Published in final edited form as:

NMR Biomed. 2008 June ; 21(5): 489–497. doi:10.1002/nbm.1216.

Amide proton transfer imaging of 9L gliosarcoma and human glioblastoma xenografts

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Abstract

Amide proton transfer (APT) imaging is a variant of magnetization transfer (MT) imaging, in which the contrast is determined by a change in water intensity due to chemical exchange with saturated amide protons of endogenous mobile proteins and peptides. In this study, eight Fisher 344 rats implanted with 9L gliosarcoma cells and six nude rats implanted with human glioblastoma cells were imaged at 4.7 T. There were increased signal intensities in tumors in the APT-weighted images. The contrast of APT imaging between the tumor and contralateral brain tissue was about 3.9% in water intensity ($1.49\% \pm 0.66\%$ versus $-2.36\% \pm 0.19\%$) for the more uniformly hypercellular 9L brain tumors, and it was reduced to 1.6% ($-1.18\% \pm 0.60\%$ versus $-2.77\% \pm 0.42\%$) for the human glioblastoma xenografts that contained hypocellular zones of necrosis. The preliminary results show that the APT technique at the protein level may provide a unique MRI contrast for the characterization of brain tumors.

Keywords

magnetization transfer; amide proton transfer; APT imaging; protein; brain tumor; 9L gliosarcoma

INTRODUCTION

Magnetization transfer (MT) is an extremely common physical phenomenon in NMR spectroscopy and imaging. Of several MT-type measurements, conventional MT in vivo (1–4) is based on the presence of a semi-solid macromolecular phase with a spectral width as large as tens of kHz. When radiofrequency (RF) irradiation is applied at a particular frequency far from the water resonance, the magnetization of immobile protons on solid-like

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macromolecules, such as cell membranes and proteins, is partially saturated, and this saturation is then transferred from the solid-like matrix to free water, causing a significant decrease in the bulk water signal intensity. The magnitude of MT is usually described by the so-called magnetization transfer ratio (MTR). Quantitatively, $MTR = 1 - S_{sat}/S_0$, where S_{sat} and S_0 are the water signal intensities measured with and without RF saturation, respectively. It was demonstrated previously (5–10) that the MTR for brain tumors is significantly lower than that for normal-appearing brain tissue, and imaging with MT may be useful for the differential diagnosis and grading of brain gliomas. In addition, gadolinium-enhanced T₁-weighted imaging with MT was reported to be able to distinguish brain tumor from surrounding peritumoral edema more accurately (11,12).

From an MR point of view, two types of cellular proteins can be distinguished: bound proteins, as detected by conventional MT (1–4), which possess solid-like properties and have protons with short T₂ (~ μs), and mobile proteins and peptides, which rotate rapidly and whose protons have relatively long T₂ (~ tens of ms). Using in vivo proton MR spectroscopy, Behar et al. (13,14) and Kauppinen et al. (15,16) first detected and identified several macromolecular peaks in brain proton MR spectra at low frequency (0–4 ppm). Subsequently, we observed a composite resonance around 8.3 ± 0.5 ppm (3.5 ppm downfield from the water resonance) in the proton spectra of cancer cells and animal brain in situ (17–19). This resonance was attributed to water-exchangeable amide protons of mobile proteins and peptides. It was recently demonstrated (19,20) that it is possible to produce endogenous mobile protein and peptide-based MRI contrast using a chemical exchange saturation transfer (CEST) enhancement scheme (21–28) for amide protons in these molecules. This approach, called amide proton transfer (APT) imaging (19,20), was shown to be sensitive to pH changes (19,29) due to the effect of pH on proton exchange, and to be able to provide brain tumor contrast (20) likely due to a higher mobile protein and peptide concentration in gliomas. In an experimental rat glial tumor at 4.7 T (20) and human brain tumor at 3 T (30), APT ratios (APTRs) in tumor were found to increase by 3–4% in the water signal intensity compared to the peritumoral brain tissue. These findings are in line with recent work by Howe et al. (31), who found that these mobile protein concentrations were higher in human brain tumors than in normal white matter, and increased with tumor grade.

Tumor xenografts derived from established glioma cell lines (e.g., 9L, C6, F98) have limitations such as genetic drift from long term in vitro culture and altered growth characteristic in animals. Glioma xenografts derived from patients and maintained in animals for a short period of time can mimic human tumor biogenesis and real growth patterns, including invasive growth and tumor necrosis. The established experimental brain tumors generally grow rapidly. For example, 9L tumors can reach a size that is detectable with MRI in one week post-implantation, and most tumor-bearing animals die in 2–3 weeks following tumor cell implantation. In contrast, human glioblastoma xenografts grow slowly and animals may live for 2–3 months post-implantation. The purpose of this study was to apply APT imaging to 9L gliosarcoma and human glioblastoma xenografts at the same experimental setup and to evaluate the capability of APT for detecting and characterizing these different experimental brain tumors.

