In vivo saturation transfer imaging of nuclear Overhauser effect from aromatic and aliphatic protons: implication to APT quantification

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Target Audience Researchers interested in the chemical exchange saturation transfer (CEST) imaging and magnetization transfer imaging.

Purpose Recent *in vivo* and phantom saturation transfer experiments at high fields showed that significant signals are originated from the Nuclear Overhauser effect (NOE) in a frequency range of -0.5 to -5 ppm from water [1,2], which is likely from aliphatic protons of macromolecules. The aliphatic proton-water NOE signal is pH-insensitive and may provide a novel contrast for mobile protein concentrations [1]. Since aromatic protons are also abundant in proteins, with a frequency range downfield of water (i.e., positive offsets) and close to the amide frequencies [3], one would also expected to have NOE from aromatic protons, similar to aliphatic proton NOE. NOE of both aliphatic and aromatic protons can impose difficulties in the assessment of the pH-sensitive amide proton transfer (APT) effect. Therefore, it is critical to evaluate 1) whether these aromatic (as well as aliphatic) protons can affect the water signal in a saturation transfer experiment through the NOE, 2) whether it is significant *in vivo*, 3) whether it overlaps with APT signal, and 4) whether it is dominated by an exchange-relayed pathway and hence sensitive to tissue pH. In this preliminary study, the NOE effects from the aromatic and aliphatic protons were examined in protein phantoms and in rats with focal brain ischemia.

Materials and methods All experiments were performed on a 9.4T Varian MRI system. 15% of bovine serum albumin (BSA) by weight was dissolved in three different solutions, namely, 100% H_2O, 10% H_2O/90% D_2O, and 5% H_2O/95% D_2O. Z-spectra were measured with a 0.25 μT and 6 s pulse, and a frequency offset range from 12 ppm to -12 ppm with uneven steps. Control images were acquired at an offset of 300 ppm for signal normalization. For *in vivo* experiments, five Sprague-Dawley rats underwent permanent middle cerebral artery occlusion (MCAO). Off-resonance irradiation was applied by a 0.8 μT and 4-s continuous wave saturation pulse, and measured with a frequency offset range from 12 ppm to -12 ppm with uneven steps. Immediately after the irradiation, spin-echo echo planar imaging images were acquired on four slices with 2.0 mm slice thickness. The field of view was 3.2 cm × 3.2 cm, and matrix size was 64 × 64. Apparent diffusion coefficient (ADC) maps were also measured to identify the ischemic regions. For quantitative data analysis, Z-spectra were obtained from the regions of interest (ROI) selected at the contralateral and ipsilateral areas, based on the ADC map.

In an *in vivo* Z-spectrum, the water signal decreases due to the direct water saturation (DS), magnetization transfer contrast from immobile macromolecules (MTC_{IM}), and magnetization transfer contrast from mobile molecules (MTC_{MM}), which includes both the chemical exchange (CE) and NOE signals from metabolites and mobile protein. To extract the MTC_{MM} (CE and NOE) signals, a model-fitted Z-spectrum (S_{fit}/S_0) was generated from experimental data of -12 to -6 ppm, -0.5 to +0.5 ppm (or -1 to 0.8 ppm for *in vivo* results), and 5 to 12 ppm ranges. The fitted Z-spectrum contains the DS effect described by a Lorentzian function in phantoms, and additionally the MTC_{IM} effect described by super-Lorentzian function for *in vivo* data. Then, each experimental Z-spectrum (S/S_0) was subtracted from S_{fit}/S_0, i.e, $MTC_{MM} = (S_{fit} - S)/S_0$, which should have only CE and NOE components.

Results and discussions It has been reported that in bovine serum albumin (BSA), the NOE signal from aliphatic protons in the -0.5 to -5 ppm range is pH-insensitive [1], while the signal in the +1 to +5 ppm range is affected by CE and therefore highly pH-sensitive. Since previous NMR spectroscopy studies had established that many aromatic protons of BSA peak at +1 to ~+4 ppm range downfield of water, one critical question is whether the NOE from aromatic protons may also affect the Z-spectrum. This was examined by measuring Z-spectrum under different deuterated water solutions. Deuterium exchanges with labile protons, effectively reducing labile proton concentration, but not with aliphatic and aromatic protons, thus selectively suppressing CE while *relatively* enhancing the NOE effects [4]. Fig. 1A showed the aliphatic NOE signals in the -0.5 to -5 ppm were enhanced with addition of D_2O, as expected [4]. The NOE dependence on different deuterated solutions is expected to be the same for aliphatic and aromatic protons, thus the differential spectra (i.e., MTC_{MM}) are normalized by aliphatic NOE peaks (Fig. 1B). Within +1 to +5 ppm, the magnitude of MTC_{MM} is highest in H_2O, and reduced, but similar for two D_2O solutions. The deuterium-induced reduction of MTC_{MM} can be attributed to CE effect from labile protons, while the remaining MTC_{MM} is likely due to NOE from the aromatic protons.

