Quantification of MRI and MRS characteristics changes in a rat model at different stage of cerebral ischemia

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Background: A better understanding the mechanisms of cerebral ischemia is important both for diagnosis and treatment.
Objective: The study aimed to quantify several characteristics of magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) to indicate the brain tissue changes at different stage of cerebral ischemia in rats.
Methods: In the present study, a rat model of cerebral ischemia was established by middle cerebral artery occlusion (MCAO) in the left hemisphere. MRI and MRS were performed on 15 Sprague Dawley rats 4 H, 24 H, and 1 W after MCAO. Apparent diffusion coefficient (ADC), relative ADC including FNR, PNR, PNF, and metabolite ratio NCC were proposed to reflect the changes of water diffusion and metabolism in brain tissue.
Results: ADCs of focal zone and penumbra zone from 1 W group were significantly larger than those from 4H group, respectively (both $p < 0.05$). PNR and PNF of 24H and 1 W groups were significantly less than 4H group (all $p < 0.01$). NCCs of focal zone and penumbra zone were significantly less than the normal zone within 4H, 24H, and 1 W groups, respectively (both $p < 0.01$). While NCCs of penumbra zone from 24H and 1 W groups were significantly larger than 4H group (both $p < 0.01$).
Conclusion: We conclude that combination of MRI and MRS characteristics can provide significant indicators for ischemic damage at different stage of cerebral ischemia in a rat model.

Keywords: Magnetic resonance imaging (MRI), Magnetic resonance spectroscopy (MRS), Cerebral ischemia, Middle cerebral artery occlusion (MCAO), Rat

Introduction

Cerebral ischemia is a condition in which there is insufficient blood flow to the brain to meet metabolic demand. This leads to poor oxygen supply or cerebral hypoxia and thus to ischemic stroke, which may involve impairments in body movement, vision and speaking. Neurological deficits are common sequelae of cerebral ischemia and neurological recovery has been accompanied by changes in brain activation patterns. What happen in the brain structure and metabolism accompany by cerebral ischemia are not very clear.

Rodent models of cerebral ischemia have contributed greatly to our understanding of the mechanisms of the occurrence and evolution of ischemic stroke. Magnetic Resonance Imaging (MRI) has been used previously in animal models to non-invasively and quantitatively evaluate the progression of focal ischemic lesions.[1,2] The neurological impairments and restoration have been linked to the presence of post ischemic lesions on T2-weighted MRI and diffusion weighted imaging (DWI).[3]

T2-weighted MRI provides a degree of certainty in assigning irreversible ischemia. DWI monitors disturbances of ion homeostasis, water distribution, and tissue microstructure.[3] The intensity of each image voxel reflects the best estimate of the rate of water diffusion at that location. DWIs visualize the ischemic core that is likely to proceed into infarction within a few hours from the onset of ischemia.[4] Following an ischemic stroke, DWI is more sensitive to the changes occurring in the lesion than the traditional MRI measurements such as T1 or T2 relaxation rates. Although the apparent diffusion coefficient (ADC) measured from DWI has been shown to decrease immediately after the onset of ischemia,[5] more investigations would be helpful to support the viewpoint that quantitative diffusion coefficient

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changes can be successfully applied for the essential distinction between the ischemic core and a potentially salvageable penumbra zone.

Several analytical approaches have been explored to predict the severity of the brain lesion from multiparametric MRI data acquired in acute stroke.[6] These studies exploited diffusion, perfusion, and T2 MR data according to the biophysical and pathologic information from the ischemic regions provided by these parameters. Multiparameter studies using quantitative T1, T2, and diffusion MRI have shown the potential to predict the infarct size with prior knowledge of the location of stroke.[7,8] These observations are significant as far as the prediction of the ischemic core is concerned in the hyperacute phase of the stroke. However, diffusion mismatch is not sufficient to predict the outcome of ischemic brain.[9,10]

Magnetic resonance spectroscopy (MRS), which is based on nuclear magnetic resonance can give information on the severity of ischemic injury by measuring metabolites after cerebral ischemia. MRS provides a non-invasive diagnostic tool for the biochemical characterization of pathophysiological processes in the brain, which allows assessment of several metabolite concentrations known to be altered following cerebral ischemia.[11,12] In brain MRS, the resonances of interest are N-acetyl aspartate (NAA), choline (Cho), creatine (Cr), myo-inositol (mI), lactate (Lac), lipids (Lip), glutamine and glutamate (Glx), and amino acids.[13] In particular, a decrease in NAA has been shown to correlate with neuronal loss which was used to predict outcome after stroke.[14,15]

Most of the previous study investigated cerebral ischemia by partial combination of MRI, immunohistochemistry, behavioral status, MRS, and histopathology.[1,2] They mainly focused on the characteristic changes in the lesion volume[3,16] or specific sensorimotor areas[2] in transient cerebral ischemia, for example, at time points between 3 and 48 h,[16,17] which is not sufficient to analyze the cerebral ischemia comprehensively.