MATERIALS AND METHODS

Animal preparation

Fisher 344 rats (n = 8) and nude rats (n = 6) weighing 200–250 g were anesthetized by intraperitoneal injection of 0.2 ml/100 g body weight of 2.5 mg/ml of xylazine and 25 mg/ml of ketamine hydrochloride. Through a small burr hole, tumor cells (25,000 9L gliosarcoma cells in 2 μl for a Fisher rat or 100,000 human glioblastoma cells in 2 μl for a nude rat) were stereotactically implanted into the forebrain of the animal on the right side (3 mm lateral to

bregma and 3 mm deep). On each of the experimental days indicated, animals implanted with tumor cells were re-anesthetized with 5% isoflurane in a mixture of 75% air and 25% O₂ in a box for about 5 minutes for induction, followed by 1.5–2% isoflurane through breathing inside an MRI volume coil during MRI procedures. While anesthetized, a PE-10 catheter was placed into the dorsal tail veins prior to MRI. The rat head and body were fixed and taped to the coil and cradle to avoid motion artifacts. Rats in the magnet were monitored online through a small-animal monitoring and gating system connected with optic fibers, and the breathing rate of the animal was kept at 40 ± 5 breaths per minute by adjusting the isoflurane ratio (1.5–2%) in the breathing mixture. At the end of the study, the anesthetized rat was sacrificed by injecting saturated KCl solution (3 ml) intravenously. After euthanasia, brains were excised and sectioned. Histological sections (10 microns thick) were stained with hematoxylin and eosin. Animal care throughout the experimental procedures in the study was in accordance with institutional guidelines.

MRI experiments

The APT imaging sequence is in form the same as the traditional MT sequences; however, some small imaging parameter adjustments are very necessary for maximizing the effect. Unlike conventional MT, APT is associated with a low-concentration proton pool of mobile proteins and peptides and takes place in a small offset range around the water resonance frequency. When RF saturation power is too strong (e.g., $>3 \mu\text{T}$), the effect may be reduced significantly or eliminated by direct water saturation and conventional MT. Therefore, a weak selective RF field should be applied to avoid too much water signal intensity attenuation. In this study, a weak continuous-wave (CW) RF field with a duration of 4 s and a power level of $1.3 \mu\text{T}$ was used for off-resonance saturation.

MRI data were acquired using a horizontal bore 4.7 T Biospec animal imager with a 4 cm I.D. volume coil for RF transmission and reception. Single-shot spin-echo echo planar imaging (EPI) was used for data acquisition. The imaging matrix was 64×64 , FOV was 28×28 or $32 \times 32 \text{ mm}^2$, and the imaging slice thickness was 2 mm. The image slice was located at the level of the caudate nucleus. Local shimming was carried out. The repetition time (TR) was 10 s, and the TE was 30 ms. Two types of experiments were performed. In the first type of APT experiments, z-spectra were acquired over an offset range of ± 6 ppm with a resolution of 0.5 ppm ($S_{\text{sat}}(\text{offset})$). One image was acquired per offset. A control image in the absence of RF saturation (S_0) was also acquired for imaging signal intensity normalization. The effects of the saturation transfer of exchangeable protons to water were subsequently identified by asymmetry analysis and compared to normal (contralateral) brain. In the second type, high signal-to-noise ratio (SNR) APT-weighted images were acquired using only frequency-labeling offsets of ± 3.5 ppm with respect to water (16 averages; $S_{\text{sat}}(\pm 3.5 \text{ ppm})$), followed by asymmetry (difference) analysis as for the first experiment. For comparison, conventional MT imaging was acquired that had the same experimental parameters as the high-SNR APT scan, except that a RF saturation frequency offset of 10 ppm (2000 Hz at 4.7 T) was used.

In addition to these MT-type experiments, several conventional imaging experiments were performed, including apparent diffusion coefficient (ADC) mapping (single-shot trace diffusion weighting (32), TR = 3 s, TE = 80 ms, b-values = 0–1000 s/mm², NA = 8); T₁ mapping (inversion recovery, predelay = 3 s, TE = 30 ms, TI = 0.05–3.5 s, NA = 4); and T₂ mapping (TR = 3 s, TE = 30–90 ms, NA = 4). About 30 minutes (4 min 40 s for z-spectrum; 8 min for high-SNR APT imaging) were used to run all these experiments.

Data analysis

In MT imaging experiments, the measured signal intensities (normalized with respect to unsaturated, S_{sat}/S_0) are often displayed as a function of saturation frequency offset relative to

water, which is assigned to be at 0 ppm. This was originally referred to as an MT spectrum or a z spectrum (3), or more recently as a CEST spectrum (22), in which the effect of saturation transfer at a specific irradiation frequency can be identified readily. It is not straightforward to demonstrate CEST effects on the water signal in tissue because of MT interference. In addition, many CEST effects occur close to the water frequency and direct water saturation effects may interfere, especially at low magnetic fields. The effect of direct saturation is also called spillover (33,34). Because the shape of the direct water saturation region of z spectra depends on T_2 and T_1 , changes in blood-oxygen-level-dependent (BOLD) effects or water content will also influence the CEST spectrum (35). To reduce the interference of other saturation effects concurrent with APT measurements, it is useful to define an MTR asymmetry (MTR_{asym}) parameter by subtracting MT ratios obtained at the negative offset with respect to water from those at the corresponding positive offset (19–21):

$$MTR_{\text{asym}} = MTR(+\text{offset}) - MTR(-\text{offset}) = S_{\text{sat}}(-\text{offset})/S_0 - S_{\text{sat}}(+\text{offset})/S_0. \quad (1)$$

