Monitoring therapeutic response on GBM in chemotherapy by amide proton transfer (APT) imaging in mice

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Introduction: Amide proton transfer (APT) imaging is one of the chemical exchange transfer (CEST) imaging methods. With this method the exchange between protons of free water and the amide groups (-NH) of endogenous mobile proteins and peptides is imaged (1). The previous work demonstrated that APT ratio (APTR) was a useful imaging biomarker for monitoring the therapeutic effects of radiotherapy and distinguishing radiation necrosis from tumor recurrence (2). The objective of our study is to investigate whether APTR is useful for monitoring treatment responses of the brain tumor in chemotherapy. In the present study, we compared the temporal changes in APTR of glioblastoma multiforme (GBM) in a mouse model with and without chemotherapy by Temozolomide (TMZ) which is widely used for the treatment of GBMs.

Materials and Methods: *Animal Protocol:* We have developed an orthotopic model in which GBM tissue is taken directory from the patients and injected into NOD-SCID mouse brains, which has been serially repeated for maintaining as the orthotopic tumor lines without ever having been in culture. MRI screening was performed from 4 weeks after implantation. Mice were subjected to APT imaging study and divided into two groups when the tumor was detected at the size of 3-5 mm. In the treated group (n = 6), mice underwent a course of chemotherapy (TMZ 80mg/kg i.v. for 3 days and then rest for 4 days; 7 days total) after the baseline imaging. Another group was served as the control group (n = 5). The same MRI session was repeated 7 days after the baseline. The brains were harvested after the final MRI session for histology.

MRI: MR imaging was conducted in a 7-T small animal MR system (Varian, Inc, Palo Alto, CA) with a 40 mm (I.D.) radiofrequency (RF) coil. All animals were anesthetized with 1%-2% isoflurane (AERRANE, Baxter Healthcare Corporation, IL) mixed in 100% oxygen. First, low-resolution localizer imaging was performed to confirm reproducible positioning. High-resolution axial multislice T1-weighted images (T1WI) and T2-weighted images (T2WI) were obtained on entire brain using a fast spin sequence (TR/TE = 500/10.3 msec for T1WI, 2500/60 msec for T2WI; FOV = 25.6×25.6 mm; matrix size = 256×256; slice thickness = 1 mm; gapless; NEX = 4). On a single 1-mm-slice delineating the tumor, APT imaging was performed as follows: Gradient echo images were obtained following a presaturation pulse (continuous-wave block pulse, B1 = 2.3 μT, duration = 5 s) which was applied at 29 frequency offsets from 7 to -7 ppm with an interval of 0.5 ppm. Other imaging parameters were: TR/TE = 6.52/3.16 ms, flip angle = 20°, FOV = 25.6 × 25.6 mm, matrix = 128 × 64 (reconstructed to 256 × 256), NEX = 8. A control image was obtained with the presaturation pulse at 300 pm. Total acquisition time for each animal was approximately 40 min.

Image Analysis: Tumor volumes were measured on the T2WI. The Z-spectra were fitted through all offsets on a pixel-by-pixel basis according to the procedure using the 12th-order polynomial fitting followed by the correction for B_0 inhomogeneity as Salhotra et al. reported (3). MTR asymmetry (MTR_{asym}) was defined as: $MTR_{asym} = S_{sat}(-offset)/S_0 - S_{sat}(+offset)/S_0$, where S_{sat} and S_0 are signal intensities on the images with presaturation pulse at 7 to -7 ppm and control (300 ppm), respectively. The calculated MTR_{asym} map at the offset of 3.5 ppm is called the APT-weighted image (APTWI). Region-of-interests (ROIs) were carefully placed around the edge of the tumors on APTWI to measure APTR. APTR was also measured in contralateral normal appearing brain for a reference in each mouse. Corrected APTR was calculated as the difference between these two APTRs (tumor – normal).

Histopathology: Hematoxylin/eosine (HE) staining was performed for microscopic examination.

Results and Discussion: There was no significant difference in volume between two groups both at baseline (Pre) and 7 days after (Post) although the tumors tended to grow more rapidly in the control group. Figure 1 shows the temporal change in corrected MTR_{asym} in both groups. The corrected MTR_{asym} values at any given frequency decreased after treatment, with a significant difference seen at 3.5 ppm (2.50 ± 0.44 % vs. 2.04 ± 0.21 %, $P < 0.05$) in the treated group. By contrast, the values increased in the control group. Figure 2 shows representative APTWIs (Pre and Post) in the treated and control groups. APT signal of the tumor (arrow) decreased in the treated group whereas it increased in the control group. The percentage (%) change of the corrected APTR relative to baseline was reduced in the treated group, however, it increased in the control group (Fig. 3), affording significant difference between the groups (-16.98 ± 12.53 % vs. 36.53 ± 11.47 %, $P < 0.0001$). Histological evaluation revealed pleomorphic cells with enlarged nuclei and cytoplasm in the treated group, and monomorphic population of cells, mitosis and highly infiltration in the control group. We postulated that the decrease of APT effects after chemotherapy might reflect inhibited production of endogenous mobile proteins and peptides responding to the chemotherapy. The present study suggests that APTR could be a useful biomarker for monitoring treatment responses of the brain tumor in chemotherapy.

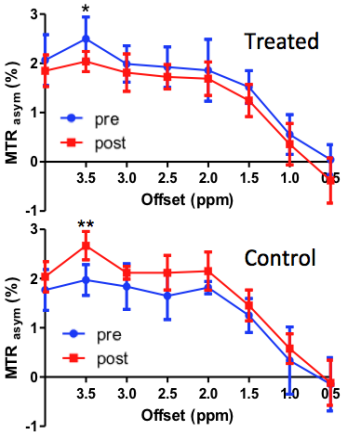

Fig 1. Temporal change in corrected MTR_{asym} in the treated and control groups. MTR_{asym} lowers at any given frequency after treatment while it increased in the control. *, $P < 0.05$ **, $P < 0.01$ by paired t-test at 3.5 ppm.

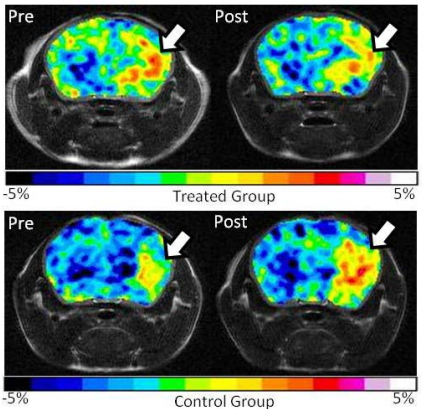

Fig 2. Typical APT-weighted images of the treated group (top) and the control group (bottom). The signal of tumors (arrows) decreased after treatment while it increased in the control.

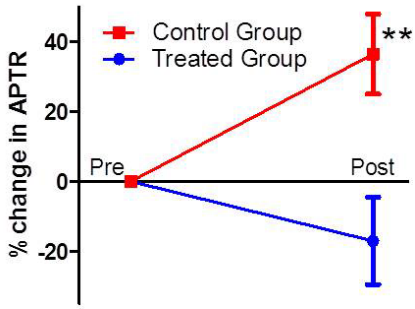

Fig 3. Percentage change of the corrected APTR (MTR_{asym} at 3.5 ppm) relative to baseline. The APTR reduced in the treated and increased in the control groups. **, $P < 0.0001$ by Student's t-test

References: 1. Zhou J et al. Nat Med 9:1085-90 (2003). 2. Zhou J et al. Nat Med 17:130-134 (2011). 3. Salhotra A et al. NMR Biomed 21:489-97 (2008).

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