Published in final edited form as:

Magn Reson Med. 2016 August ; 76(2): 635–644. doi:10.1002/mrm.25893.

Influence of water compartmentation and heterogeneous relaxation on quantitative magnetization transfer imaging in rodent brain tumors

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Abstract

Purpose—This study investigates the influence of water compartmentation and heterogeneous relaxation properties on quantitative magnetization transfer (qMT) imaging in tissues, and in particular whether a two-pool model is sufficient to describe qMT data in brain tumors.

Methods—Computer simulations and *in vivo* experiments with a series of qMT measurements before and after injection of Gd-DTPA were performed. Both off-resonance pulsed saturation (pulsed) and on-resonance selective inversion recovery (SIR) qMT methods were used, and all data were fit with a two-pool model only.

Results—Simulations indicate that a two-pool fitting of four-pool data gives accurate measures of PSR (pool size ratio of macromolecular vs free water protons) when there is fast transcytolemmal exchange and slow R_1 recovery. The fitted *in vivo* PSR of both pulsed and SIR qMT methods showed no dependence on R_1 variations caused by different concentrations of Gd-DTPA during wash-out, while the fitted k_{ex} (MT exchange rate) changed significantly with R_1 .

Conclusion—A two-pool model provides reproducible estimates of PSR in brain tumors independent of relaxation properties in the presence of relatively fast transcytolemmal exchange, while estimates of k_{ex} are biased by relaxation variations. In addition, estimates of PSR in brain tumors using the pulsed and SIR qMT methods agree well.

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Keywords

qMT; PSR; SIR; transcytolemmal water exchange; Gd-DTPA; tumor

Introduction

Magnetization transfer in tissues (1) originates from the cross-relaxation between macromolecular and free water protons, via dipolar interactions and/or chemical exchange. The semi-quantitative metric, magnetization transfer ratio (MTR), has been adopted to indirectly image variations in macromolecular proton pool sizes, which often correlate with variations in tissue composition in pathophysiological conditions. MTR imaging has been successfully applied to characterize neurological disorders (2), neuromuscular diseases (3), and cancer (4–8). However, MTR is also sensitive to hardware choices (e.g. magnetic field strength) and pulse sequence parameters (e.g. RF irradiation power, frequency offset), and hence its specificity and sensitivity to specific pathophysiological changes are reduced. Moreover, MTR as a single metric is inherently sensitive to multiple tissue properties simultaneously, such as tissue relaxation rate, MT exchange rates, and the size of macromolecular proton pool. Therefore, variations in measured MTR may originate from various sources including multiple tissue properties and acquisition approaches, which increase difficulty to interpret MTR data. To increase the specificity to individual tissue properties and reduce the influence of hardware settings, quantitative magnetization transfer (qMT) imaging methods have been developed to quantify the intrinsic proton relaxation rates, MT exchange rate, and the pool size ratio (PSR) of macromolecular protons to free water protons. Several qMT methods using different models, analyses and acquisitions have been proposed, such as continuous-wave (CW) saturation (9), pulsed saturation (10), selective inversion recovery (SIR) (11), stimulated-echo-based (12), and steady-state free precession based (13) methods. Among these, the pulsed saturation (pulsed MT) method has been the most widely adopted. The pulsed method applies saturation pulses with varying frequency offsets and saturation powers systematically. A variety of quantitative analyses have been proposed to describe the pulsed qMT data, including those developed by Sled and Pike (10), Ramani et al. (14), Yarnykh (15), and Portnoy and Stanisiz (16). The SIR qMT method (11) applies an on-resonance inversion pulse to invert the magnetization of the free-water pool, and the resulting transient longitudinal magnetization is measured and fit to a bi-exponential function. Unlike the pulsed saturation method, SIR is based on the transient response of the sample, and hence provides a fundamentally different way of probing effects of multiple compartments on two-pool qMT fittings. A number of variants of the SIR approach have also been developed and applied in small animal (17–19) and human *in vivo* imaging (20,21) at different field strengths.