If conventional MT effects were symmetric with respect to the water resonance, any contribution due to proton exchange should result in a positive MT difference. However, the solid-like MT effect is somewhat asymmetric (19,36) with respect to the water resonance, with a center frequency in the aliphatic range. As a consequence, the resulting MTR_{asym} curve for offsets 0–5 ppm from water has a shape that depends on the inherent MTR'_{asym} of the solid-phase MT effect (MTR'_{asym}) as well as on the proton transfer ratio (PTR) of the contributing exchangeable protons:

$$MTR_{\text{asym}}(\text{offset}) = MTR'_{\text{asym}}(\text{offset}) + PTR(\text{offset}). \quad (2)$$

For the amide protons of mobile protein and peptides, we abbreviate the amide PTR as $APTR$ below.

All data processing procedures were performed by Interactive Data Language (IDL, Research Systems, Inc., Boulder, CO, USA). The experimentally measured z-spectra (1st type, 25 offsets) were fitted through all offsets using a 12th-order polynomial (the maximal order available with IDL) on a pixel-by-pixel basis. After this, the fitted curve was interpolated using an offset resolution of 1 Hz (namely, 2401 points). The actual water resonance was assumed to be at the frequency with the lowest signal intensity. The deviation from 0 ppm, $\Delta\nu_0 (= \gamma\Delta B_0/(2\pi))$, where γ is the proton gyromagnetic ratio; in the unit of Hz), was used as a measure for the B_0 field inhomogeneity. To correct for the field inhomogeneity effects, the measured MT curve for each pixel was interpolated to 2401 points and shifted correspondingly along the direction of the offset axis. Finally, the realigned z-spectra were interpolated back to 25 points, and the outermost points of ± 6 ppm were excluded in the display. On the other hand, the high-SNR APT-weighted images (2nd type) were calculated according to:

$$MTR_{\text{asym}}(3.5\text{ppm}) = S_{\text{sat}}(-3.5\text{ppm})/S_0 - S_{\text{sat}}(3.5\text{ppm})/S_0. \quad (3)$$

The calculation is straightforward, and no B_0 inhomogeneity correction was added to these APT-weighted images, which is not possible for these two-offset data ($\pm 3.5\text{ppm}$).

For conventional MRI, data analysis was performed as follows: $MTR(10\text{ppm}) = 1 - S_{\text{sat}}(10\text{ppm})/S_0$. The spin-lattice relaxation time of water, T_1 , was fitted with a three-parameter equation: $I = A + B \exp(-TI/T_1)$. The spin-spin relaxation time of water, T_2 , was fitted using $I = I_0 \exp(-TE/T_2)$. The average apparent diffusion coefficient of water, $ADC_{\text{av}} = \text{Trace}(D) /$

3, was fitted by $I = I_0 \exp(-b \cdot ADC_{av})$. As done by most of the investigators, the background noise was not taken into account in the fitting.

Finally, quantitative analysis of imaging was carried out. As done previously (20), APT, ADC_{av} , and histology images were used as a basis for the definition of regions of interest (ROIs). Based on increased APT and increased ADC_{av} , we could easily find a well-contrasted area of tumor for all rats. The ROIs of tumor covered as large tumor regions as possible. For all rats, these ROIs were about 40–50 pixels, depending the tumor size. The same ROIs were applied to all images (including z-spectrum analysis) for each rat. In this study, MRI data were acquired on the coronal plane. Unlike our previous work in the axial plane (20), peritumoral edema (increased ADC_{av} but normal APT) could not be identified for many cases, and peritumoral regions were not analyzed here.

RESULTS

Figure 1 shows z-spectra, MTR_{asym} spectra, and MTR_{asym} -difference spectra in an offset range of ± 5.5 ppm acquired on the 9L ($n = 6$) and human glioblastoma ($n = 2$) brain tumor models. An upward shift of the tumor z-spectra is visible for both tumor models (Fig. 1A and B). Whereas the effects of conventional MT and direct water saturation dominate the z-spectra of the narrow offset range, careful observation indicates the existence of the small APT effects that are visible as a very small dip in z-spectra at a frequency offset of about 3.5 ppm downfield from the water resonance (dotted vertical line). Because the chemical shift of water is 4.75 ppm, the frequency of this dip corresponds to ~ 8.3 ppm in the proton NMR spectrum, where mobile protein and peptide amide protons resonate (17–19). The presence of APT effects becomes more pronounced when applying an MT asymmetry analysis to reduce the influence of direct saturation and the symmetric part of the conventional MT effects. The resulting curves in Fig. 1C and D show a varying MT asymmetry that is initially positive and then negative. This finding supports a previous report (36) that the solid-like MT effect is asymmetric with respect to the water resonance. Note that $MTR_{asym}(3.5 \text{ ppm})$ is consistently lower in this study due to lower saturation power used here (1.3 μT compared to the previous 2 μT (20)). When taking the difference between the tumor and contralateral asymmetry spectra, a large positive signal difference covering the offset range from about 2 to 5 ppm becomes clearer. The difference in $MTR_{asym}(3.5 \text{ ppm})$ between tumor and contralateral normal brain tissue is smaller in the human glioblastoma tumor model than in the 9L tumor model.

