Neuroscience 284 (2015) 400–411

INTERNAL CAPSULE STROKE IN THE COMMON MARMOSET

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Abstract—White matter (WM) impairment and motor deficit after stroke are directly related. However, WM injury mechanisms and their relation to motor disturbances are still poorly understood. In humans, the anterior choroidal artery (AChA) irrigates the internal capsule (IC), and stroke to this region can induce isolated motor impairment. The goal of this study was to analyze whether AChA occlusion can injure the IC in the marmoset monkey. The vascular distribution of the marmoset brain was examined by colored latex perfusion and revealed high resemblance to the human brain anatomy. Next, a new approach to electrocoagulate the AChA was developed and chronic experiments showed infarction compromising the IC on magnetic resonance imaging (MRI) scanning (day 4) and histology (day 11). Behavioral analysis was performed using a neurologic score previously developed and our own scoring method. Marmosets showed a decreased score that was still evident at day 10 after AChA electrocoagulation. We developed a new approach able to induce damage to the marmoset IC that may be useful for the detailed study of WM impairment and behavioral changes after stroke in the nonhuman primate.

Key words: anterior choroidal artery, internal capsule, motor impairment, nonhuman primate, white matter stroke.

INTRODUCTION

Stroke is a devastating disease, being the major cause of acquired disabilities around the world (Donnan et al., 2008). To understand the injury mechanisms and develop new strategies aimed to improve the motor conditions of stroke survivors, several animal models have been developed (Canazza et al., 2014). Owing to the heterogeneous nature of stroke and additional features such as age, sex, race and comorbidities that vary among patients, there is no ideal animal model of human stroke (Mergenthaler and Meisel, 2012); however, the developed models have tried to mimic as much as possible the human condition. Because the middle cerebral artery (MCA) is the most commonly affected artery among stroke patients (Rordorf et al., 1998), one of the most common models for stroke research is MCA occlusion (MCAO) in rodents (Tamura et al., 1981; Kohno et al., 1995). Although these models have helped to unveil the effects of cortical ischemia (Astrup et al., 1981; Neumann-Haefelin et al., 2000; Dijkhuizen et al., 2001), they mislead the researchers' attention to gray matter (GM) injury. Owing to the fact that the GM/white matter (WM) ratio found in the rat neocortex (GM:WM = 87:13) is significantly higher than in humans (GM:WM = 61:39, Zhang and Sejnowski, 2000), the rodent MCAO stroke model induces large infarcts affecting mainly the GM. This feature has inspired the development of neuroprotective agents focused on GM protection aiming for neuron rescue; although such therapies succeed in the rodent recovery after stroke, they fail in clinical trials (Xu and Pan, 2013). This discrepancy between rodent models and human trials has drawn the attention to the essential brain structural differences between both species: the WM ratio.

Recent imaging studies done in stroke survivors have highlighted the importance of WM damage, demonstrating that corticospinal tract (CST) integrity can be considered as a reliable predictor of stroke severity and clinical outcome (Thomalla et al., 2004; Puig et al., 2011; Rosso et al., 2013). Additionally, there is evidence that motor dysfunction after MCA stroke is more dependent on WM than GM damage (Rosso et al., 2011). Because both GM and WM differ importantly in the initial

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Abbreviations: ACA, anterior cerebral artery; AChA, anterior choroidal artery; AChAO, anterior choroidal artery occlusion; AMG, autometallographic; BA, basilar artery; CST, corticospinal tract; DW, distilled water; FA, flip angle; FOV, field of view; FS, Freret neurologic score; GM, gray matter; HSD, honestly significant difference; IC, internal capsule; ICA, internal carotid artery; MCA, middle cerebral artery; MCAO, middle cerebral artery occlusion; MNS, marmoset neurologic score; MRI, magnetic resonance imaging; NHP, nonhuman primate; OR, orbital rim; OT, optic tract; PB, phosphate buffer; PCA, posterior cerebral artery; PcomA, posterior communicating artery; SCA, superior cerebellar artery; TE, echo time; TM, temporal muscle; TR, repetition time; WM, white matter.

http://dx.doi.org/10.1016/j.neuroscience.2014.10.015
0306-4522/

responses to ischemia (Hughes et al., 2003), some researchers suggest that WM ischemia may have a longer therapeutic window (Muñoz Maniega et al., 2004; Koga et al., 2005). Therefore, it is imperative to deepen the research on WM ischemia owing to its potential for the development of new therapies for stroke patients.

To investigate the effect of subcortical WM injury, different animal models have been developed; a rodent model (Frost et al., 2006; Lecrux et al., 2008) that induces direct damage on the internal capsule (IC), and a mini-pig model (Tanaka et al., 2008) that attempts to impair the IC by occlusion of the anterior choroidal artery (AChA). Although both approaches induced motor impairment, the damage was subtle and transient in contrast to human strokes that compromise the AChA territory (Derflinger et al., 2013). Stroke of the AChA in the human brain can lead to infarction of the posterior limb of the IC, which induces isolated motor deficits due to disruption of the CST (Rascol et al., 1982; Nelles et al., 2008; Likitjaroen et al., 2012). The development of relevant animal models to study this condition may help to identify critical factors related to WM changes after ischemia and for the development of new approaches focused on the rescue of WM (Sozmen et al., 2012).

