NEUROSCIENCE
INTERNATIONAL BRAIN IBRO RESEARCH ORGANIZATION

RESEARCH ARTICLE

J. Lin et al. / Neuroscience xxx (2017) xxx–xxx

Cerebral Venous Collagen Remodeling in a Modified White Matter Lesions Animal Model

Jing Lin,[a] Linfang Lan,[b] Dilong Wang,[a] Baoshan Qiu[a] and Yuhua Fan[a]*

[a] Department of Neurology, First affiliated Hospital, Sun Yat-sen University, Guanghzou, China

[b] Department of Medicine and Therapeutics, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong

Abstract—To mimic the expected pathological changes of white matter lesions (WMLs) and increase the stability, we applied modified two-vessel occlusion (modified 2VO) (1-week interval bilateral carotid artery occlusion) in stroke-prone renovascular hypertensive rats (RHRSP) and established a modified WML model (RHRSP/modified 2VO) that compared their phenotypes with RHRSP and sham-operated rats. In addition, we tried to differentiate small veins from small arteries through the presence of smooth muscle to study the pathological changes of small veins detailed in the model. RHRSP/modified 2VO rats showed higher stability and more extensive white matter damage without an obvious increase in mortality rate at 12 weeks after the modified 2VO operation compared to RHRSP rats. RHRSP/modified 2VO rats showed more severe small venous collagen deposition than RHRSP rats, and the majority of the deposition was collagens I and IV rather than collagen III. In addition, RHRSP/modified 2VO rats possessed cognitive impairment, mild wall thickness and blood–brain barrier disruption. Our findings suggest that the modified WML model (RHRSP/modified 2VO) mimics cognitive impairment and small vessel pathological changes similar to WMLs in humans. Differentiating small veins from small arteries through smooth muscle is feasible, and marked small venous deposition may play an important role in the hypertensive white matter lesions.

Key words: white matter lesions, venous collagen deposition, stroke-prone renovascular hypertensive rat, modified bilateral carotid artery occlusion, smooth muscle, cognitive impairment.

INTRODUCTION

White matter lesions (WMLs), also known as leukoaraiosis (LA) or white matter hyperintensities (WMHs), are often seen as hyperintensities on T2-weighted magnetic resonance or fluid-attenuated inversion recovery imaging (Wardlaw et al., 2013). As one of the typical changes of cerebral small vessel disease, WMLs are associated with cognitive impairment and poor functional outcome (Au et al., 2006; Herrmann et al., 2008). However, the pathogenesis of WMLs has not been well elucidated. Impairment of cerebral blood flow autoregulation due to vascular risk factors such as hypertension and chronic cerebral hypoperfusion resulting from blood supply watershed may underlie the white matter changes (Pantoni and Garcia, 1997; Birns et al., 2009). "Jugular venous reflux" has been recently proposed as being involved in WMLs through the hemodynamic impact on cerebral veins (Chung et al., 2011). Previous investigations have failed to demonstrate the importance of pathological venous changes in WMLs because it is difficult to distinguish small cerebral veins from arteries.

To mimic the expected pathological changes and explore the underlying mechanisms of WMLs, various animal models have been developed. Bilateral common carotid artery occlusion (BCCAO) or the two-vessel occlusion (2VO) model in rats represents the characteristic features of white matter damage and cognitive impairment (Wakita et al., 1994; Jiwa et al., 2010). Its drawbacks include high mortality rate due to acute ischemia and lack of hypertension (Otori et al., 2003). The model of stroke-prone spontaneously hypertensive rats (SHRSP) is accompanied by the features of human small vessel disease and is widely used (Bailey et al., 2011). This model, however, possesses an inherent limitation in that genetic factors that cannot be ignored play a role in SHRSP (Lin et al., 2001; Brittain et al., 2013).

*Corresponding author.
E-mail address: fansusan@126.com (Y. Fan).

Abbreviations: 2k2c, two kidney-two clip; AP, alkaline phosphatase; BBB, blood–brain barrier; BCCAO, bilateral common carotid artery occlusion; LA, leukoaraiosis; MBP, myelin basic protein; modified 2VO, modified two vessel occlusion; RHRSP, stroke-prone renovascular hypertensive rats; SBP, systolic blood pressure; SHRSP, stroke-prone spontaneously hypertensive rats; WMHs, white matter hyperintensities; WMLs, white matter lesions; α-SMA, α-smooth muscle actin.

https://doi.org/10.1016/j.neuroscience.2017.10.031
0306-4522/

In our previous study, we established RHRSP as a WML animal model (Fan et al., 2015). The incidence and severity of WMLs were not very high by 24 weeks post-operation of a two-kidney, two-clip (2k2c) procedure, and the mortality rate increased as the duration of hypertension extended. In the present study, we seek to test the modified bilateral carotid artery occlusion (1-week interval, modified 2VO) in stroke-prone renovascular hypertensive rats (RHRSP) and establish the RHRSP/modified 2VO model, and to differentiate small veins from small arteries through the presence of smooth muscle to detect the pathological changes of small veins in the modified WMLs animal model. Our hypothesis is that this model may increase the model stability and more precisely mimic small vessel pathological changes, and that small venous collagen remodeling plays an important role in WMLs.

