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Investigating microbleeding in cerebral ischemia rats using susceptibility-weighted imaging

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ABSTRACT

Purpose: To detect the distribution and prevalence of cerebral microbleeding (CMB) in rats after acute ischemic stroke using susceptibility-weighted imaging (SWI).
Method: After middle cerebral artery occlusion, 3 T MR scanning was performed on 10 rats at 4 h and 24 h after ischemia. T2-weighted images (T2WI), T2 maps and diffusion-weighted images (DWI) were generated to estimate the severity of the brain ischemia. The SWI data were used to analyze location counts, size and distribution of the CMBs. The brain injury was evaluated and determined by hematoxylin and eosin (H&E) staining.
Result: At 24 h after onset of ischemia, 13 CMBs were found in seven of the nine ischemic rats, whereas only two CMBs were found at 4 h after ischemia onset in one of the nine ischemic rats. All visible CMBs detected in the SWI data were located in the ischemic lateral cortex, with diameters ranging from 0.2 to 0.5 mm. In addition, we observed thickened vessels near the CMBs in the ischemic hemisphere in the SWI minimum intensity projections that did not appear in the symmetrical regions on the contralateral hemisphere. Histopathological results confirmed the CMBs, and increased microvascular density was observed in the ipsilateral hemisphere.
Conclusion: SWI technique allows the detection of CMBs and the accompanying thickened vessels in vivo in a rat model of cerebral ischemia, which appear to be challenging tasks using T2WI and DTI. The results reported in this work provide a better understanding of the pathophysiological mechanism of acute stroke.

1. Introduction

Cerebral microbleeding (CMB) is the manifestation of sub-clinical neurological impairment in patients with blood components in brain tissues [1]. CMBs commonly occur among the elderly. They are caused by brain microvascular disease and are closely correlated with aging, blood pressure and heart disease. Recently, increases in the measurements of clinical CMBs have been interpreted as chronic small vessel ischemia and perivascular demyelination [1–3]. Stroke is one of the most common causes of mortality and disability in people throughout the world; ischemic stroke accounted for 86% of all stroke. Cerebral ischemia causes dramatic changes in the structure and function of the blood–brain barrier (BBB), such as vascular brain edema, which leads to the opening of tight junctions between capillary endothelial cells in the brain and a significant increase in the permeability of the BBB. BBB damage associated with the presence of small vessel disease leakage is present during biphasic BBB openings. It is thought that CMBs are the result of extravasations of blood components caused by abnormal BBB permeability; a number of articles have described CMBs in acute ischemic stroke patients [4,5].

To enable better prognosis and treatment of cerebral ischemia, it is essential to more precisely predict the extent and severity of histological brain damage caused by cerebral ischemia in individual subjects. Therefore, the estimation of CMBs in experimental settings would be an important contribution to a better assessment of acute ischemic stroke. Experimental animal models of stroke have provided evidence for biphasic BBB openings during periods of cerebral ischemia from 1 to 4 hours and 24 to 48 hours [6]. However, there has been little research on CMBs in acute stroke animal models with BBB opening.

In recent years, with the rapid development of MRI, increasing attention has been paid to small-vessel disease, and it has been demonstrated that gradient-echo (GRE) T2*-weighted MRI is

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particularly sensitive for the detection of CMBs [1,7,8]. T2* dephasing occurs due to inhomogeneities in the local magnetic field caused by hemosiderin deposition [7]. CMBs in the human brain are described as ellipsoid areas of signal abnormality with a diameter smaller than 5 mm and no peripheral edema, and they are caused by the rupture of the microvasculature in the basal ganglia and subcortex [9]. However, CMBs in animal models, such as rats, are much smaller and more difficult to observe using the T2*-weighted GRE sequence. A susceptibility-weighted imaging (SWI) is characterized within the high-pass-filtered phase images. The phase images can be transformed into a special phase mask, which can be multiplied to the magnitude of the images to generate a kind of new SWI image and to enhance the conspicuity of magnet-sensitive substances. This contributes to an enhanced T2* effect of paramagnetic substances, such as hemosiderin or deoxyhemoglobin. The enhanced T2* dephasing showed as hypointensity caused by hemosiderin deposition contained in CMBs allows for the detection of more numerous small microbleeds, which are more conspicuous than they are in T2*-weighted sequences [10,11]. An MRI examination at different time points provides a more informative and precise assessment of brain injury without further impairment; another examination was also performed after treatment. The objectives of the present study were to (1) perform SWI in a rat model of acute ischemic stroke to determine the distribution and prevalence of CMBs and (2) identify the correlation between the presence and distribution of CMBs and biphasic BBB opening.

2. Materials and methods

2.1. Animal model

Sixteen healthy adult SD rats aged 8 to 12 weeks and weighing 250 to 300 g were used in this study. All rats were cage reared, provided sufficient food and drinking water and were housed under light for 12 hours per day at room temperature (23 ± 1) °C. According to the method described by Longa [12], a nylon intraluminal suture was placed in the left cervical internal carotid artery (ICA), the post cerebral artery (PCA) and the middle cerebral artery (MCA) sequentially, and the suture was advanced into the intracranial part of the artery to block the left MCA without reaching the anterior cerebral artery (ACA). In this way, 10 out of 16 adult SD rats were subjected to ischemic stroke in the left hemisphere of the brain due to permanent MCA occlusion (MCAO), and the other 6 healthy rats were used for control experiment. A heat source was used to ensure that the rectal and temporalis temperatures were (37 ± 0.5) °C with a brain temperature of 30 ~ 33 °C. All of the animal studies were performed according to a protocol approved by the Institutional Animal Care and Use Committee of East China Normal University.