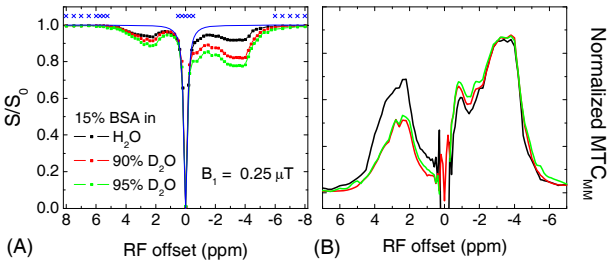

Fig. 1. (A) Z-spectra of 15% BSA in H_2O (black), 90% D_2O and 95% D_2O. The blue curve is a fit of the DS effect, and its difference with the experimental data (= MTC_{MM}) is normalized by the peak of NOE of aliphatic protons at ~ -3.5 ppm. (**B**) Normalized MTC_{MM} spectra show similar aliphatic NOE pattern for all solutions. In a downfield range (+1 to +5 ppm), the spectrum of BSA in H_2O (black) contains both CE and non-CE contributions. In both D_2O solutions, the CE signal is suppressed, and the remaining signals are likely from NOE of aromatic protons.

The *in vivo* experimental Z-spectrum (circles, Fig. 2A) was compared with a model-fitting Z-spectrum (blue curve). The MTC_{MM} contains a large broad peak in -1 to -5 ppm (right inset plot) due to NOE of aliphatic protons, and a narrow peak at +3.6 ppm on top of a large broad peak in the +1 to +5 ppm (left inset plot) range, which was similarly observed in human 3 T studies [5]. The magnitude of the MTC_{MM} at 3.6 ppm is ~6%, which is about 100% larger than the APT effect of ~3% reported in previous literature, suggesting a large non-APT component. Indeed, when the differential spectrum of ipsilateral versus contralateral ROIs were compared, the ischemic lesion only induces a small reduction of MTC_{MM} signal (mainly in the 3 to 4 ppm range), while the majority of the spectra in the downfield region and the whole spectra in the upfield region are almost unchanged. These pH-insensitive MTC_{MM} signal in downfield and upfield frequencies are likely from NOE of aromatic and aliphatic protons, respectively.

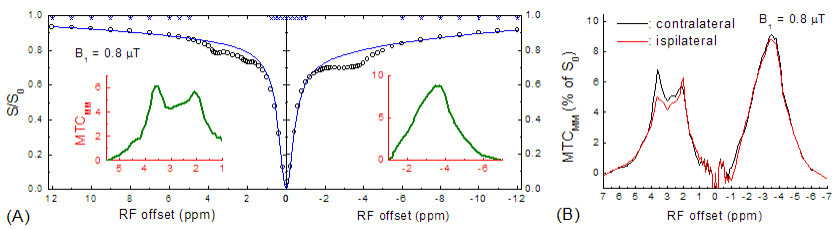

Fig. 2. (A) Averaged experimental (circles) vs. model-fitted (blue curve) Z-spectrum on the contralateral ROI of MCAO rats (n = 5). A model function includes both the DS and MTC_{IM} using data points whose offsets are indicated by blue crosses in top of the figure. The green curves in insets show the difference between the fitted model curve and the experimental data. (**B**). The differential spectra from the contralateral and ipsilateral ROIs showed minimal difference in the upfield aliphatic NOE signal. In the downfield region, the difference is also small and mainly limited in the offset range of 3-4 ppm, indicating a large pH-insensitive component.

Conclusions Both protein phantoms and *in vivo* experiments show significant NOE signals from aromatic protons, in a range of +1 to +5 ppm from water, which will affect the quantification of APT effects at ~ 3.6 ppm. Similar to aliphatic NOE, these aromatic NOE signals are insensitive to pH, and further studies would be necessary to understand their signal properties such as the dependence on irradiation pulse power.

References [1]. Jin, T et al., MRM in press, DOI: 10.1002/mrm.24315 (2012). [2]. Jones, CK et al., Proc 19th ISMRM, p.2735, (2011). [3]. Sadler PJ et al., Eur J Biochem 205:631, (1992). [4]. Ling, W et al, PNAS 105:2266 (2008). [5]. Jones, CK et al., MRM 67:1579 (2012).