Morphologic Changes of Cerebral Veins in Hypertensive Rats: Venous Collagenosis Is Associated with Hypertension

Min Zhou, MD,* Lijuan Mao, MD, MS,† Ying Wang, MD, PhD,* Qian Wang, MD,* Zhiyun Yang, MD, PhD,† Shurong Li, MD, MS,† and Ling Li, MD, PHD*

Background: The aims of this study were to determine whether arterial hypertension could affect the venous system of brain and to find out the consequent pathologic changes of cerebral veins. *Methods:* Thirty male Sprague–Dawley rats were divided into 2 groups: a sham-clipped group and a stroke-prone renovascular hypertensive rat group. A 2-kidney 2-clip rat model was used to induce renovascular hypertension in the hypertensive group. Systolic blood pressure was measured by tail cuff once each week. Susceptibility-weighted imaging (SWI) was performed at 12, 16, and 20 weeks after surgery. All the rats were sacrificed after the SWI examination at 20 weeks after surgery. The brains were extracted and embedded in paraffin for histologic examination. Masson trichrome staining was performed to identify venous collagenosis. *Results:* The sham group demonstrated less prominence of cerebral veins compared with hypertensive groups ($P < .01$); the hypertensive group showed significant venous collagenosis in cerebral venous walls compared with the sham group ($P < .01$). *Conclusions:* The increased visibility of cerebral veins on SWI as a sign of venous hypertension and the thickened cerebral venous walls (venous collagenosis), which may play a role in cerebral ischemia and/or infarction, are both consequences of long-term hypertension in hypertensive rats. **Key Words:** Susceptibility-weighted imaging—stroke-prone renovascular hypertensive rat—hypertension—cerebral venous change—venous collagenosis.

Hypertension is a major risk factor for cerebral vascular disease (CVD), and it is closely associated with both ischemic and hemorrhagic CVD. In the clinic, we found numerous cases of CVD accompanied by high blood pressure, including cerebral infarction and brain hemorrhage, which cannot be fully explained by hypertensive insults of cerebral arteries. Previous investigations have only focused on cerebral artery disease and have failed to attach importance to the role of the cerebral venous system in CVDs. In the present study, we observed changes in cerebral veins in renovascular hypertensive rats using susceptibility-weighted imaging (SWI) and histopathology method, looking for a new direction for the further study of CVD.

Stroke-prone renovascular hypertensive rat (RHRSP), introduced in 1998,[1] is an animal model with acquired hypertension independent of genetic deficiency. Because the morphologic and physiological changes of cerebral

From the *Department of Neurology, Guangdong Key Laboratory for Diagnosis and Treatment of Major Neurological Diseases, National Key Clinical Department, National Key Discipline; and †Department of Radiology, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China.

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Address correspondence to Ling Li, MD, PhD, Department of Neurology, The First Affiliated Hospital of Sun Yat-sen University, 58 Zhongshan Road II, Guangzhou, China. E-mail: april1987@live.cn.

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vessels are similar to those in hypertensive patients, the rats represent a more than 60% incidence of spontaneous stroke. The advantages of this animal model are 100% development of hypertension without gene modification, high similarity to human hypertension in cerebrovascular pathology and physiology, and easy establishment with low cost. Thus, the model has been extensively used in the investigation of ischemic stroke and has been shown as a reliable animal model.[2,3]

SWI has developed into a powerful clinical tool to visualize venous structures and iron in the brain and to study diverse pathologic conditions.[4-7] SWI venography allows for the detailed visualization of cerebral veins without using an exogenous contrast agent on the basis of a magnetic susceptibility difference between oxygenated and deoxygenated hemoglobin.[8] Previous studies have shown that the large cerebral vein changes of rats that had traumatic brain injury could be detected by SWI.[9,10] In another study, SWI was used to evaluate hypoxic–ischemic injury by examining each patient's cerebral "prominence of vein" (POV) on susceptibility-weighted image.[11]

The aims of this study were to determine whether arterial hypertension could affect the venous system of brain and to find out the consequent pathologic changes of cerebral veins.