The purpose of this study was to quantify and compare both MRI and MRS characteristics of focal, penumbra, and normal zones 4 h, 24 h, and 1 week after the onset of ischemia in rats. It helps to elucidate the mechanisms underlying brain impairment and variation, and aids in optimization of diagnostic methods.

Materials and methods

Animals

Adult male Sprague–Dawley (SD) rats aged 8–10 weeks, weighing 200–250 g were purchased from BEIJING HFK BIOSCIENCE CO., LTD in this experiment (License No. SCXK (Beijing), 2009- 0007). They were housed in transparent Makrolon cages during 12 h day/night cycle in a temperature-controlled room (25 °C) with free access to food and water. This study received permission from the Animal Care and Research Committee of Beijing University of Technology, Beijing, China.

Middle cerebral artery occlusion

Rats were anesthetized by an intramuscular injection of 10% chloral hydrate (0.4 ml/100 g, provided by department of pharmacology, Peking union medical college hospital) and placed in the prone position on a stereotaxic system (DW-5, Chengdu Taimeng Technology CO., Ltd., China). The rat's left carotid region was exposed through a midline cervical incision (about 20 mm). The left common carotid artery (CCA), internal carotid artery (ICA), and external carotid artery (ECA) were isolated from the surrounding tissue. A nylon suture (product code: 2432-50, Beijing ShaDong biological technology CO., LTD) coated with poly-l-lysine, with its tip shaped round, was inserted into the left ICA and advanced to a point approximately 18–20 mm distal to the bifurcation of ECA and ICA, thereby occluding the origin of the middle cerebral artery (MCA). During surgery, the core temperature of the rat was maintained at 37 ± 1 °C by a heating lamp.

After recovery from anesthesia, rats were scored as follows: (0) no neurological deficit was observed, (1) failure to extend the forepaw of the right side, (2) circling to the right side, (3) falling to the right, and (4) unable to walk and had a depressed level of consciousness. Only the rats with a Longa's score 1–2 were selected for our study.[18]

Group of rats

The qualified 15 SD rats were selected and randomly divided into three groups: 4 h after MCAO (4 H group), 24 h after MCAO (24 H group), and 1 week after MCAO (1 W group) with 5 rats in each group.

Magnetic resonance scanning

MRI experiments were performed in a horizontal 7 T magnet (Bruker, Germany). A surface coil (2.3 cm × 1.5 cm) was used for brain imaging. Multislice coronal spin-echo diffusion-weighted images (DWI) were acquired with an echo planar (EP) diffusion-trace MRI sequence (matrix size = 108 × 128, TR = 4500 ms, TE = 35 ms, FOV = 33 × 40 mm^2, FA = 90°, b0 = 600 s/mm^2).

T2-weighted images were acquired using a SE sequence (TE = 41 ms, TR = 3140 ms, FOV = 40 × 40 mm^2, matrix size = 240 × 320, slice thickness = 0.7 mm, FA = 180°).

Spectra acquisition was performed using a chemical shift imaging (CSI) (TE/TR = 135/1500 ms) spectroscopy method. Measurements were made in the regions of interest (ROI).

Data analysis

The normal, penumbra and focal zone were determined on T2WI by an experienced radiologist. Focal zone was selected in the highlight region and normal zone was selected in the contralateral region.