Because nonhuman primates (NHPs) are phylogenetically closer to the human, where the WM volume is larger than rodents' (GM/WM ratio: 79/21. Zhang and Sejnowski, 2000; Bailey et al., 2009; Okano et al., 2012), the development of an alternative model of WM ischemia in such species may provide relevant information about WM responses after stroke and improve further translational research. The common marmoset (*Callithrix jacchus*) is a NHP similar to *Homo sapiens*, with a brain five times larger than the rat's, representing approximately 2.7% of its body weight, which is equivalent to human proportions (Abbott et al., 2003; Okano et al., 2012). Moreover, the neocortical GM/WM ratio is smaller in comparison with rodents (Zhang and Sejnowski, 2000), and marmoset ergonomics are closer to the human's. We consider that the similarities in WM proportions and ergonomics between marmosets and humans may offer a promising scenario for the study of WM changes after ischemia.

The aim of this study was to establish whether a vessel homologous to the human AChA exists in the marmoset brain and to evaluate the effect of its occlusion on the IC. To our knowledge, there is no established method to induce an infarct in the marmoset IC as a model of WM stroke.

EXPERIMENTAL PROCEDURES

Animals

Twenty-two laboratory-bred adult common marmosets (*C. jacchus*) ~4.5 years old at the start of the experiments were used. Two already euthanized marmosets (fixed and long-term freeze-preserved: cadaveric preparations) were used for carotid artery cannulation and colored latex intravascular perfusion (Alvernia et al., 2010). Twelve marmosets were used to evaluate brain vascular anatomy (non-operated side) and test the reproducibility of the AChA occlusion (AChAO; operated-side) by the injection of colored latex perfusion after surgery (acute experiments). The remaining eight marmosets were divided into two groups to perform AChAO ($n = 5$) and sham operation ($n = 3$). These animals were observed for 11 days before euthanasia (chronic experiments). All monkeys were kept within a large colony to allow good visual and auditory interaction with other marmosets. All procedures were performed in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the Animal Research Committee at the National Institute of Neurosciences in Tokyo, Japan.

AChA identification

To identify the vascular anatomy of the marmoset, liquid latex was used as previously described (Alvernia et al., 2010), with some modifications as follows: For cadaveric preparations, the animals were unfrozen at room temperature, and bilateral dissection of the common carotid arteries was performed. Cannulation was achieved using an 18-gauge catheter (18G × 2'' catheter; Nipro, Osaka, Japan), and both catheters were perfused with tap water followed by liquid red latex solution (Ward's Natural Science 37-2571, Columbus Chemical Industries, Columbus WI, USA) using a 10-cc syringe until leakage from the contralateral carotid artery and vertebral arteries was evident. After 20 min, the brain was dissected carefully and vascular exploration was performed. To evaluate the consistency of the vascular patterns, pictures were taken, hand drawings from the right side of the intracranial vessels emerging from the internal carotid artery (ICA) were performed, and the distance between the AChA and ICA bifurcation was measured. The same evaluation was performed for the non-operated side of animals used to test the surgical procedure accuracy (see below). In total, 14 animals were used for the evaluation of the vascular pattern.

Surgical procedures

Surgical preparation. Marmosets were anesthetized with Isoflurane (1–2% (v/v); Mylan Pharmaceutical Co., Ltd. Morgantown WV, USA) delivered initially via an animal face mask, then through endotracheal intubation (6 Fr catheter, length 6.5 cm). Two g/kg of D-Mannitol (20% (w/v); Yoshindo Inc., Toyama, Japan) were slowly injected from the catheterization of the femoral vein (26G × 3/4'' catheter; Nipro, Osaka, Japan) as a bolus, followed by continuous infusion (0.7 ml/h) containing Remifentanil (0.18 μg/h; Ultiva 5 mg; Janssen Pharmaceutical, Tokyo, Japan) and Rocuronium Bromide (24 μg/h; Eslax, 25 mg/2.5 ml; MSD Co., Ltd., Tokyo, Japan) before starting artificial ventilation (A.D.S. 2000; Engler, Hialeah FL, USA) (flow rate: 1.65 ± 0.4 l/min; peak inspiratory pressure: 15 cm of H_2O; respiratory rate: 9.5 ± 1 breaths per minute). During surgery, heart rate (189.7 ± 16.4 beats per minute) and arterial oxygen saturation (SaO2: 96.4 ± 2.5%) were monitored with a pulse oximeter (8600V NONIN medical Inc., Plymouth MN, USA). Electrocardiographic traces