Most qMT methods assume a simple two-pool model that consists of a macromolecular proton pool and a free water proton pool (9). However, this is an oversimplification because real biological tissues may contain multiple distinct water and macromolecular (solid) pools. For example, white matter (WM) has been reported as a system consisting of multiple water/solid components, including myelin water, intra-/extra-axonal water, myelin solids (macromolecular protons), and non-myelin solids (22–24). Different diseases may alter

different water/solid components in different ways, and hence the simplified two-pool model may not characterize pathophysiological variations adequately. For example, demyelination can cause decreases of macromolecular proton pools, while inflammation can cause increases of free water proton pools. Although both of these two different pathological changes cause decreases of PSR values, the underlying mechanisms are very different. In order to improve the accuracy of model fits, more complex four-pool models have been developed (22–25). However, these models also include assumptions and more importantly, acquiring and fitting data are time-consuming, which limits their practical implementation.

In addition to applications in neurodegenerative diseases, MT imaging, especially MTR, has been used extensively for imaging cancer, e.g. brain (5), prostate (6), pancreas (7), and breast cancer (8). There has also been increasing interest in applying qMT imaging to characterize solid tumors (15,19,26,27) to overcome the intrinsic limitations (discussed above) of MTR. Again, these qMT studies used a two-pool model to simplify data analyses and speed up acquisitions for practical *in vivo* use. However, given the fact that tumors contain multiple water compartments, e.g. intra- and extra-cellular spaces, with heterogeneous relaxation properties, the validity of fitting qMT data of tumors using such a two-pool model has not been fully investigated. Moreover, anti-cancer treatments may alter the water compartmentation (28) and selectively change intra- or extracellular macromolecular contents (28–30) which are anticipated to cause relaxation property variations within tumors, which may confound the interpretation of derived qMT parameters in complex ways. Therefore, it is plausible to investigate the influence of multiple water compartments and heterogeneous relaxation properties on qMT measurements in tumors. Note that, different from normal neural tissues, tumor cells have been reported to have a relatively high transcytolemmal water exchange rate, which may simplify the requirements for accurate qMT modelling. For example, the apparent transcytolemmal exchange rate has been reported up to $2.9 \pm 0.8 \text{ s}^{-1}$ in human solid brain tumors, much larger than those in human white matter (range from $0.8 \pm 0.08 \text{ s}^{-1}$ in the internal capsule to $1.6 \pm 0.11 \text{ s}^{-1}$ in frontal white matter) (31). Moreover, if apoptosis occurs after effective anti-cancer treatments, the transcytolemmal water exchange rate in tumors can increase further to $6.8 \pm 3.9 \text{ s}^{-1}$ (32), four times that of normal white matter. For comparison, the longitudinal relaxation rate, R_1 , has been reported as $0.63 \pm 0.03 \text{ s}^{-1}$ in rat white matter and $0.47 \pm 0.07 \text{ s}^{-1}$ in rat brain tumor at 9.4T (19), much smaller than the transcytolemmal exchange rate in tumors. If the freely diffusing water molecules in different water compartments (intra- and extracellular spaces) are well mixed during qMT measurements in tumors, a two-pool model would be sufficient to describe qMT data. This would significantly simplify data analysis and make accurate qMT imaging feasible for practical studies.

In the current study, the influence of water compartmentation and relaxation on qMT measurements in tumors was investigated via comprehensive numerical simulations and *in vivo* experiments using two widely used qMT approaches, i.e. pulsed (off-resonance) and SIR (on-resonance) methods. Without any assumption of the distribution of macromolecules in tumors, numerical simulations based on two-, three-, and four-pool tissue models with various transcytolemmal water exchange rates and proton relaxation rates were performed to predict the results of both pulsed and SIR acquisitions. After that, irrespective of the number of pools used in the simulations, the simple two-pool model was used to fit all simulated MT

signals to investigate whether two-pool models are sufficient to describe qMT data in tumors. Subsequent *in vivo* experiments imaged rats bearing 9L brain tumors and implemented a series of repeated qMT measurements after Gd-DTPA (gadolinium-diethylenetriamine pentaacetic acid) injections to estimate qMT parameters in the presence of different relaxation rates in tumors. Because Gd-DTPA selectively alters the extracellular relaxation properties without altering transcytolemmal water exchange rate, PSR or k_{ex} (MT exchange rate between the free water and macromolecular pools based on a two-pool model), this manipulation provides an opportunity to investigate how much derived qMT parameters in tumors vary with relaxation changes. QMT parameters were fit with a two-pool model only and compared without and with different concentrations of Gd-DTPA. In addition, the qMT parameters obtained using pulsed and SIR methods were compared to see whether these two different qMT methods produce similar results.

