apparatus consisted of a swimming pool made of black painted fiberglass (90 cm diameter, 35 cm height). For the tests, the tank was filled with water maintained at 23 ± 2 °C. The target platform (10 cm^2) was made of transparent acrylic and submerged 1 cm beneath the surface of the water. The starting points for the animals were marked on the outside of the pool as