Detection of Early Response to proteasome inhibitor treatment in a Rat Glioma Model with Amide Proton Transfer (APT) Imaging

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INTRODUCTION

Recently, a new magnetization transfer (MT)-based contrast mechanism for MRI, called chemical exchange-dependent saturation transfer (CEST),[1] has emerged in the field of cellular and molecular imaging. This technique has now evolved into several different variants[2-4] with nomenclature based on magnetic properties of new CEST contrast agents (diamagnetic and paramagnetic) and on the particular exchangeable groups or compounds being detected. In one of these, dubbed amide proton transfer (APT) imaging,[4] endogenous mobile proteins and peptides, such as those in the cytoplasm, are detected through selective saturation of a variety of amide protons in the peptide bonds. APT-MRI has the potential to expand the range of molecular MRI techniques to the endogenous protein and peptide level. Proteasomes make up nearly 1% of cellular proteins and regulate intracellular protein stability.[5] Proteasomes degrade the bulk of cellular proteins, and inhibition of proteasome function is expected to lead to a progressive accumulation of cellular proteins. We investigated whether APT imaging could detect changes in tumor protein levels after exposure to the proteosome inhibitor bortezomib in a rat glioma model.

MATERIALS & METHODS

Three Fischer 344 rats received 9L gliosarcoma cells (25,000 cells/2 μl) by stereotactic injection to right caudate/putamen. The rats were treated with 0.2 mg/kg bortezomib i.v. on post-implantation days 10 and 14. MRI was performed at pre-treatment, 6 hours, one day, two days, and four days post-treatment, as well as 6 hours and 15 hours after the second treatment. At each time point of the MRI measurements, isoflurane anesthetized rats were scanned using a Bruker 4.7T animal imager. Single-shot SE EPI was used for data acquisition (matrix 64×64, FOV 32×32 mm^2, slice thickness 1.5 mm). The APT imaging sequence employed low-power continuous-wave RF saturation (power 1.3 μT, time 4 sec, TR 10 sec). A full MT-spectrum (offsets -6 to 6 ppm, interval 0.5 ppm) was acquired (S_{sat}). One unsaturated image (no saturation pulses added) was acquired for control (S_0). The APT effect was quantified using the MT-ratio asymmetry parameter at a frequency offset of 3.5ppm from water: $MTR_{asym}(3.5ppm) = [S_{sat}(-3.5ppm)-S_{sat}(3.5ppm)]/S_0$. The APT contrast between tumor and contralateral normal-appearing brain tissue was calculated for all time points and then normalized to pre-treatment. Two other MRI scans were acquired for comparison: (i) T_2 map (spin echo, TR 3 s, TE 30-90 ms, NA 4) and (ii) ADC_{av} map (single-shot trace diffusion weighting, TR 3 s, TE 80 ms, b-value 0-1000 s/mm^2, NA 8).

RESULTS AND DISCUSSION

Figure 1 shows the normalized $MTR_{asym}(3.5ppm)$, T_2, and ADC contrasts in bortezomib-treated rats as a function of post-treatment time. It can be seen clearly that the $MTR_{asym}(3.5ppm)$ in treated tumor increased approximately **6-24 hours** after bortezomib treatment, and then started to decrease. All three rat tumors showed increased APT signals after injection, but the time to peak enhancement was variable, causing a large standard deviation. In contrast, the average tumor T_2 and ADC for the same three rats were overall more stable. The three rats treated with bortezomib showed slower tumor growth and increased survival than controls (about 3 weeks versus 2 weeks).

Proteasomes maintain cellular functions by degrading misfolded proteins to prevent protein aggregation. Proteasome inhibitors have been of increasing interest in anti-cancer therapy regimens due to recognition of the critical role that proteasomes have in the regulation of cell cycle proteins. Bortezomib is a proteosome inhibitor recently emerging for anti-cancer, and other treatments.[5] The tumor showed an increased APT signal (more saturation transfer) following bortezomib treatment, which was attributed to increased protein concentration in the tumor. These results are consistent with the notion that cancer cells are more susceptible to proteasome inhibitors than normal cells and inhibition of proteasome activities can, therefore, lead to an increase in cellular cytosolic protein concentration with a larger increase in tumor than in normal tissue. Our findings are in agreement with the previous observations that the proteasome activity in tumor was reduced 1 hr after receiving bortezomib and gradually returned toward baseline in a day.[5] The average tumor T_2 and ADC were relatively more stable. Taken together, the data suggests that bortezomib does in fact have a unique mechanism from standard cytotoxic agents, such as BCNU, that cause large T_2 and ADC changes 2-3 days post-treatment.[6]

CONCLUSONS

The preliminary data suggests that APT imaging is able to detect the specific protein alterations associated with bortezomib therapy on brain tumors.

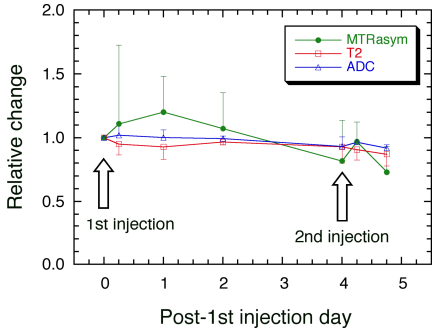

Fig. 1. Time course of tumor APT (or $MTR_{asym}(3.5ppm)$), T_2, and ADC measures following bortezomib therapy (n = 3). The drug was injected twice (i.v. 0.2 mg/kg) on post-implantation days 10 and 14. Tumor APT increased after treatment, but T_2 and ADC showed relatively small changes.

REFERENCES

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