Figure 2 shows the $MTR_{asym}(3.5 \text{ ppm})$ (i.e., APT-weighted) image along with several other MRI types acquired on the 9L gliosarcoma tumor model (post-implantation day-12). It can be observed that T_1 , T_2 , and ADC_{av} are all increased in the tumor region. The MTR maps at offsets 3.5 and 10 ppm are both dark (hypointensity) in the tumor region, which may be due to increased water content, while the $MTR_{asym}(3.5 \text{ ppm})$ images are bright (hyperintensity), presumably due to higher protein and peptide concentration in gliomas (31). Namely, the $MTR_{asym}(3.5 \text{ ppm})$ and conventional MT images display different MRI contrasts between tumor and contralateral normal tissue. Figure 3 is an example of MRI for the human glioblastoma tumor model (7 weeks post-implantation). Roughly, the MRI contrast appears similar to the 9L glioma model, namely increased intensity in tumor in the APT-weighted image and decreased intensity in tumor in the MTR map. It is important to notice that the hyperintense (T_2 , T_1 , ADC) or hypointense (MTR) regions on several conventional MR images are generally larger than that on the APT-weighted image. Similar to our previous work (20), we assigned regions as being peritumoral when exhibiting increased ADC_{av} and approximately normal $MTR_{asym}(3.5 \text{ ppm})$. The peritumoral regions also showed markedly increased T_2 and T_1 , and decreased MTR with respect to the normal brain. These regions may be associated with white matter tract edema (37), which could easily be identified in some human glioblastoma tumor cases (Fig. 3).

A comparison of the MRI parameters measured for 9L tumors ($n = 8$) and for human glioblastoma tumors ($n = 6$) is given in Table 1. The MRI contrasts between tumor and contralateral normal tissue are all lower for the human glioblastoma tumor model than for the rat 9L tumor model. Furthermore, the measured MTR values at 10 ppm are larger for the human glioblastoma xenografts than for the 9L tumors ($P \leq 0.001$), and the other MRI parameters are all smaller in the human glioblastoma than in the 9L gliomas (T_1 , $P \leq 0.01$; T_2 , ADC_{av} , and $MTR_{asym}(3.5 \text{ ppm})$, $P \leq 0.001$), while the difference for contralateral brain tissue is less significant. These results confirm our above qualitative observations based on the z-spectra and MR images. In particular, we should notice increased $MTR_{asym}(3.5 \text{ ppm})$ but decreased $MTR(10 \text{ ppm})$ in the tumor with respect to the contralateral brain tissue. The difference between the $MTR_{asym}(3.5 \text{ ppm})$ values obtained from z-spectra and those obtained from APT-weighted images may be partially due to the presence of the B_0 field inhomogeneity in the uncorrected APT-weighted images.

We asked whether there were prominent differences in the histopathological features of these two glioma models that might explain the differences in MRI parameters. A comparison of hematoxylin and eosin stained tumor sections (Fig. 4) revealed that both tumor types consisted of relatively well demarcated hypercellular neoplasms. However, a substantial portion of the deeper regions of the human glioblastoma xenografts contained hypocellular zones of necrosis surrounded by a pseudopalisading rim characteristic of highly aggressive human glioblastomas. These regions of necrosis were distinctly absent from the 9L tumors.

DISCUSSION

It is important to compare the physical mechanisms that underlie conventional MT imaging and APT imaging. Conventional MT can be detected over a very large frequency range, on the order of about 100 kHz. This frequency range is determined by the dipolar-dipolar interaction and chemical shift anisotropy for semi-solid macromolecules in tissue. Contrary to MT contrast, APT effects are detected mainly in the offset range 2–5 ppm and maximized at about 3.5 ppm downfield from the water signal. The main differences between MT and APT are the frequency specificity of the saturation transfer effects and the approximately symmetric appearance of conventional MT with respect to the water resonance. The effect of APT originates from backbone amide protons associated with endogenous mobile, cytosolic proteins and peptides in biological tissue and depends purely on hydrogen exchange, which is mainly base-catalyzed in the physiological condition (37 °C, pH 7–7.4). There are two possible molecular mechanisms (38,39) responsible for conventional MT. The first pathway is through dipolar coupling from protons of the immobilized macromolecular phase to protons of hydration water on the macromolecular surface to protons of the unbound bulk water. The second pathway is through the exchangeable protons of some macromolecular side groups (e.g., -OH, -SH, and -NH), which mix with water protons via fast chemical exchange. Therefore, APT and conventional MT are two different MRI contrast mechanisms, even though both can be assessed in a saturation transfer experiment. In this paper, it is shown that the APT and MT contrasts between tumor and normal-appearing brain tissue are different (hyperintensity on the APT-weighted image versus hypointensity on the MTR map, corresponding to increased APT effect but decreased conventional MT effect in tumor with respect to contralateral brain tissue).