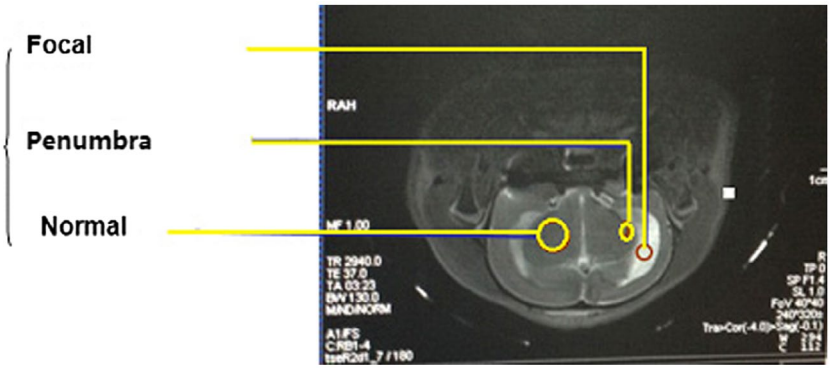

Figure 1 Example of focal, penumbra, and normal zone in T2 image

In pathology and anatomy, the penumbra was the area surrounding an ischemic focal zone, shown in Fig. 1.

ADC reflects the degree of diffusion of water molecules through different tissues. ADCs of three ROIs selected, respectively, in the normal, penumbra and focal zone were calculated and averaged by software Functool (GE AW 4.2). Relative ADCs were proposed to overcome the between-rat differences and reflect the MRI characteristics at different stage of ischemic stroke. Here, three parameters were defined as:

$$\mathrm{FNR} = \frac{\mathrm{FZ} - \mathrm{NZ}}{\mathrm{NZ}} \tag{1}$$

$$\mathrm{PNR} = \frac{\mathrm{PZ} - \mathrm{NZ}}{\mathrm{NZ}} \tag{2}$$

$$\mathrm{PNF} = \frac{\mathrm{PZ} - \mathrm{NZ}}{\mathrm{FZ}} \tag{3}$$

Where FZ, NZ, and PZ are ADCs in focal, normal, and penumbra zones, respectively.

FNR reflects the ADC in focal zone relative to normal zone, PNR reflects the ADC in penumbra zone relative to normal zone and PNF reflects the ADC in penumbra zone relative to normal zone normalized by focal zone. FNR, PNR, and PNF were calculated in 4H, 24H, and 1 W group, respectively.

Choline(Cho), N-Acetylaspartate (Naa) and Creatine (Cr) of three ROIs selected, respectively, in the normal, penumbra, and focal zone were measured on MRS and averaged. Then a new parameter NCC was defined to reduce the effect of between-rat variability.

$$\mathrm{NCC} = \frac{\mathrm{Naa} - \mathrm{Cho}}{\mathrm{Cr}} \tag{4}$$

Statistical analysis

Experimental measurement data was expressed as Mean ± SD and was statistically processed by SPSS 18.0 software (IBM Corporation, New York, United States) using one-Way ANOVA analysis. The ADCs were compared within and between groups and the relative parameters of focal and penumbra zones were compared between the three groups. $p < 0.05$ was considered statistically significant.

Results

MRI assessment

ROI selection

Figure 2 shows the DWI (left column) and T2WI (right column) in 4H, 24H and 1 W groups. The circles in DWI are ROIs selected in normal, penumbra, and focal zones, in which 1, 2, 3 are ROIs in focal zone, 4, 5, 6 are in normal zone and 7, 8, 9 are in penumbra zone.

Comparison of ADC between the three ROIs

Table 1 shows that the ADC in focal zone was significantly smaller than normal zone within 4H, 24H, and 1 W groups (all $p < 0.05$). ADC in penumbra zone was significantly smaller ($p < 0.05$) than normal zone within 4H and 24H group, but not significantly different in 1 W group.

ADC changes with ischemic time following MCAO for the three ROIs

From Table 1, it can also be seen that the ADCs in focal and penumbra zones from 1 W group were significantly larger than those from 4H group, respectively (both $p < 0.05$).

Relative ADC changes with ischemic time following MCAO

The relative ADCs are summarized in Table 2. FNR was not significantly different between 4H, 24H, and 1 W groups ($p > 0.05$). PNR and PNF of 24H and 1 W groups were significantly less than 4H group (all $p < 0.01$).

The results indicated that FNR increased, PNR and PNF decreased with ischemic time following MCAO.

MRS changes with ischemic time following MCAO

Figure 3 gives an example of MRS and its corresponding ROI from penumbra zone in 4 H, 24H, and 1 W group, where ROI was marked using a red rectangle.

