Reversible Loss of N-acetyl Aspartate after Transient Middle Cerebral Artery Occlusion in Rats: A Longitudinal Study by Proton Magnetic Resonance Spectroscopy

J. Qian[1,2], and H. Lei[1]

[1]State Key Laboratory of Magnetic Resonance and Atomic and Molecular Physics, Wuhan Institute of Physics & Mathematics, Chinese Academy of Sciences, Wuhan, Hubei, China, People's Republic of, [2]MR R&D, Philips and Neusoft Medical Systems Co., Ltd., Shenyang, Liaoning, China, People's Republic of

INTRODUCTION Numerous previous studies with in vivo ^{1}H MR spectroscopy (MRS) have shown that cerebral ischemia, in both clinical stroke patients and animal models, results in significantly decreased N-acetyl aspartate (NAA) contents in the affected brain regions[1]. It is widely accepted that NAA can be used a biochemical marker to assess neuronal viability/integrity in cerebral ischemia[1]. However, this concept has recently been questioned, based on evidence showing that, the initial NAA loss observed in certain stroke and multiple sclerosis (MS) patients and in a photothrombotic cortical occlusion permanent ischemia model can reverse spontaneously over a period of time[1,2]. Few previous studies have measured the chronic changes of NAA and other cerebral metabolites in the transient cerebral ischemia models. In this study, we measured the longitudinal changes of NAA and other metabolites in the brain of rats subjected to a 15-min transient middle cerebral artery occlusion (MCAO) using in vivo ^{1}H MRS, and correlated the spectroscopic results to histological/immunohistological findings.

MATERIALS and METHODS The right middle cerebral arteries (MCA) of male Wistar rats, weighing 200-250 g, were occluded by suture insertion for 15 minutes[3]. MR examinations were carried out at 1d, 3d, 7d, 14d, 4 weeks, 8 weeks and 16 weeks (n=6, 5, 5, 6, 6, 5 and 3, respectively) post-ischemia. Isoflurane (1.5-2%) anesthetized rats were subjected to MRI/MRS examinations performed on a Bruker Biospec 4.7 T /30 cm spectrometer. T_2-weighted imaging was performed with FOV 4 cm×4 cm, matrix size 128×128, slice thickness 0.8 mm, TR 2500 ms and TE 30-180 ms. A PRESS sequence was used to acquire localized ^{1}H spectra from the ipsilateral and the contralateral striatum with voxel size 2.5 cm×2.5 cm×2.5 cm, TR 1 ms, TE 136 ms and 128-512 averages. Integrated peak areas were measured and normalized to that of the Cr resonance at 3.02 ppm in the contralateral striatum. After MR examinations, the rats were transcardially perfused with 4% paraformaldehyde. The brains were removed and cut into sections, which were then stained with glial fibrillary acidic protein (GFAP) and hematoxylin and eosin (H&E). The number of surviving viable-looking neurons in the ischemic striatum was estimated from H&E stained brain sections, and the surviving neuronal ratio (%) was calculated by dividing the average number of viable-looking neurons in the ipsilateral dorsolateral striatum (DL Str) by that in the contralateral side. Statistical comparisons were carried out, as appropriate, by two-tailed Student's paired t-tests and one-way ANOVA followed by post hoc Tukey's test.

RESULTS Relative to the T_2 of the contralateral DL Str, the T_2 of the ischemic DL Str increased significantly ($p<0.05$) by about 12% at 1d and 3d post-ischemia, but thereafter decreased significantly between 7d and 16 weeks ($p<0.05$). The relative NAA peak area in the ipsilateral striatum decreased significantly by about 40% at 1d, recovered gradually thereafter, and was no longer statistically different from that in the contralateral striatum at 14d (Fig.1). The surviving neuronal ratio in the ipsilateral DL Str was 11.9±2.3% at 1d, and had a trend to decrease further between 1d and 16 weeks. The GFAP immunoreactivity in the ischemic DL Str started to be visible at 1d, increased significantly with time up to 4 weeks and persisted until 16 weeks.

DISCUSSION The longitudinal T_2 changes and hisological/immunohistological findings in the ischemic lesions observed in this study agreed well with the observations of previous studies using the same MCAO ischemia model[4]. In the presence of extensive selective neuronal loss that persisted between 1d and 16 weeks, the NAA level in the ischemic lesion decreased acutely, recovered gradually with time and normalized to the control level by 2 weeks after ischemia. The time course of NAA recovery coincided with that of GFAP-immunoactivity increase observed post-ischemia. These results give further support to the hypothesis that nonneuronal cells, particularly the reactive oligodentrocytes and astrocytes, may contribute to NAA and/or *N*-acetyl aspartyl-glutamate (NAAG) production in the subacute and chronic stages of cerebral ischemia[2].

REFERENCES [1] Demougeot, C. et al, *J Neurochem*, **90** (2004) 776-783; [2] Demougeot, C. et al, *J Cereb Blood Flow Metab*, **23** (2003) 482-489; [3] Wang, X.X.et al, *Magn Reson Med*, **56** (2006) 474-480; [4] Fujioka, M. et al , *Ann Nerol*, **54** (2003) 732-747.

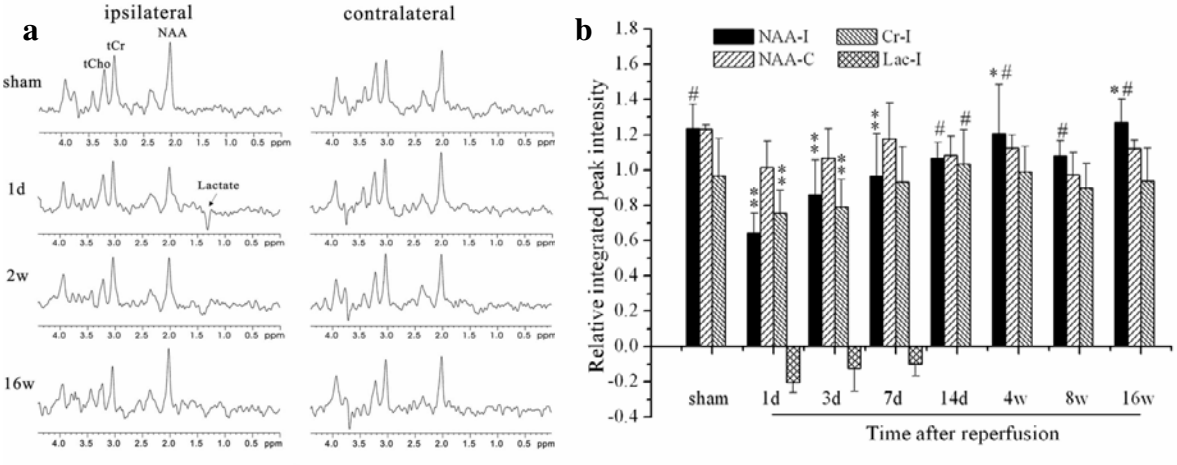

Fig 1. (a) ^{1}H MR spectra acquired from a sham rat and an ischemic rat at 1d, 2 weeks (2w) and 16w post-ischemia; (b) Changes of relative signal intensities of ipsilateral (-I) NAA, creatine (Cr) and lactate (Lac) and contralateral (-C) NAA with time. **p<0.05, compared to contralateral; #p<0.05, compared to 1d; and *p<0.05, compared to 3d.