2.2. Magnetic resonance imaging measurements

T2-weighted images (T2WI), T2 maps, diffusion-weighted images (DWI) and SWI scans of the rat brain were collected using a 3 T Siemens whole body scanner (MAGNETOM Trio, Erlangen, Germany) at 4 h and 24 h after induction ischemia; these time points are believed to correspond to BBB opening [6]. Each rat's head was positioned inside a 5-cm-diameter, 5-cm-long dual-tuned, dual-quadrature, birdcage radiofrequency mouse coil. The scanning parameters were as follows: (1) to observe ischemic injury in the brain, T2WI was performed with a T2 spin echo (SE) sequence, a 50 × 50 mm^2 field of view (FOV), a spatial resolution of 0.2 × 0.2 × 1.0 mm^3, a repetition time/echo time (TR/TE) of 3330/68 ms, a flip angle of 120°, parallel imaging used integrated parallel acquisition techniques with an acceleration factor of 2 (iPAT = 2), and a total of 18 slices. The acquisition time (AT) was 5:21. (2) To measure the development of tissue water/edema, T2 maps were acquired using a T2 multi-echo sequence, an FOV of 50 × 50 mm^2, a spatial resolution of 0.3 × 0.3 × 1.0 mm^3, a TR/TE of 3330/16, 32, 48, 64, 80, and 96 ms, a flip angle of 180°, iPAT = 2, and a total of 15 slices. The AT was 6:04. (3) To detect changes in cerebral ischemia, DWIs were acquired by an echo-planar pulse sequence with nine orthogonal directions at different 'b' values (0, 1000 s/mm^2), a TR/TE of 3300/99 ms, an FOV of 67 × 67 mm^2, a spatial resolution of 0.7 × 0.7 × 2.0 mm^3, iPAT = 2, and a total of 8 slices. The AT was 6:21. (4) To detect CMBs, SWI data (including SWI images, SWI filtered phase images, magnitude images and minimum intensity projections) were acquired using a 3D-GRE sequence with an FOV of 50 × 50 mm^2, a spatial resolution of 0.2 × 0.2 × 1.0 mm^3, TR/TE = 32/20 ms, a flip angle of 20°, iPAT = 2, and a total of 24 slices. The AT was 8:55.

2.3. Image analysis

All MRI images were analyzed using SPIN (Signal Processing in NMR, MRI Institute of Biomedical Research, Detroit). We used only T2WI and DWI data for visual examination of the occurrence and location of cerebral ischemia. According to the theory of the measurement of T2 relaxation time, we used the multi-echo T2 map results to reconstruct T2 map images. On the reconstructed T2 maps, all of the T2 relaxation time was acquired from a single coronal slice of the septal hippocampus (visually located at 6–7 mm from the front tip of the frontal cortex, which based on the rat brain atlas) [13]. The selected slice displayed the largest area of edema. Measurement of the T2 relaxation time was performed in two regions of interest (ROI) on this coronal slice: the ischemic ipsilateral hemisphere and the contralateral hemisphere, excluding the ventricles (Fig. 1A, B, C, D).

On the SWI and SWI-filtered phase images, the CMBs were numbered sequentially from the rostral-most slice to the caudal-most slice on all coronal slices over the entire brain of each rat. The CMBs on the phase images appeared as small spherical regions of hypointensity. Because of the volume averaging, the small CMBs could not be ignored with 1-mm slice thickness. The locations of the CMBs and the areas with T2 hyperintensity/reduced ADC were observed on T2WI, ADC maps, SWI and anatomical drawings within similar slices. Using minimum intensity projections (mIPs), the CMBs were easily distinguished from a transverse section of a vessel. Two observers independently evaluated the CMBs. The CMBs stated in the results were agreed upon by the two observers.

2.4. Histopathological evaluation

All rats were anesthetized with 10% chloral hydrate (0.33 ml/100 g) and then perfused transcardially with 0.1 M phosphate buffer saline (PBS) followed by 4% paraformaldehyde (PFA) in 0.1 M PBS after MRI scanning. The brains were post-fixed overnight in 4% PFA and cryoprotected in 20% and 30% sucrose; then, 30-μm slices were cut using a freezing stage microtome. The locations of the brain specimens corresponded to the scanning direction of MRI slices.

Hematoxylin and eosin (H&E) staining was used to validate the results of the MRI scanning. The selected slices, which correspond to the position of the MRI slices, were examined and recorded using digital photomicrography (Olympus AH-3) under 100× and 200× magnifications. The edema and morphological changes in the entire brain were recorded using a digital single-lens reflex camera (Canon EOS 550D). The CMBs and cell nucleus of the H&E histological images were measured at the regions corresponding to the ROIs of T2WI and SWI data.