When $(2\pi\Delta\nu_0 T_2)^2 \gg 1$ and spins are isolated, the frequency offset at half maximum direction saturation (saturation width) can be described by (40):

$$\nu_{1/2} = \frac{\gamma B_1}{2\pi} \sqrt{\frac{T_1}{T_2}} \quad (4)$$

In this study, the RF saturation field strength was 1.3 μ T. Using water T_1 and T_2 at 4.7 T in Table 1, the saturation width for water is calculated to be 270–290 Hz. Notice that the presence of conventional MT makes the actual width much larger (~ 7 ppm, namely, ~ 1400 Hz at 4.7 T, see Fig. 1). The T_1 and T_2 values for amide protons may be equal to or a little shorter than those of water, respectively, but the exact values are not available this time. We expect that the saturation width for amide protons is as large as 200–300 Hz. In our experiments, the B_0 field inhomogeneity ($\Delta\nu_0$) was found to be within ± 50 Hz for all pixels. Therefore, the possibility that the 3.5 ppm signal was missing during RF radiation in the two-offset high-SNR APT imaging experiments was very low.

The characteristic of proton transfer effects becomes clearer when applying an MT asymmetry analysis to remove direct saturation and conventional MT effects. The resulting curves (Fig. 1C and D) show a varying MTR asymmetry that is initially positive and then negative, and the contrast of MTR_{asym} between tumor and normal-appearing tissue is maximized at the offsets of 2–5 ppm, where the amide protons resonate. When subtracting the normal MTR_{asym} plot from the tumor curve, a maximum signal change is found at the offset of ~ 3.5 ppm from water (Fig. 1E and F), indicating that this difference originates predominantly from the amide protons of the mobile proteins and peptides. Therefore, similar to Eq. [2], we have:

$$MTR_{\text{asym}}(3.5 \text{ ppm}) = MTR'_{\text{asym}}(3.5 \text{ ppm}) + APTR. \quad (5)$$

Since $MTR_{\text{asym}}(3.5 \text{ ppm})$ consists of two parts of $MTR'_{\text{asym}}(3.5 \text{ ppm})$ and APTR, the $MTR_{\text{asym}}(3.5 \text{ ppm})$ -based images should be referred to as APT-weighted images. We should notice that MTR'_{asym} may include the effects of B_0 field inhomogeneity (including susceptibility shifts) and improper water resonance frequency (including time-related shifting) (27). When comparing lesions or physiological alterations such as acute ischemia in the rat brain following middle cerebral artery (MCA) occlusion, APTR may be assessed under the assumption that MTR'_{asym} remains unaltered (19). However, care should be taken for explaining the APT contrast of brain tumors, because the MTR'_{asym} difference between tumor and contralateral normal brain tissue may exist.

As described before (20), increased APTR in the tumor with respect to normal brain can result from increased cellular amide proton content, increased proton exchange rates (or pH), and several other factors. It is possible that the observed differences in APT between the human glioblastoma and rat gliosarcoma models may be associated with many cellular, biochemical, and molecular determinants. Even though more MRI studies with histological correlations are needed in the future, our preliminary observations suggest that the presence of hypocellular zones of necrosis in the human glioblastoma xenografts may be the foremost factor. It is known (41) that many types of tumor cells proliferate rapidly and might accumulate defective proteins at a much higher rate than normal cells. Using in vivo proton MR spectroscopy, it was demonstrated recently by Howe et al. (31) that the MRS-detectable mobile macromolecular proton concentration for several upfield resonances is higher in human brain tumors than in normal white matter, and increases with tumor grade. Assuming each of the human glioblastoma and rat gliosarcoma cells has similar protein content, the presence of substantial hypocellular zones of necrosis below MRI resolution would lead to a decreased cellular protein concentration in the human glioblastoma xenografts. Furthermore, the hypoxic pseudopalisades that surround the necrosis might have a lower cellular pH (42). Therefore, the human glioblastoma xenografts that contained the necrotic zones and adjacent hypoxic pre-necrotic zones might differ enough in pH and cytosolic amide proton content from the more uniformly hypercellular 9L tumors and generate the observed APT signal differences between

these two tumor xenografts. While somewhat speculative, these findings suggest that APT imaging might have the potential to provide clinically relevant information pertaining to tumor viability and cell density.

CONCLUSIONS

APT imaging was applied to 9L gliosarcomas and human glioblastoma brain tumor models under the field strength of 4.7 T. It was found that the effect of APT (sensitive to mobile proteins and peptides) is larger in tumor than in contralateral normal-appearing brain tissue, which is different from the contrast between tumor and normal tissue in conventional MT images (sensitive to semi-solid macromolecules). The results show that APT provides a unique MRI modality for identifying tumor regions and mapping tumor distribution. The APT mechanism opens a completely new avenue of MRI research. This non-invasive approach to image cellular events at the protein level through high-resolution in vivo MRI may have clinical applications in the future.