EXPERIMENTAL PROCEDURES

Animals

The experimental protocol conformed to the Animal Welfare Act Guide for Use and Care of Laboratory Animals, and was approved by the local ethics committee for animal research, Sun Yat-sen University, China. All surgical procedures involving animals were monitored according to institutional guidelines in compliance with the regulations. All efforts were made to minimize the number of animals used and their suffering.

Study design

One hundred sixteen Sprague–Dawley rats (Guangzhou China) weighing 80–100 g were randomly divided into a hypertension group ($n = 80$) and a sham-operated group ($n = 36$). The hypertension group underwent a 2k2c procedure, and systolic blood pressure (SBP) was measured 12 weeks later (tail-cuff sphygmomanometer BP-98A, Softron, Japan). Rats (SBP $\geq$ 180 mm Hg) in the hypertension group were randomly divided into a RHRSP/modified 2VO group and a RHRSP group. The RHRSP/modified 2VO group rats were subjected to modified bilateral carotid artery occlusion operation (1-week interval). The Morris water maze test was conducted pre-operation, and at 4, 8, and 12 weeks post-operation of modified 2VO (9–10 animals per group). After the Morris water maze test, the rats were euthanized, and transmission electron microscopy (3 animals per group) and histological evaluation were performed (5–6 animals per group) (Fig. 1).

Surgical procedure

The method of 2k2c to establish RHRSP was described previously by Zeng et al. (Zeng et al., 1998). Rats were anesthetized with 1% pentobarbital (50 mg/kg) and then underwent a median longitudinal incision on the abdominal skin; the renal arteries were exposed. Ring-shaped silver clips (0.3 mm in diameter) were placed around the roots of both right and left renal arteries. The sham-operated group received the same surgical procedure without the renal artery clips. In the RHRSP/modified 2VO group, rats were given a modified 2VO operation. Rats were anesthetized with 1% pentobarbital (50 mg/kg) via an intraperitoneal injection. A neck ventral midline incision was performed, and the bilateral carotid arteries were carefully separated from their sheaths. The right carotid artery was gently banded with a 5–0 silk suture, and the left carotid artery was occluded in the same way 1 week later. The sham-operated group and the RHRSP group received the same surgical procedures without carotid artery ligation.

Morris' water maze test

Rats underwent adaptive training the day before the test. The rats were allowed to swim freely in a pool (120-cm diameter) for 120 s. In the navigation test, the rats were placed in water from four positions sequentially, and the latency of escaping onto the platform was recorded (maximum swimming time 60 s). All rats remained on the platform for 10 s at the end of each trial. In the probe trial, the platform was removed and a single 60-s probe trial was conducted. The number of times crossing the former platform and the time ratio of the target quadrant were recorded. All procedures were monitored using a video tracking system (DMS-2, Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, China).

Transmission electron microscopy

Rats were euthanized with sodium pentobarbital and perfused transcardially with 0.9% saline. White matter was removed and postfixed in Karnovsky's fixative. Tissues were cut into thin slices according to the standard procedure for the electron microscopy. Ultrastructural changes of the blood–brain barrier (BBB) were observed in 200 kV using a Tecnai transmission electron microscope.

Histological evaluation of WMLs and small vessel changes

Rats were euthanized with sodium pentobarbital and perfused transcardially with 0.9% saline, followed by 4% paraformaldehyde in 0.1 mol/L phosphate buffer (PB, pH 7.4). The brains were removed and tissue block was postfixed for 24 h. Coronal brain blocks were embedded in paraffin and cut into 3-μm sections (from +1.8-mm bregma to −4.5-mm bregma). Hematoxylin–eosin (H&E) staining was used to observe the overall morphology of small vessels, large infarcted focus, and pyknotic neurons in the hippocampus. Luxol fast blue staining was used to examine myelin loss. The degree of white matter changes was graded as normal (grade 0), disarrangement of the nerve fibers (grade 1), the formation of marked vacuoles (grade 2), and the disappearance of myelinated fibers (grade 3), according to Wakita et al. (Wakita et al., 1994). Immunohistochemistry was performed to observe myelin basic protein (MBP). For immunohistochemical staining, brain sections