High Resolution Imaging of Myo-Insitol in Alzhemier's Disease Pathology

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Introduction:
Myo-Inositol (MI) is one of the most abundant metabolites present in the human brain. MI is a marker of glial cells proliferation and functions as an osmolyte in brain. The concentration of MI has been shown to change in various brain pathologies. Proton magnetic resonance spectroscopy (^{1}HMRS) studies have shown increased MI concentration in Alzheimer's disease (AD). Glial cells proliferation/activation is associated with the pathological changes in AD[1]. ^{1}HMRS has been widely used to monitor MI changes in AD, however, it suffers from poor resolution and also does not provide information about the region with the higher glial cells proliferation/activation. Recently, high-resolution mapping of MI in human brain has been performed by exploiting exchangeable –OH protons present on MI using a technique commonly known as chemical exchange saturation transfer imaging (CEST)[2]. In the current study, for the first time we perform MI CEST on transgenic AD mice (APP/PS1) to evaluate the changes in MI concentration compared to wild type mice.

Materials and Methods:
Under an approved IACUC protocol three transgenic AD mice and two age matched control wild type mice were imaged at 9.4T horizontal bore small animal scanner (Varian, Palo Alto, CA) using a 20-mm diameter commercial quadrature proton coil (Imaging Corp., Cleveland, OH). Animals were kept under anesthesia (1.5% isoflurane in1 liter/min oxygen) and their body temperature maintained with the air generated from a heater (SA Instruments, Inc., Stony Brook, NY). Respiration and body temperature was continuously monitored using a MRI compatible small animal monitor system (SA Instruments, Inc., Stony Brook, NY). CEST imaging of the rat brain was performed using a custom-programmed segmented RF spoiled gradient echo (GRE) readout pulse sequence with a frequency selective continuous wave (CW) saturation preparation pulse. The sequence parameters were: field of view =20×20 mm^2, slice thickness = 2 mm, flip angle=15^0, GRE readout TR=6.2 ms (64 segments), TE =2.9 ms, matrix size=128×128, number of averages=2. For every 8s one saturation pulse and 64 acquisition segments were applied. CEST images were collected using a 5 second saturation pulse at peak B1 of 75Hz with varying frequencies from 0-1.5 ppm in step size of 0.1ppm. MI CEST contrast was calculated at ±0.6ppm by normalizing with 20ppm signal using the equation:-MICEST=100*(S_{-ve}-S_{+ve})/S_0, where S_{-ve}, S_{+ve} and S_0 are the acquired MR signals at -0.6ppm, +0.6ppm and 20ppm respectively. B1 and B0 maps were also acquired and used to correct the MI CEST contrast as described previously[2].
Single voxel spectra (SVS) were performed with stimulated echo acquisition mode (STEAM) using a vendor (Varian) provided pulse sequence with following parameters: voxel size= 5mm×5mm×2mm (Voxel volume 50 µL), spectral width=4 kHz, Number of points=4006, average=264, TE=8 ms, Tm=7 ms, and TR=5s. A water suppression pulse sequence with variable pulse power and optimized relaxation delays (VAPOR) was pre-encoded to STEAM sequence. Localized shimming was performed to obtain localized water line width values of 0.075ppm or less. Unsuppressed water spectrum was also acquired using the same parameter for the purpose of normalization. SVS spectra were obtained from the raw free induction decay data by exponential apodization (20Hz), Fourier transformation, phase correction and baseline removal. Peak integrals of MI peak and unsuppressed water peak were calculated using a non-linear least squares fitting (MATLAB "nlinfit" routine) program. The MI integration value was normalized with unsuppressed water integration value.

Results and Discussion:
Brain MICEST maps from wild type and AD mouse are shown in figure 1 and 2. Higher MICEST contrast was observed both in gray and white matter in AD than wild type mice suggestive of higher concentration of MI as well as active glial cells proliferation in AD mice.^{1}H MRS spectra for the voxels as shown in the anatomical images in both figure 1 and 2 clearly show increase MI concentration in AD compared to wild type mice. Both the spectra were displayed on the same vertical scale. Normalized MI integration with water integration value for the voxels placed on the anatomical images in both wild type and AD mouse as shown in figure 1A and 2A was 42% higher in AD than wild type mice, and for the same voxels an average of 44% increased MICEST contrast was observed in AD compared to wild type mice. Both the spectral and MICEST changes were in good agreement with each other as shown in figure 3.
The initial study showed that detection of change in MI concentration in AD pathology is possible using MI CEST technique. AD is associated with the glial cells proliferation/activation and MI is a marker of glial cells proliferation. The only method available to monitor the glial cells proliferation/activation in AD is histochemical analysis. While using the current method brain region with active glial cells proliferation can be mapped at high resolution, a further validation is required through histopathology. Gradual increase in the MI concentration has been shown from control to MCI to full onset of AD[3]. Once these results are validated using large statistically significant number of animals MICEST may provide a quantitative method of monitoring the molecular changes associated with disease progression in AD patients and may help to improve their clinical management.

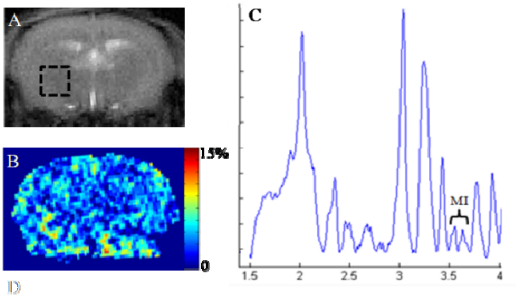

Figure 1: A shows the anatomical image from a wild type mouse. B is showing the corresponding CEST map obtained by using 75 Hz peak B1 and 5s saturation duration at ±0.6ppm. The MI peaks are marked by arrow.

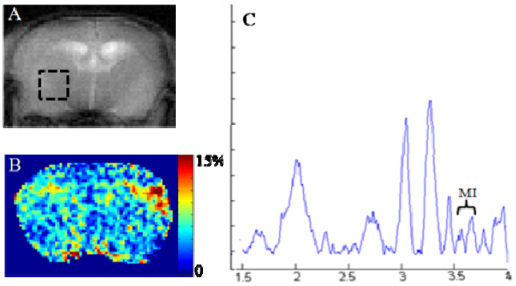

Figure 2: A. Shows the anatomical image from a Alzheimer,s Disease (APP/PS1) mouse. B is showing the corresponding CEST map obtained by using 75 Hz peak B1 and 5s saturation duration at ±0.6ppm. The MI peaks are marked by arrow.

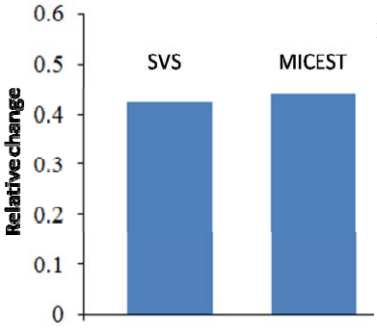

Figure 3: Bar graphs are showing the relative change in myo-inositol (MI) in AD mice compared to wild type mice. Relative change in MI quantified using single voxel spectroscopy (SVS) is in good agreement with the relative MICEST change.

References:
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2. Haris et.al. Neuroimage. 2010 Oct 14,
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