Abbreviations used

ADC	apparent diffusion coefficient
APT	amide proton transfer
APTR	amide proton transfer ratio
BOLD	blood-oxygen-level-dependent
CEST	chemical exchange saturation transfer
CW	continuous-wave
EPI	echo planar imaging
MCA	middle cerebral artery
MT	magnetization transfer
MTR	magnetization transfer ratio
NA	number of accumulations
PTR	proton transfer ratio
RF	radiofrequency
ROI	region of interest
SNR	signal-to-noise ratio

Acknowledgments

The authors thank Dr. Charles G. Eberhart (JHU) for help on histological explanation, and Dr. C. David James (Mayo Clinic) for providing the human glioblastoma glioma xenograft model used in this study. This work was supported in part by grants from NIH (EB02634 and EB02666) and the Whitaker Foundation.

REFERENCES

1. Wolff SD, Balaban RS. Magnetization transfer contrast (MTC) and tissue water proton relaxation in vivo. *Magn. Reson. Med* 1989;10:135–144. [PubMed: 2547135]
2. Balaban RS, Ceckler TL. Magnetization transfer contrast in magnetic resonance imaging. *Magn. Reson. Q* 1992;8:116–137. [PubMed: 1622774]
3. Bryant RG. The dynamics of water-protein interactions. *Annu. Rev. Biophys. Biomol. Struct* 1996;25:29–53. [PubMed: 8800463]

4. Henkelman RM, Stanisz GJ, Graham SJ. Magnetization transfer in MRI: a review. *NMR Biomed* 2001;14:57–64. [PubMed: 11320533]
5. Yousem DM, Montone KT, Sheppard LM, Rao VM, Weinstein GS, Hayden RE. Head and neck neoplasms - magnetization-transfer analysis. *Radiology* 1994;192:703–707. [PubMed: 8058937]
6. Kurki T, Lundbom N, Kalimo H, Valtonen S. MR classification of brain gliomas: value of magnetization transfer and conventional imaging. *Magn. Reson. Imag* 1995;13:501–511.
7. Quesson B, Bouzier A-K, Thiaudiere E, Delalande C, Merle M, Canioni P. Magnetization transfer fast imaging of implanted glioma in the rat brain at 4.7T: interpretation using a binary spin-bath model. *J. Magn. Reson. Imag* 1997;7:1076–1083.
8. Okumura A, Takenaka K, Nishimura Y, Asano Y, Sakai N, Kuwata K, Era S. The characterization of human brain tumor using magnetization transfer technique in magnetic resonance imaging. *Neurol. Res* 1999;21:250–254. [PubMed: 10319332]
9. Vonarbourg A, Sapin A, Lemaire L, Franconi F, Menei P, Jallet P, Le Jeune JJ. Characterization and detection of experimental rat gliomas using magnetic resonance imaging. *MAGMA* 2004;17:133–139. [PubMed: 15503254]
10. Pui MH, Wang Y. Diffusion and magnetization transfer MRI of brain infarct, infection, and tumor in children. *Clin. Imag* 2005;29:162–171.
11. Mehta RC, Pike GB, Haros SP, Enzmann DR. Central nervous system tumor, infection, and infarction: detection with gadolinium-enhanced magnetization transfer MR imaging. *Radiology* 1995;195:41–46. [PubMed: 7892492]
12. Hawighorst H, Debus J, Schreiber W, Knopp MV, Engenhart-Cabillic R, Essig M, Brix G, van Kaick G. Contrast-enhanced magnetization transfer imaging: improvement of brain tumor conspicuity and delineation for radiosurgical target volume definition. *Radiother. Oncol* 1997;43:261–267. [PubMed: 9215785]
13. Behar KL, Ogino T. Assignment of resonances in the ^1H spectrum of rat brain by two dimensional shift correlated and J-resolved NMR spectroscopy. *Magn. Reson. Med* 1991;17:285–303. [PubMed: 1676483]
14. Behar KL, Ogino T. Characterization of macromolecule resonances in the ^1H NMR spectrum of rat brain. *Magn. Reson. Med* 1993;30:38–44. [PubMed: 8371672]
15. Kauppinen RA, Kokko H, Williams SR. Detection of mobile proteins by proton nuclear magnetic resonance spectroscopy in the guinea pig brain *ex vivo* and their partial purification. *J. Neurochem* 1992;58:967–974. [PubMed: 1738003]
16. Kauppinen RA, Palvimo J. Contribution of cytoplasmic polypeptides to the ^1H NMR spectrum of developing rat cerebral cortex. *Magn. Reson. Med* 1992;25:398–407. [PubMed: 1614325]
17. Mori S, Eleff SM, Pilatus U, Mori N, van Zijl PCM. Proton NMR spectroscopy of solvent-saturable resonance: a new approach to study pH effects *in situ*. *Magn. Reson. Med* 1998;40:36–42. [PubMed: 9660550]
18. van Zijl PCM, Zhou J, Mori N, Payen J, Mori S. Mechanism of magnetization transfer during on-resonance water saturation. a new approach to detect mobile proteins, peptides, and lipids. *Magn. Reson. Med* 2003;49:440–449. [PubMed: 12594746]
19. Zhou J, Payen J, Wilson DA, Traystman RJ, van Zijl PCM. Using the amide proton signals of intracellular proteins and peptides to detect pH effects in MRI. *Nature Med* 2003;9:1085–1090. [PubMed: 12872167]
20. Zhou J, Lal B, Wilson DA, Laterra J, van Zijl PCM. Amide proton transfer (APT) contrast for imaging of brain tumors. *Magn. Reson. Med* 2003;50:1120–1126. [PubMed: 14648559]
21. Guivel-Scharen V, Sinnwell T, Wolff SD, Balaban RS. Detection of proton chemical exchange between metabolites and water in biological tissues. *J. Magn. Reson* 1998;133:36–45. [PubMed: 9654466]
22. Ward KM, Aletras AH, Balaban RS. A new class of contrast agents for MRI based on proton chemical exchange dependent saturation transfer (CEST). *J. Magn. Reson* 2000;143:79–87. [PubMed: 10698648]
23. Goffeney N, Bulte JWM, Duyn J, Bryant LH, van Zijl PCM. Sensitive NMR detection of cationic-polymer-based gene delivery systems using saturation transfer via proton exchange. *J. Am. Chem. Soc* 2001;123:8628–8629. [PubMed: 11525684]

