Contamination of Neurometabolite Amine-Water Proton Exchange to Amide Proton Transfer MRI

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Target audience: Researchers interested in the signal sources and applications of *in vivo* chemical exchange saturation transfer MRI contrasts.

Purpose: The chemical exchange (CE) between water and endogenous amide protons from the backbone of proteins and peptides can be exploited as a useful MRI contrast in various disease conditions. Especially, a change in pH, occurring in ischemia, can be detected by the amide proton transfer (APT) technique, which relies on slow exchanging amide and water proton transfer. However, APT-weighted signals might be contaminated by other labile protons, such as the amine protons, whose resonance frequency is close to amide and whose line width is broadened due to their fast exchange rates with water. To investigate this issue, we measured chemical exchange signals of nine major metabolites in the brain and estimated their contributions to the APT-weighted signals.

Methods: Two experiments were performed on a 9.4 T system: 1) *in vivo* APT and localized proton spectroscopy studies of ischemic rats, and 2) phantom studies at two pH values. Detunable volume-exciting (6.4-cm diameter) and surface-receiving coils (2.2-cm diameter) were used for animal studies, while a 3.8-cm ID quadrature volume coil was used for phantom studies. All images were obtained with a spin-echo echo planar imaging (EPI) sequence. APT-weighted maps were measured following magnetization preparation with an off-resonance square wave pulse with the following parameters: 3-s duration, 63-Hz irradiation power, frequency offsets 3.5 ppm (amide frequency), -3.5 ppm (reference frequency) and 300 ppm (control frequency) relative to water proton resonance, TE/TR = 28/7600 ms. APT-weighted maps were calculated as $APTw = MTR_{asym} = (S_{-3.5ppm} - S_{3.5ppm})/S_{300ppm}$. 1) Sixteen male Sprague-Dawley rats weighing 280 – 410 g were studied with approval by the Institutional Animal Care and Use Committee. Middle cerebral artery occlusion (MCAO) was carried out to induce permanent ischemia in the left hemisphere [1]. Water-suppressed localized ^{1}H spectra were measured with a short-TE localized stimulated-echo acquisition mode (STEAM) sequence with TE/TR = 4/3000 ms and voxel size 3×3×3 mm^3 [2]. The unsuppressed water signal was used as a reference for quantification. The concentrations of nine metabolites (lactate (Lac), gamma aminobutyric acid (GABA), glutamate (Glu), glutamine (Gln), taurine (Tau), total Cr (tCr), myo-Inositol (Ins), N-acetylaspartate (NAA), and phosphorylcholine (PCho)) were quantified using LCModel [3]. 2) Six phosphate-buffered saline (PBS) solutions of 20 mM Glu, 20 mM Tau, 20 mM (GABA), 20 mM (Gln), 10 mM Cr and phosphocreatine (PCr) each, and 10 mM Ins and NAA each were prepared. The solutions were titrated to pH values of 7.0 or 6.5. All solutions were doped with 0.1 mM of manganese chloride for shortening their T_1 and T_2 values (resulting in $T_1 \approx 1.3$ s and $T_2 \approx 125$ ms at 37 °C). The phantoms were kept at a temperature of 37 °C during measurements.

Results and Discussion: The APT-weighted maps showed darkening in the ischemic region within 1 hr after ischemia onset (Fig. 1), consistent with earlier observations [4]. The darkening was explained by the reduced APT signal in the ischemic region with slowing down of amide proton exchange at reduced pH. Ischemia also induced marked changes in the concentrations of several metabolites (Table 1). Concentrations of lactate and GABA were higher on the lesion side compared to the contralateral side while the concentrations of Glu and Tau were lower on the lesion side. Tissue pH can be estimated from its linear relationship with Lac concentration and was found to be 6.1±0.2 (mean±STD) under our conditions [5]. Earlier studies found pH values in the range of 6.3 – 6.8 in ischemic rat brains [4,5]. Thus, the pH of 6.5 in our phantoms lied within the possible pH range of tissues under ischemia. Phantom data are shown in Fig. 2. Although Glu and GABA contain only amine protons their MTR_{asym} values were largest among the phantoms studied (Fig. 2A). Their MTR_{asym} increased at lower pH, consistent with the theoretical expectation of increased CE signal at slower exchange rate for fast exchanging amine protons [6]. The MTR_{asym} in NAA was negligible, despite the presence of amide protons in them, which might be explained by the fact that the resonance frequency of the amide protons in NAA (3.1 ppm) is away from the 3.5-ppm saturation pulse frequency used in the APT experiment and their exchange rates are small [7]. The MTR_{asym} signals in amine-containing metabolites Tau and tCr were also very small, possibly due to unfavorably fast or slow exchange rates, respectively. Gln contains both fast-exchanging amine protons and slowly-exchanging amide protons, and its MTR_{asym} is negligible at pH = 7 but increases at pH = 6.5. This suggests negligible contribution from the amide protons at pH = 7 while the contributions from amine proton exchange might become important at low pH. Ins contains the exchangeable hydroxyl group and does not have any significant MTR_{asym}. Based on the measured *in vivo* metabolite concentrations and assuming pH = 6.5 in the ischemic regions and the exchange rates are similar in the phantoms and in brain, the contributions of the metabolite to the observed *in vivo* APT-weighted contrast (ΔMTR_{asym}) can be estimated after correcting for the T_1 difference between the phantoms and brain tissues and are plotted in Fig. 2B. Contributions of metabolite changes induced by ischemia alone would produce positive ΔMTR_{asym}, opposite to the observed negative ΔMTR_{asym}. Thus, the true ΔAPT due to amide-proton exchanges should be larger than the observed *in vivo* ΔMTR_{asym} value of 1.8±1.2%. The contribution of GABA is maximal among the metabolites, which is due both to the increased GABA concentration and reduced pH in the ischemic regions.

