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Research report

Glial-associated changes in the cerebral cortex after collagenase-induced intracerebral hemorrhage in the rat striatum

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ABSTRACT

Striatum and the cerebral cortex are regions susceptible to secondary injury after intracerebral hemorrhage (ICH) and glial cells in tissue adjacent to the hematoma may modulate cellular vulnerability after brain damage. Nonetheless, while the glial- associated changes occurring in the cerebral cortex after ICH may be important in maximizing brain recovery, they are not fully understood. The aim of this study was to evaluate the temporal profile of glial-associated changes in the cerebral cortex after ICH. First, the motor consequences of ICH and its relation to the lesion volume were analyzed. Secondly, glial cell proportion (GFAP+ and S100B+ astrocytes, CD11+ microglia) in the ipsilesional sensorimotor cortex and striatum, using flow cytometry were evaluated. ELISA was used to measure GFAP and S100 B content in these structures as well as S100 B levels in serum and cerebral spinal fluid. Main results revealed that ICH induced a delayed increase in GFAP+ cells in the sensorimotor cortex, as compared to the striatum, although the pattern of GFAP expression was similar in both structures. Interestingly, the time-curve patterns of both S100 B and CD11+ microglial cells differed between the cortex and striatum. Altogether, these results suggest a different dynamics of glial-associated changes in the cerebral cortex, suggesting it is a vulnerable structure and undergoes an independent secondary process of reactive glial plasticity following intracerebral hemorrhage.

1. Introduction

Intracerebral hemorrhage (ICH) is a devastating stroke subtype characterized by bleeding into the brain parenchyma (Manno, 2012) resulting in cell death and long term functional disability (Broderick et al., 1993; Lo et al., 2003). While the majority of ICH studies have focused on the primary lesion site, i.e., the striatal damage, knowledge of the secondary cortical changes is limited. Given that striatal damage can disrupt corticostriatal and corticospinal connectivity (Alexander et al., 1986; Pennartz et al., 2009) which are central to recovery following ICH, understanding of glial-induced changes in the cerebral cortex (Sukumari-Ramesh et al., 2012; Taylor and Sansing, 2013) may be an important step towards developing new therapeutic strategies (Fig. 1).

Astrocytes play an important role in promoting brain homeostasis and providing structural support to neurons within the brain parenchyma. They contribute to the formation and maintenance of the blood–brain barrier (BBB) as well as to regulation of cerebral blood flow (Ransom et al., 2003; Zhao and Rempe, 2010), and after a brain insult reactive astrocytes restrict the lesion area to protect the surrounding tissue (Sofroniew and Vinters, 2010). Reactive astrocytosis is a complex response involving cellular and molecular changes; glial fibrillary acidic protein (GFAP) and S100 calcium-binding protein B (S100B) are biomarkers used to determine the reactive phenotype of astrocytes and to predict the extent of brain injury (Mori et al., 2010; Sofroniew and Vinters, 2010). Although GFAP is not homogeneously expressed in a normal post-matured central nervous system (CNS), it labels most astrocytes responding to an injury (Yang and Wang, 2015). Similarly, S100 B is shown to be critical for astrocyte shape and migration after injury, exerting paracrine and autocrine effects on neurons and glial cells (Brozzi et al.,2009; Rothermundt et al., 2003).

Microglial cells also mediate reactive gliosis following brain injury by secreting inflammatory cytokines (Taylor and Sansing, 2013). While microglia are essential for hematoma resolution, they are also associated with BBB disruption, inflammation and delayed neuronal loss (Block and Hong, 2005). Therefore, death or dysfunction of glial cells

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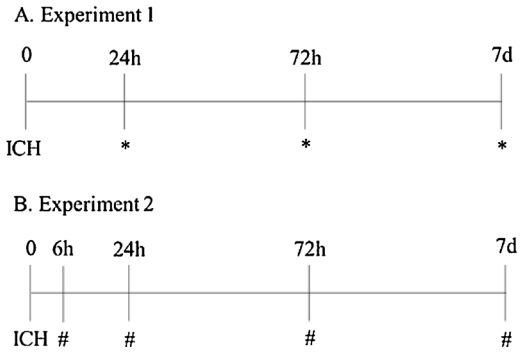

Fig. 1. Experimental design. * Motor behavior analysis and histology, N = 42 (A). # Biochemical analysis, N = 34 (B).

can affect neuronal function, reducing the possibility of motor recovery (Sukumari- Ramesh et al., 2012; Wasserman and Schlichter, 2007).

Considering the importance of glial responses on development of ICH injury, and the fact that the cerebral cortex share anatomical and functional connectivity with striatum, the present study aims to evaluate the glial-associated changes in the cerebral cortex after intrastriatal hemorrhage in the rat. For this purpose, astrocyte and microglial cells proportion were assessed by flow cytometry as well as the content of the GFAP and S100 B proteins by ELISA at 6 h, 24 h, 72 h and 7 days after a collagenase-induced intracerebral hemorrhage in the rat striatum (Mestriner et al., 2011).

