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Original contribution

Altered white matter integrity and functional connectivity of hyperacute-stage cerebral ischemia in a rat model

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ABSTRACT

Ischemic stroke is accompanied by structural deformation and functional deficits in the affected hemisphere. Within a couple of hours after symptom onset, the accurate identification of brain characteristics is critical to design the therapeutic strategies and it can potentially improve overall brain tissue viability by minimizing irreversible brain damage. In this study, white matter integrity and functional connectivity within 2–4 h after right middle cerebral artery occlusion in rats were investigated using multimodal magnetic resonance imaging. During this stage, diffusion tensor image (DTI) revealed that fractional anisotropy along the ipsilesional external capsule was slightly increased as compared with preoperative baseline. Resting state functional MRI (rs-fMRI) showed that the inter-hemispheric functional connectivities from primary motor (M1), primary somatosensory of forelimb (S1FL), and barrel field (S1BF) seeds were considerably reduced at the hyperacute stage. Fractional amplitudes of low frequency fluctuations (fALFF) from rs-fMRI were significantly enhanced at the hyperacute stage in the frequency spectrum between 0.01 and 0.08 Hz. In addition, the changes in fALFF were negatively correlated with the number of functionally connected voxels in M1, S1FL and S1BF. Our results suggest that these techniques are useful tools to evaluate remarkable brain changes in the hyperacute stage of ischemic stroke.

1. Introduction

Ischemic stroke accounts for approximately 80–88% of all strokes [1,2]. A clinical diagnosis of ischemic stroke should be made within a couple of hours of symptom onset by accurately identifying the exact lesion and extent of brain damage in order to facilitate treatment. Such rapid response can effectively improve overall brain tissue viability by minimizing irreversible brain damage. Therefore, rapid and accurate assessment of disease status within clinically available time employing non-invasive diagnostic methods such as neuroimaging tools is essential. Multimodal magnetic resonance imaging (MRI) is widely used in clinical and experimental research to assess the extent of brain damage in patients with cerebral ischemic stroke, providing information on pathophysiology of the disease including vascular patency, areas of hypo-perfusion, metabolism, and structural damage [3,4]. A combination of perfusion-weighted imaging (PWI) and diffusion-weighted imaging (DWI) is commonly used for grading clinical severity as well as assessing the viability of ischemic brain tissue [5,6]. This approach (i.e., mismatch model) utilizes extrinsic geometrical legion boarders to classify the infarct core, penumbra, and peri-infarct area, depending on MRI contrast differences. However, this approach has limitations for interpreting disease status and progress of cerebral ischemic stroke because reperfusable tissue within the perfusion deficit area is sometimes revealed in PWI, and the hyperintense region revealed on DWI is often capable of being recovered after reperfusion [7–9]. Therefore, there is demand for additional parameters to diagnose cerebral ischemic stroke, especially during the early stage of the disease.

In this work, we investigate white matter (WM) integrity and functional connectivity using diffusion tensor imaging (DTI), resting state functional MRI (rs-fMRI), and blood oxygen level dependent functional MRI (BOLD-fMRI), which can evaluate neuronal interactions and activities that occur at the hyperacute stage of cerebral ischemic stroke. DTI provides insight into microstructural organization by measuring diffusive characteristics of water molecules within tissue and can be used to evaluate WM integrity. Using a diffusion tensor model, multiple contrast images, such as fractional anisotropy (FA) and mean diffusivity (MD) are generated to represent the diffusion

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properties within a voxel [10]. Since DTI is mainly associated with WM, which is a highly oriented structure with neuronal fiber, these properties can allow us to gain useful information for WM fiber integrity in the brains of patients with cerebral ischemia [11–13].

BOLD-fMRI is extensively studied for understanding brain function [14–16]. BOLD contrast originates from the intra-voxel magnetic field heterogeneities induced by paramagnetic deoxyhemoglobin. Such regional perturbations occur due to enhanced neuronal activity and metabolism during overt tasks or experimental input in the sensory [17], motor [18], and cognitive [19] domains, which allows us to image brain function using MRI. In most studies of ischemic stroke using animal models, functional response using BOLD-fMRI was investigated mostly during acute (<24-h after onset) and chronic stages (>2-weeks after onset), which often complicates the prediction of functional outcome [20–22]. The functional response during the hyperacute stage (within 2 to 4-h after the onset) may be necessary, which might provide valuable information for identifying disease status as well as predicting functional outcome. However, it is complicated to manipulate experimental design and regulate physiological condition to obtain reliable BOLD-fMRI signal responses for the hyperacute stage of disease. The alternative is to use rs-fMRI, which is a recently updated method from which functional connectivity between distant brain regions is extracted based on spontaneous low-frequency fluctuations. Although the meaning of the rs-fMRI signal remains controversial [23], evidence has suggested that resting state low frequency fluctuations are related to neuronal activation task performance [24]. The methodological advantage of rs-fMRI is that it can be performed without requiring an overt task or external input. Therefore, it is applicable to experimental animals that are extremely fragile, such as those during the hyperacute stage of ischemic stroke [25–27]. For analysis of rs-fMRI, functional connectivity based on temporal correlation is routinely employed using seed-based analysis [23] and/or multivariate decompositions such as independent component analysis (ICA) [28,29]. Recently, besides temporal correlation, amplitudes of low frequency fluctuations (ALFF) have attracted considerable attention as a quantitative measurement [30,31], in which the fluctuations of the rs-fMRI signals are generally present between 0.01 and 0.08 Hz frequency bands. According to ALFF, the amplitude of resting state BOLD fluctuations reflects spontaneous regional neuronal activities [32]. ALFF is usually calculated in the low frequency band, and is scaled by the global mean ALFF value across voxels. However, ALFF is prone to noise from physiological sources, particularly near the ventricles and large blood vessels. To address this problem, Zuo and his colleagues [33] proposed fractional ALFF (fALFF), which is insensitive to noise sources compared to the ALFF. Therefore, along with functional connectivity, fALFF can be helpful to identify spontaneous coherent BOLD signals and to characterize healthy brain function as well as dysfunction.