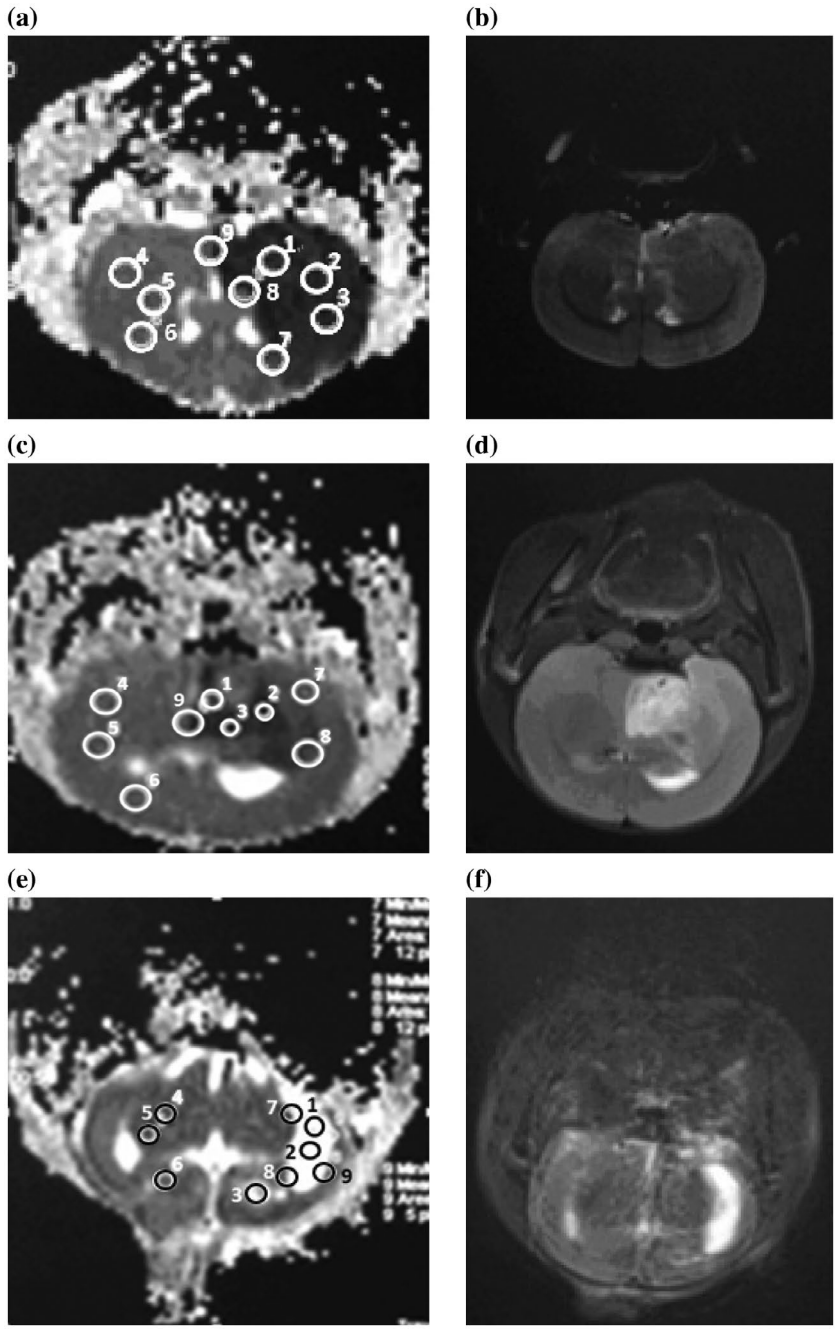

Figure 2 DWI and T2 from (a) & (b) 4H group; (c) & (d) 24H group; (e) & (f)1 W group. 1, 2, 3 are in focal zone 4, 5, 6 are in normal zone and 7, 8, 9 are in penumbra zone

Table 1 ADC in focal, penumbra and normal zones from 4H, 24H, and 1 W groups

Group	Focal zone	Penumbra zone	Normal zone
4H	492 ± 21	593 ± 33	791 ± 13
24H	517 ± 83	644 ± 39	756 ± 46
1 W	588 ± 93*	764 ± 42*	714 ± 24

*$p < 0.05$ with comparison referred to the 4H group.

Table 2 Relative ADC changes with ischemic time following MCAO

Group	FNR	PNR	PNF
4H	37.8 ± 3.2	25.0 ± 1.2	40.3 ± 2.7
24H	44.1 ± 2.1	14.8 ± 1.2*	26.5 ± 1.3*
1 W	47.7 ± 5.3	6.9 ± 0.9*	8.3 ± 0.3*

*$p < 0.01$, with comparison referred to the 4H group.