Materials and Methods

Animal Treatment

The study was approved by the ethics committee of the Sun Yat-Sen University. Thirty male Sprague–Dawley rats, weighing 80-100 g, were obtained from the experimental animal center of Guangdong province and were randomly divided into 2 groups the sham-clipped group (sham, n = 12) and the RHRSP model group (RHRSP, n = 18).

The Sprague–Dawley male rats were fed ad libitum and housed under conventional conditions of controlled temperature (23 ± 2°C), humidity (55 ± 10%), and light (12-hour light/12-hour darkness). All animal treatments were strictly in accordance with international ethical guidelines and the National Institutes of Health Guide on the Care and Use of Laboratory Animals.

An RHRSP model was used to induce renovascular hypertension.[1-3] Under anesthesia with 3% sodium pentobarbital (intraperitoneally at 36 mg/kg body weight), a midline laparotomy was used for the bilateral placement of a partially occlusive silver clip (.3-mm internal diameter) on the renal arteries of RHRSPs. The ring part of the clip was placed around the root of each artery, and then, the outer gap of the clip was shut. The rats of the sham surgery group underwent laparotomy and the isolation of the bilateral renal arteries without clip placement. Beginning after 4 weeks of recovery from surgery, the systolic blood pressure (SBP) was measured by tail cuff once each week.

Magnetic Resonance Imaging and Grading

Rats were placed in a prostrate position and anesthetized by an intraperitoneal injection of 3% sodium pentobarbital at 36 mL/kg body weight. Magnetic resonance imaging (MRI) was performed at 12, 16, and 20 weeks after surgery. The 3 T MRI (SIEMENS VERIO 3.0 T, Munich, Germany) and a rat radiofrequency coil (4 channel; Chenguang, Shanghai, China) were used. The brain MRI was taken with the sequence parameters set for SWI (repetition time, 32 milliseconds; echo time, 20 milliseconds; flip angle, 15°; field of view, 60 × 48.6 mm; matrix size, 320 × 320; thickness, 1 mm; and number of excitations, 2).

The SWI sequence was used to evaluate the cerebral venous system. Based on a previous study,[8] we have developed an SWI categorical grading scale for the cerebral venous system of rats. Each SWI sequence was evaluated with a categorical grading scale, ranging from 0 to 3 (Table 1 and Fig 1). Two researchers, blinded to each rat's blood pressure status, assigned POV scores to each rat's SWI sequence. When disagreement occurred between the 2 researchers with regard to the POV score of a rat, an average score was used for the analyses.

Histopathology

Under deep anesthesia, the thoracic cavity of the rats was opened, and at room temperature, phosphate-buffered saline (300 mL for rats) was infused for 15 minutes and then with a fixative consisting of cold 4% paraformaldehyde and .1 mol/L phosphate buffer at pH 7.4 (300 mL) via the left ventricle. The heart was spontaneously beating on the initiation of infusion, and the animals were simultaneously allowed to exsanguinate via right atrium puncture.

The brains were extracted and postfixed for 24 hours in 4% paraformaldehyde in .1 mol/L phosphate buffer and then dehydrated in a program-controlled automatic dewaterer. Coronal brain blocks were embedded in paraffin for histologic examination. The fixed brains were sliced into 4-μm-thick coronal sections.

Table 1. *Grading scale of cerebral veins demonstrated on susceptibility-weighted imaging sequences*

Grade	Appearance of cerebral veins by susceptibility-weighted imaging
0	No visible cerebral veins in any plane
1	Several definite, fine, light gray, deep cerebral veins or occasionally dark, distinct, large veins
2	Dark distinct cerebral veins in both medullary and cortical areas or enlarged internal cerebral veins
3	Global, numerous, thick, dark cerebral veins and dilated internal cerebral veins