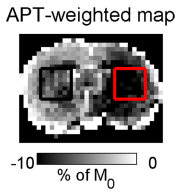

Figure 1: APT-weighted map in a representative rat 1 h after permanent MCAO. The red and black squares denote the voxel locations of the spectroscopy study on the lesion and contra-lateral sides, respectively.

	Lac	GABA	tCr	Glu	NAA	Tau	Gln	Ins	PCho
Ischemia (μmol/g)	22.1±6.0	6.7±1.6	7.9±1.0	8.3±1.2	8.9±0.8	7.5±2.1	2.4±1.6	6.1±1.3	1.4±0.6
Contralateral (μmol/g)	0.8±1.0	3.8±1.0	8.4±0.9	13.8±1.8	10.3±1.0	11.9±2.8	4.6±0.9	5.4±1.2	1.8±0.4

Table 1: Metabolite concentrations in the ischemic and contralateral areas 1 h after MCAO. The errors are standard deviations across rats.

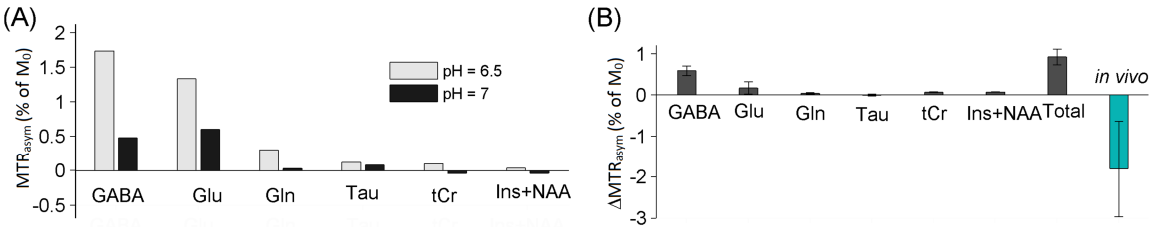

Figure 2: (A) MTR_{asym} values in metabolite phantoms at pH of 7 and 6.5. (B) Estimated MTR_{asym} contrast due to changes in metabolite concentration and pH (from 7 to 6.5). The two bars on the right denote the sum of the contributions from metabolites and the *in vivo* contrast, respectively.

Conclusions: The fast exchanging amine protons from GABA, Glu, and Gln can significantly contribute to the APT-weighted signal, which show opposite pH dependence to the true APT signal due to amide proton exchange. Thus, possible contaminations from those metabolites should be considered when interpreting the underlying sources of APT contrast under pathological conditions.

References: 1. Kiozumi, J., *et al.*, The Japanese Journal of Stroke **8**, 1 (1986). 2. Tkac, I., et al., Magn Reson Med, **41**, 649 (1999). 3. Provencher, S.W. Magn Reson Med, **30**, 672 (1993). 4. Zhou, J. *et al*., Nature Medicine **9**, 1085 (2003). 5. Jokivarsi, K.T. *et al*., Magn. Reson. Med. **57**, 647 (2007). 6. Jin, T. *et al.*, Magn Reson Med 65, 1448 (2011). 7. Mori, S., *et al*., Magn. Reson. Med. **40**, 36 (1998).