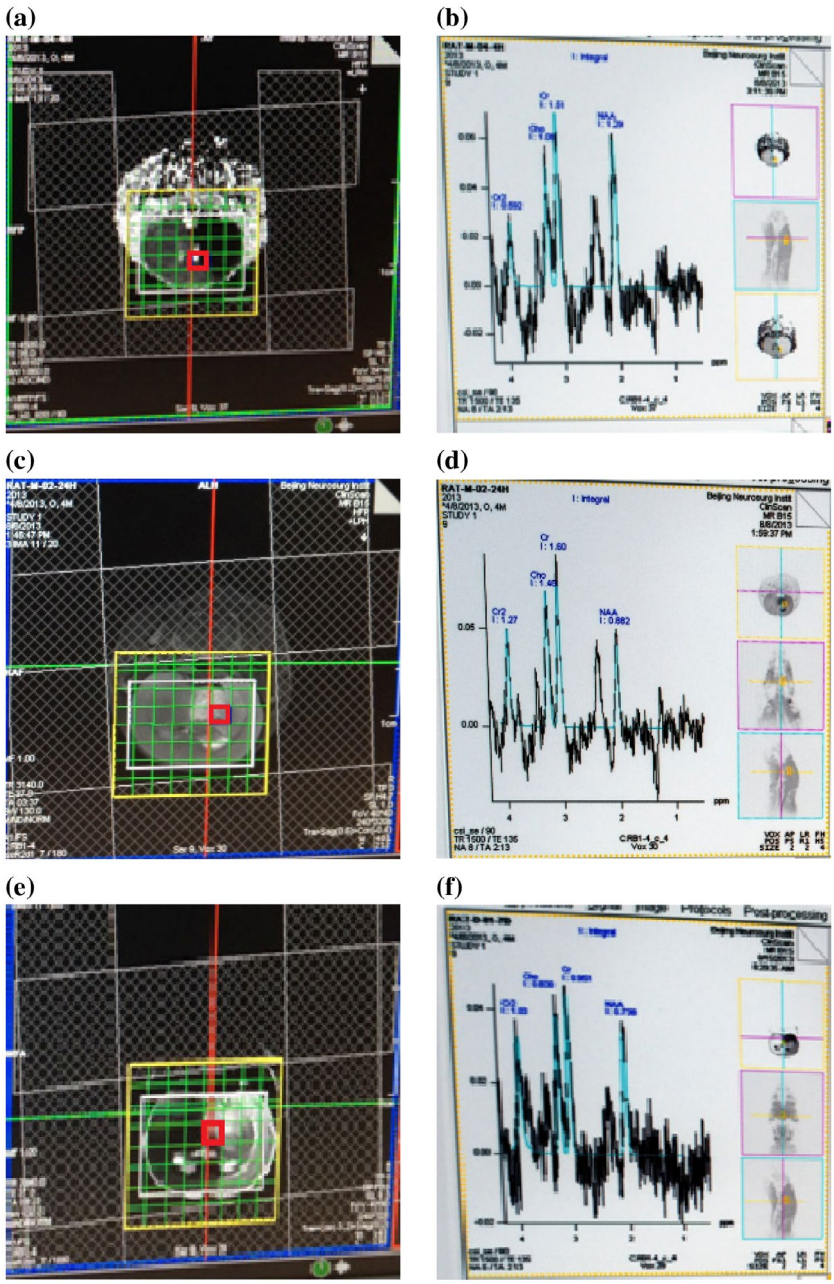

Figure 3 ROI from penumbra zone and its corresponding MRS. (a) and (b) 4H group; (c) and (d) 24H group; (e) and (f)1 W group

Table 3 NCC in focal, penumbra and normal zones from 4H, 24H, and 1 W group

Group	Focal zone	Penumbra zone	Normal zone
4H	3.4 ± 0.3	7.1 ± 1.2	102.8 ± 5.3
24H	4.4 ± 0.2	29.0 ± 2.3*	107.8 ± 7.5
1 W	7.7 ± 1.3	148.0 ± 13.1*	160.8 ± 11.1

*$p < 0.01$ with comparison referred to the 4H group.