24. Zhang S, Winter P, Wu K, Sherry AD. A novel europium(III)-based MRI contrast agent. *J. Am. Chem. Soc* 2001;123(7):1517–1578. [PubMed: 11456734]
25. Aime S, Barge A, Delli Castelli D, Fedeli F, Mortillaro A, Nielsen FU, Terreno E. Paramagnetic Lanthanide(III) complexes as pH-sensitive chemical exchange saturation transfer (CEST) contrast agents for MRI applications. *Magn. Reson. Med* 2002;47:639–648. [PubMed: 11948724]
26. Zhang S, Merritt M, Woessner DE, Lenkinski R, Sherry AD. PARACEST agents: modulating MRI contrast via water proton exchange. *Acc. Chem. Res* 2003;36:783–790. [PubMed: 14567712]
27. Zhou J, van Zijl PC. Chemical exchange saturation transfer imaging and spectroscopy. *Progr. NMR Spectr* 2006;48:109–136.
28. Terreno E, Cabella C, Carrera C, Castelli DD, Mazzon R, Rollet S, Stancanella J, Visigalli M, Aime S. From spherical to osmotically shrunken paramagnetic liposomes: An improved generation of LIPOCEST MRI agents with highly shifted water protons. *Angew. Chem. Int. Ed* 2007;46:966–968.
29. Jokivarsi KT, Grohn HI, Grohn OH, Kauppinen RA. Proton transfer ratio, lactate, and intracellular pH in acute cerebral ischemia. *Magn. Reson. Med* 2007;57:647–653. [PubMed: 17390356]
30. Jones CK, Schlosser MJ, van Zijl PC, Pomper MG, Golay X, Zhou J. Amide proton transfer imaging of human brain tumors at 3T. *Magn. Reson. Med* 2006;58:585–592. [PubMed: 16892186]
31. Howe FA, Barton SJ, Cudlip SA, Stubbs M, Saunders DE, Murphy M, Wilkins P, Opstad KS, Doyle VL, McLean MA, Bell BA, Griffiths JR. Metabolic profiles of human brain tumors using quantitative in vivo ^1H magnetic resonance spectroscopy. *Magn. Reson. Med* 2003;49:223–232. [PubMed: 12541241]
32. Mori S, van Zijl PCM. Diffusion weighting by the trace of the diffusion tensor within a single scan. *Magn. Reson. Med* 1995;33:41–52. [PubMed: 7891534]
33. Spencer RGS, Horska A, Ferretti JA, Weiss GH. Spillover and incomplete saturation in kinetic measurements. *J. Magn. Reson. B* 1993;101:294–296.
34. McMahon MT, Gilad AA, Zhou J, Sun PZ, Bulte JWM, van Zijl PCM. Quantifying exchange rates in chemical exchange saturation transfer agents using the saturation time and saturation power dependencies of the magnetization transfer effect on the magnetic resonance imaging signal (QUEST and QUESP): pH calibration for poly-L-lysine and a starburst dendrimer. *Magn. Reson. Med* 2006;55:836–847. [PubMed: 16506187]
35. Zhou J, Payen J, van Zijl PCM. The interaction between magnetization transfer and blood-oxygen-level-dependent effects. *Magn. Reson. Med* 2005;53:356–366. [PubMed: 15678541]
36. Pekar J, Jezzard P, Roberts DA, Leigh JS, Frank JA, McLaughlin AC. Perfusion imaging with compensation for asymmetric magnetization transfer effects. *Magn. Reson. Med* 1996;35:70–79. [PubMed: 8771024]
37. Witwer BP, Moftakhar R, Hasan KM, Deshmukh P, Haughton V, Field A, Arfanakis K, Noyes J, Moritz CH, Meyerand ME, Rowley HA, Alexander AL, Badie B. Diffusion-tensor imaging of white matter tracts in patients with cerebral neoplasm. *J. Neurosurg* 2002;97:568–575. [PubMed: 12296640]
38. Liepinsh E, Otting G. Proton exchange rates from amino acid side chains-implication for image contrast. *Magn. Reson. Med* 1996;35:30–42. [PubMed: 8771020]
39. Gochberg DF, Kennan RP, Maryanski MJ, Gore JC. The role of specific side groups and pH in magnetization transfer in polymers. *J. Magn. Reson* 1998;131:191–198. [PubMed: 9571092]
40. Henkelman RM, Stanisz GJ, Kim JK, Bronskill MJ. Anisotropy of NMR properties of tissues. *Magn. Reson. Med* 1994;32:592–601. [PubMed: 7808260]
41. Adams J. The proteasome: a suitable antineoplastic target. *Nat. Rev. Cancer* 2004;4:349–360. [PubMed: 15122206]
42. Okunieff P, Urano M, Kallinowski F, Vaupel P, Neuringer LJ. Tumors growing in unirradiated tissue: oxygen, metabolic state, and pH. *Int. J. Rad. Oncol. Bio. Phys* 1991;21:667–673.