Although the uses of DTI and fMRI for stroke related studies have independently been well established, the relationship between WM integrity and functional connectivity has not been explored in detail in experimental stroke animal models. In this study, we simultaneously investigate WM integrity and functional connectivity in rats with cerebral ischemia during the hyperacute stage. In addition, BOLD-fMRI was applied to compare functional outcomes acquired from rs-fMRI. Our hypothesis is that using combined data for WM integrity and functional connectivity can provide useful information to identify specific changes associated with neuronal interactions and activities during the hyperacute stage of ischemic brain stroke.

2. Materials and methods

2.1. Animal preparation

All animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the Samsung Biomedical Research Institute (SBRI) and experiments were performed in accordance with the guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International) and Institute of Laboratory Animal Resources (ILAR). Adult male Sprague–Dawley rats (6–7 weeks old, n = 12, 300 to 350 g) were subjected to focal cerebral ischemia by transient middle cerebral artery occlusion (t-MCAO) [34]. Rats were initially anesthetized with 5% isoflurane and maintained with 1% to 2% isoflurane in a mixture of O_2 and air gases (30%:70%) under spontaneous respiration during surgery. Blood oxygen saturation (SpO_2) and heart rate were continuously monitored with a pulse oximeter (SurgiVet® Inc., Norwell, MA, USA) and body temperature was maintained at 36 ± 1 °C. The blood flow of the right middle cerebral artery (MCA) was blocked for 90 min with 5.0 silk thread (Ethicon Inc., Somerville, NJ, USA). After 90 min, the inserted silk thread was removed and reperfusion was performed.

For MRI experiments, rats that underwent the orotracheal intubation were anesthetized initially using 5% isoflurane and maintained by using an artificial ventilation system that supplied 1.4% isoflurane in a mixture of O_2 and air gases (30%:70%). Rats were laid on a water circulating heating pad to maintain body temperature (36 ± 1 °C). Each rat head was carefully fixed with earplugs and a tooth bar to reduce head motion artifacts during imaging. The physiological conditions such as end-tidal CO_2 ($EtCO_2$), arterial oxygen saturation (SpO_2), heart rate, and respiration rates were consistently monitored with capanograph combined with pulse meter (SurgiVet® Inc.) during the entire MRI experiments.

2.2. MRI data acquisition

MRI measurements were performed with a 7 T MR System (Bruker-Biospin, Fallanden, Switzerland). A quadrature birdcage coil (72 mm i.d.) (Bruker-Biospin) and an actively decoupled phased array head coil were used for signal excitation and reception, respectively. The rats underwent MRI under preoperative control conditions as the baseline measure, and less than 4 h post-stroke as the hyperacute-stage measure. For anatomical imaging, T2 weighted MR images (T2WI) were acquired using a fast spin-echo sequence with the following parameters: repetition time (TR)/echo time (TE) = 3000/60 ms, number of excitations (NEX) = 4, echo train length = 6, in-plane resolution = 120 × 120 μm^2, slice thickness = 1.5 mm, number of slices = 8 coronal slices, and acquisition time = 8 min 24 s. DTI for WM integrity and rs-fMRI for functional connectivity were sequentially performed. DTI was acquired using diffusion-weighted spin-echo echo planar imaging sequences (EPI): TR/TE = 4500/37 ms, NEX = 3, field of view (FOV) = 30 × 30 mm^2, matrix = 192 × 192, in-plane resolution = 156 × 156 μm^2, slice thickness = 0.75 mm, number of slices = 18 coronal slices, gradient direction = 30, diffusion gradient duration (δ) = 5 ms, diffusion gradient separations (Δ) = 15 ms, b-values = 1000 s/mm^2, and acquisition time = 31 min 30 s. rs-fMRI was acquired within less than 4 h using a single-shot gradient-echo EPI sequence with the following parameters: TR/TE = 1000/15 ms, flip angle = 45°, NEX = 1, FOV = 30 (readout) × 15 (phase encoding) mm^2, matrix = 64 × 32, in-plane resolution = 469 × 469 μm^2, slice thickness = 1.5 mm, number of slices = 8, and number of repetitions = 300.

BOLD-fMRI was also performed using electrical forepaw stimulation for comparisons with the rs-fMRI data. BOLD-fMRI was acquired within less than 4 h using the same acquisition parameters as rs-fMRI for each rat. Electrical stimulation was generated by a constant current generator (Pulse-master TMA300 Multi-Channel Stimulator; World Precision Instruments, Sarasota, FL, USA). We derived electrical stimulus pulses (1.0 ms pulse width and 1.4 mA current) at a frequency of 12 Hz. Two needle-electrodes were inserted into the forepaw of the rat under the plantar skin between digits two and four. Each stimulus run consisted of a 20 s