The NCCs are summarized in Table 3. NCCs of focal zone and penumbra zone were significantly less than the normal zone within 4H, 24H, and 1 W groups, respectively ($p < 0.01$). Both NCCs of focal zone and normal zone presented no significant difference between 4H, 24H, and 1 W groups ($p > 0.05$). While NCCs of penumbra zone from 24H and 1 W groups were significantly larger than 4H group ($p < 0.01$). NCC increased with ischemic time following MCAO.

Discussion

Brain edema is one of the main complications in cerebral ischemia.[3] In DWI, the intensity of each image voxel reflects the best estimate of the rate of water diffusion at that location. Because the mobility of water is driven by thermal agitation and highly dependent on its cellular environment, diffusion changes are correlated to the clinical deficit and are potentially useful for early diagnosis and

longitudinal evaluation. For instance, DWI is more sensitive to early changes after a stroke than more traditional MRI measurements such as T1 or T2 relaxation rates. To elucidate diffusion and relaxation effects on image contrast, one may obtain quantitative images of the diffusion coefficient, or more exactly the ADC.

In our study, the reduced ADC of water in focal and penumbra zones are in agreement with the previous studies.[17] The relative ADC ratios presented are helpful to reflect the water diffusion of focal and penumbra zone relative to normal zone after MCAO. It was observed that the relative ADC in the focal zone (FNR) did not change significantly as time elapsed, which indicated the injury in the focal zone might be irreversible. Immediately following the stroke, blood flow and therefore oxygen transport is reduced locally, leading to hypoxia of the cells near the location of the original insult. This can lead to hypoxic cell death (infarction) and amplify the original damage from the ischemia; however, the penumbra area may remain viable after an ischemic event due to the collateral arteries that supply the penumbral zone. PNR and PNF being significantly decreased with time suggested that water diffusion of penumbra zone approached to normal zone. Penumbra is under an elevated risk of infarction, and monitoring its variation may provide important information for clinicians.[19]

N-acetylaspartate (Naa) is the second most abundant metabolite in the human central nervous system (CNS). Naa emits the strongest signal in MRS of the human brain, which has clinical significance. Many neurological disorders involving neuronal loss or dysfunction result in reductions in brain Naa levels. Choline (Cho) is the precursor molecule for the neurotransmitter acetylcholine, which is involved in many functions including memory and muscle control. It is used in the synthesis of the constructional components in the body's cell membranes. Creatine (Cr) is a nitrogenous organic acid that occurs naturally in vertebrates and can be a reservoir to store energy as phosphocreatine.[20]

Hirt's study compared NAA, taurine, Glu, and Gln between 3 h and 8 h and considered NAA + Glu + Tau as a predictor of an irreversible lesion.[16] To reduce the effect of between-rat variability, our study focused on the within-rat ratio of NAA, Cho, and Cr and their changes with time (4H, 24H, and 1 W). The proposed NCC in this study shows the evident differences of normal, penumbra, and focal zones within 4H, 24H, and 1 W groups and indicates the significant changes in penumbra zone between 24H, 1 W, and 4H groups, which may be useful to reflect the metabolic changes at different stage of cerebral ischemia.

MRI and MRS characteristics can be performed in animal models of 'lacunar' stroke, which may have practical implications to the treatment of cerebral small vessel disease with drugs to improve endothelial function given the non-atherothrombotic etiology of small vessel disease.[21] The prior presence of a transient ischemic attack (TIA) is associated with an early good outcome in non-lacunar ischemic strokes, which suggesting a neuroprotective effect of TIA possibly by inducing a phenomenon of ischemic tolerance.[22] TIA model will be studied in the next step, and the penumbra location and focal size could be measured and investigated combined with the present characteristics in a further study.

In summary, our investigation illustrates that ADC measured using DWI is a viable surrogate marker of the damage and variation of brain tissue after a stroke. The relative ratio of Naa, Cho, and Cr can reflect the metabolic changes in normal, focal, and penumbra zones at different stage of cerebral ischemia. The combination of MRI and MRS techniques provides a good understanding of the evolution of ischemic damage and to probe the efficacy of future therapeutic interventions in a rat model of stroke.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

The authors give special thanks to Dr Wangsheng Lu for his instruction to MCAO operation.

Funding

This work was supported by Beijing Natural Science Foundation [grant numbers 7132028 and 7132021] and National Natural Science Foundation of China [grant number 81441053].

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