In vivo ^{1}H MRS measurements of acetate in mouse striatum after permanent focal middle cerebral artery occlusion

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INTRODUCTION Studying ischemic models including transgenic modified mice would help us identifying potential biomarkers and treatment agents. MR techniques are ideal tools for *in vivo* studies. Higher magnet fields have been well-established to sustain sufficient sensitivity not only to locate ischemia core but also to monitor neurochemical alterations after transient middle cerebral artery occlusion (tMCAO) in mice (1 and references therein). Therefore, studying neurochemical constituents (19 metabolites) after permanent MCAO (pMCAO) would be feasible and thus shred insights in better understanding ischemia induced cascading damages. However, previous in vitro ^{1}H MRS studies suggested that two hydrolytic metabolites from neuronal marker N-actylaspartate(NAA), asparate (Asp) and acetate (Ace), presented noticeable concentrations after pMCAO (2). Unlikely, methyl proton chemical shifts of Ace at physiological conditions presented one single resonance at 1.90ppm (3) and very close to one of γ-aminobutytic acid (GABA) resonances (1.89ppm) (3). Although LCModel has been shown robustly quantify MR spectra with pre-known metabolite information (4), the overlapping might remain problematic by introducing systematic errors to quantify Ace. Alternatively, ischemic stroke induced accumulation in GABA (5), which could severe problems in measuring Ace using short echo ^{1}H MRS. We noticed that GABA, one of J-coupled metabolites, could be minimized at TE values ~1/2J due to its J-modulation (6). Therefore, the measurements of Ace with minimal GABA contribution can be reached at a moderate echo time. The primary aim of this study was to estimate the quality of measurement of Ace using short echo time ^{1}H MRS by comparing to MR spectra acquired at a moderate echo time.

METHODS All experiments were performed on a 14.1T/26cm horizontal magnet inserted with a 12-cm gradient coil (400mT/m, 120μs). A home-built quadrature coil with two physically decoupled 12mm-diameter loops in a half volume shape was used as RF transceiver. Previous studies suggested T_2 of GABA at 14.1T was in the range of 70-100ms, phantom (NAA:GABA:Ace = 10:5:1) study was performed at 2.8, 20, 40, 60, and 100 ms to estimate an initial minimal echo time to eliminate GABA. All animal experiments were approved by the local veterinary authorities. Four adult mice (male, 22-35g) were subjected to endoluminal middle cerebral artery occlusion (MCAO) by the filament technique at 0-hr (1). Throughout the entire procedures and measurements, animals were anesthetized under 1-2% isoflurane and well-maintained rectal temperature in the range of 35-37°C. Localized ^{1}H MRS on ipsilateral striatum at 1, 3, 8 and 24 h post pMCAO using SPECIAL (1.8×2×1.8mm^3; TE=2.8ms; TR=4000ms; NT=360, 1 and references therein). The spectral data were processed and quantified using LCModel (Ace in the data basis set) as described previously (1 and references therein). Since previous studies suggested the peak of Ace was ~6h after pMCAO (2), we acquired the spectra at short echo time (2.8ms) and at moderate echo time ~8 h after pMCAO. T_2 relaxation time of Ace was assumed to be identical to that of NAA CH3 (2.01ppms), the Ace/NAA was evaluated for further comparison.

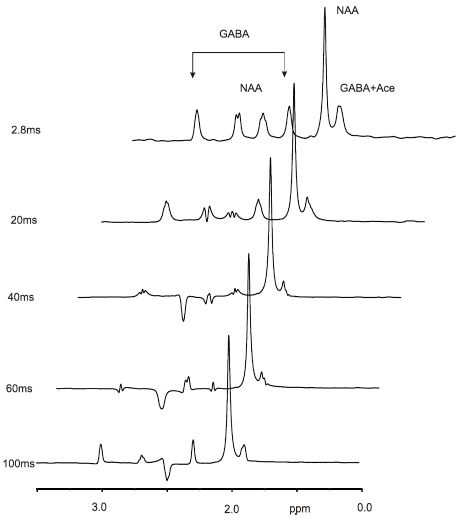

Figure 1 MR spectra on a phantom containing NAA, GABA and Ace (10:5:1, pH=7.4) at different TEs (2.8, 20, 40, 60, 100 ms). The spectra were processed with lb=12Hz to mimic *in vivo* conditions. The noticeable shoulder at 1.89ppm was minimized at ~40ms

RESULTS AND DISCUSSION The signal of the J-coupled metabolite, GABA, was observed minimal at ~40ms (Figure 1), which is consistent with previously reported J-modulation in the coupled spin systems (6). In consequence, *in vivo* MR spectra at such echo time allowed us visually identifying Ace despite the presence of high GABA content (Figure 2) and resulted in quantifying Ace with substantial improvements. For instance, substantial narrower standard deviations of Ace/NAA ratio at 40ms, i.e. 0.21±0.04, was obtained when compared to 0.25±0.12 obtained at 2.8ms. We noticed that MR spectra acquired with 2.8ms echo time remained sufficient to quantify Ace (CRLBs<15%) while a significant amount of GABA was presented after pMCAO and sustained additional information including another hydrolytic metabolite, Asp (Figure 3). The resulting Asp and Ace are consistent with previous in vitro results (2). In addition, the sum of NAA and Ace remained constant until 8h after pMCAO (Figure 3). In conclusion, Ace could be observed in vivo after pMCAO using the moderate echo ^{1}H MRS at high magnetic fields. Short echo time ^{1}H MRS remained reliable measurement methods in combination of LCModel and delivered other metabolic information.

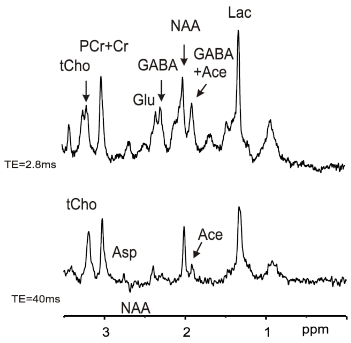

Figure 2 *In vivo* MR spectra of striatum at 8hr post pMCAO. The top trace was acquired with TE=2ms and the bottom trace was acquire with TE= 40ms. A comparable shorter echo time than 140ms (Figure 1) was sufficient to eliminate all possible GABA contributions at 1.89ppm. Abbreviations: Cr: creatine; Glu; glutamate; PCr: phosphylcreatine; Lac; lactate; tCho: total choline.

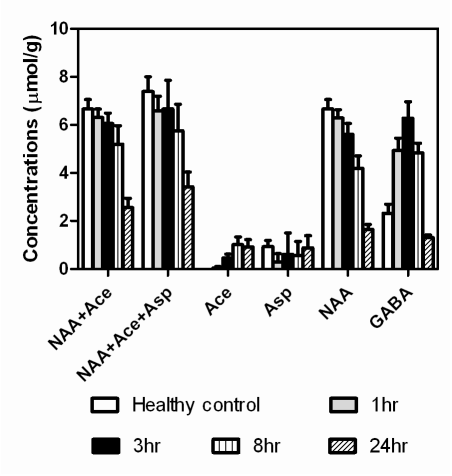

Figure 3. Evolution of GABA, NAA, Asp and Ace after permanent MCAO

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