2. Materials and methods

All procedures and experimental design were approved by the University Ethics Committee (project number 23976) and were in accordance with the Guidelines for Care and Use of Laboratory Animals adopted by the National Institute of Health (USA) and with National Animal Experimentation Control Board (CONCEA-Brazil). Efforts were made to minimize animal suffering and reduce the number of animals needed.

2.1. Experimental design

Adult male Wistar rats ($N = 76$) were assigned to two experiments. They were obtained from a local colony at approximately 3 months of age (300–350 g) and housed in groups of 4–5 in Plexiglas cages under standard laboratory conditions (12 h light/dark cycle with lights off at 7:30 p.m. and a controlled temperature of 22 ± 2 °C). Water and standard laboratory chow were provided *ad libitum*.

2.1.1. Experiment 1

The time-dependent effect of ICH on motor behavior performance and the lesion volume, space-occupying effect and ipsilateral cortical volume were evaluated. This experiment was run to characterize behavioral and histological changes observed during the ICH development. Rats were divided into six groups: 24 h, 72 h and 7-day post-ICH and their respective sham controls. The neurological score, grasping and cylinder tests, and all the histological parameters were assessed at the same endpoints by an investigator blinded to the rat's experimental condition. A total of 42 animals were used for behavioral and histological analysis.

2.1.2. Experiment 2

The temporal profile of S100 calcium-binding protein B (S100 B − astrocyte marker), glial fibrillary acidic protein (GFAP − reactive astrogliosis marker), alpha component of integrin (CD11–microglia marker) in the ipsilesional striatum and sensorimotor cortex was determined. Furthermore, S100 B levels in the serum and cerebrospinal fluid (CSF) were also measured. For this experiment, rats were divided into five groups: control, 6 h, 24 h, 72 h and 7 days post-ICH. The control group was composed by naïve animals since it is recognized that sham rats (surgical control) show significant increase of S100 B in serum and CSF (Thelin et al., 2016). A total of 34 animals were used for biochemical analysis.

2.2. Intracerebral hemorrhage

Animals were anesthetized with halothane (70% N2O and 30% O2; 4% induction, 2% maintenance) and placed in a stereotaxic frame for ICH surgery. A burr hole was drilled in the skull and a 26-G needle (Hamilton, Reno, NV, USA) was inserted into the dorsolateral striatum in the following coordinates: 0.0 mm anterior (AP), 3.6 mm lateral (ML) and 6.0 mm ventral (DV) (in relation to Bregma in the left side) (Paxinos and Watson, 2007). Bacterial collagenase type-IVs (Sigma-Aldrich, USA) was infused to produce the ICH lesion in a concentration of 0.2 U per 1.0 μL of saline buffer. In sham surgeries, collagenase was replaced with sterile saline in same concentration of lesioned animals. The needle was kept in position for an additional 5 min period and then slowly removed to prevent backflow (MacLellan et al., 2006a, 2006b; MacLellan et al., 2008; Rosenberg et al., 1990; Szymanska et al., 2006). After surgery, animals received topical application of lidocaine (10% of Xylocaine®) and their behavior were monitored. Body temperature was maintained between 36.5 °C and 37.5 °C throughout the surgery using a self- regulating heating blanket (Letica, Spain). In this study, six animals destined to experiment 2 died due to surgery/stroke complications.

2.3. Behavioral assessment (experiment 1)

2.3.1. Grip strength test

The grip strength test was performed as previously described (Jeyasingham et al., 2001). Briefly, the animal was held by the abdomen and lowered until the body was horizontal and the rat grasped a metal bar attached to a strain gauge. Then, the animal was steadily pulled by the tail until it released the bar. The values of 3 trials were recorded at each endpoint and the average of all readings was calculated as the grip strength score (g) (Cabe et al., 1978).

2.3.2. Neurological score

The neurological score (beam walk ability and spontaneous ipsilateral body rotation) was used to rate motor dysfunction. The beam walk ability was performed to assess the capability of the rat to traverse a narrow, elevated beam wooden till the home cage. This test was repeated three times for an adaptation period (before the surgery) and, then performed in a single trial at each evaluated endpoint. The beam walk ability was rated into five degrees of severity: 0 − normal walking to home cage; 1 − minor dysfunction such as long stride or unbalanced walking; 2 − mild dysfunction with frequent slip of the limb from the beam; 3 − moderate dysfunction with inability to walk on a beam; and 4 − severe dysfunction, falling from beam within ten seconds. Spontaneous ipsilateral rotation was assessed with the rat being held by the tail for 10 s. This test was also rated into five degrees of severity: 0 − normal symmetric rotation; 1–a tendency to ipsilateral rotation; 2–ipsilateral rotation with occasional contralateral turns; 3–frequent ipsilateral rotation without contralateral rotation; and 4–continuous ipsilateral rotation (Altumbabic et al., 1998; Imamura et al., 2003). For the final result, the sum of the points in each test was counted and expressed as medians/interquartile range.

2.3.3. Asymmetrical forelimb use (cylinder test)

Animals were placed into a plexiglas cylinder (20 cm diameter × 40 cm high) on a glass tabletop and video-recorded from below with an angled mirror. Spontaneous ipsilateral and contralateral forelimb wall contacts were recorded for 2 min. The average number of contralateral forelimb contacts was divided by the sum of contacts of

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