Figure 1.

Z-spectra (A, B), MTR_{asym} spectra (C, D), and MTR_{asym} -difference spectra (E, F) for the 9L brain tumors (left column, 12 days post-implantation, n = 6) and the human glioblastoma brain tumors (right column, 7 weeks post-implantation, n = 2). The data from the same post-implantation days were averaged. The offsets of ± 6 ppm were excluded in the display. Tumor: open circles; contralateral normal brain tissue: solid circles. MTR_{asym} difference = $MTR_{\text{asym}}(\text{tumor}) - MTR_{\text{asym}}(\text{normal})$. The APT effects are visible at the offset of 3.5 ppm in the z-spectra and MTR_{asym} spectra. The effect is stronger in tumor than in the contralateral normal-appearing region.

Figure 2.

MR images and histology for the 9L gliosarcoma tumor model in a Fisher 344 rat (12 days post-implantation). The tumor is visible in all of the MR images, as confirmed by histology. The APT-weighted image appears to distinguish a small, likely edematous region (arrow) from tumor. The blue dotted line on the APT-weighted image defined a tumor ROI for quantitative analysis.

Figure 3.

MR images and histology for the human glioblastoma brain tumor model in a nude rat (7 weeks post-implantation). The hyperintense (T_2 , T_1 , ADC) or hypointense (MTR) regions on several conventional MR images are larger than that on the APT-weighted image. The APT-weighted image is able to separate the likely edematous part (arrow) from tumor, and its hyperintensity profile is in good correspondence with histology. The blue dotted line on the APT-weighted image defined a tumor ROI for quantitative analysis.

Human glioblastoma

9L gliosarcoma

Figure 4. Photomicrographs of hematoxylin and eosin stained sections of orthotopic human glioblastoma (A, C) and 9L gliosarcoma (B, D). Prominent hypocellular, eosinophilic zones of necrosis (N) are evident within the deeper regions of the human glioblastoma tumors and absent within the 9L gliosarcoma model. White arrows (C) indicate pseudopalisades that surround the necrosis. Bars equal 1 mm (A, B) and 0.5 mm (C, D).

Table 1

parameters measured for the 9L brain tumor model on Fisher 344 rats (12 or 13 days post-implantation; n = 8) and for the human glioblastoma brain tumor model on nude rats (5–7 weeks post-implantation; n = 6), and the student's t-test significance of the difference between the 9L and human glioblastoma

	T ₂ (ms)		T ₁ (s)		In vivo ADC _{av} (10 ⁻⁹ m ² /s)		MTR at 10ppm ^d		MTR _{asym} at 3.5ppm from z-spectrum ^d		MTR _{asym} at 3.5ppm from APTw image ^d	
	9L	Glioblastoma	9L	Glioblastoma	9L	Glioblastoma	9L	Glioblastoma	9L	Glioblastoma	9L	Glioblastoma
Contralateral	58.3 ± 1.0	57.1 ± 1.2	1.40 ± 0.04	1.35 ± 0.04	0.72 ± 0.03	0.68 ± 0.02	31.7% ± 0.9%	31.4% ± 0.8%	-2.36% ± 0.19%	-2.77% ± 0.42%	-1.92% ± 0.66%	-2.70% ± 0.51%
Pr	83.8 ± 2.4 ^{***}	65.6 ± 3.3 ^{***}	1.97 ± 0.04 [*]	1.83 ± 0.09 [*]	1.16 ± 0.05 ^{***}	0.92 ± 0.03 ^{***}	19.2% ± 0.7% ^{***}	22.0% ± 2.1% ^{***}	1.49% ± 0.66% ^{***}	-1.18% ± 0.60% ^{***}	3.13% ± 0.83% ^{***}	-0.70% ± 0.97% ^{***}

MTR_{Rasym}(3.5 ppm) and MTR(10 ppm) are RF power dependent.

0.01,
≤ 0.001