Methods

Optimization of SIR acquisition for tumors

In a previous study (33), we used a Cramer-Rao lower bound criterion to minimize the scanning time for SIR data acquisitions, and found a minimum of five signal data points (each with one pair of inversion time t_i and delay time t_d (18)) were appropriate for SIR data analysis. However, that optimization was based on an assumption that tissue T_1 was in the range of $0.67 - 2$ s (such as in normal white matter), whereas tumor T_1 values at higher fields are usually significantly larger, e.g. up to 3 s in rodent brain tumors at 9.4T. (19). A sampling scheme of 20 pairs of t_i and t_d was implemented previously for SIR qMT imaging in rodent brain tumors for better accuracy (19), but then the total scanning time was much longer (~ 14 min for 2-shot echo-planar-imaging (EPI) acquisitions). In the current study, we performed a similar optimization as reported in (33) to minimize the scanning time required for SIR qMT imaging in tumors with a broader range of R_1 ($0.33 - 2$ s⁻¹). To enhance the stability and precision of data fitting, seven signal data points were chosen and the corresponding optimized seven pairs of t_i and t_d are shown in Table 1. The total scanning time for each SIR data acquisition was 63 s for single-shot EPI acquisitions.

Numerical Simulations

All numerical simulations and data analyses in the following sections were performed using MATLAB (Mathworks, Natick, MA). Due to a lack of precise knowledge about the distribution of macromolecules in tumors that may interact with free water, the MT process in cancerous tissues was described using two-, three-, and four-pool models, respectively. For simplicity, only the most generalized four-pool model system is shown here. More details of numerical simulations of all model systems can be found in the Supporting Materials. We need to emphasize that, despite different number of pools used in the simulations, all simulated qMT signals were fit to a two-pool qMT model only to obtain qMT parameters. By such a means, the validity and sufficiency of the time-efficient two-pool qMT model to describe complex qMT signals *in vivo* was evaluated.

Four-pool MT model for tumors—The four-pool MT model includes intracellular free (mobile) water “A”, extracellular free (mobile) water “B”, intracellular macromolecular

(immobile) protons “C”, and extracellular macromolecular (immobile) protons “D”, as illustrated in Figure 1. In addition to their intrinsic MR parameters M_{0i} , R_{1i} , R_{2i} , ($i = A, B, C$, and D , respectively), there were exchange processes between these pools, with k_{AB} noted as the transcytolemmal water exchange rate from the intracellular to the extracellular free water, k_{BA} noted as the transcytolemmal water exchange rate in the inversed direction, i.e. from the extracellular to the intracellular free water, k_{CA} noted as the MT exchange rate from the intracellular macromolecules to the intracellular free water, and k_{DB} noted as the MT exchange rate from the extracellular macromolecules to the extracellular water. Note that previous reported transcytolemmal water change rates usually indicate k_{AB} , and hence k_{AB} was mainly investigated in the current work. For the exchanges between these pools, there are balanced relations i.e. $M_{0A} \times k_{AB} = M_{0B} \times k_{BA}$, $M_{0A} \times k_{AC} = M_{0C} \times k_{CA}$, and $M_{0B} \times k_{BD} = M_{0D} \times k_{DB}$. Our choices of intrinsic parameter values mainly followed previous studies (34). To mimic the relaxation property changes caused by the injection of Gd-DTPA, R_{1B} was varied from 0.3 to 2.9 s⁻¹, stepped by 0.2 s⁻¹, and $R_{2B} = 20 + (R_{1B} - 0.4) \times 10$ s⁻¹. The transcytolemmal water exchange rate k_{AB} varied from 0 to 6 s⁻¹. Two sets of simulations were performed based on the four-pool model system: (1) different intrinsic PSRs in intra- and extracellular components with parameters of $M_{0A} = 0.705$, $R_{1A} = 0.4$ s⁻¹, $R_{2A} = 20$ s⁻¹, $M_{0B} = 0.24$, $M_{0C} = 0.05$ and $M_{0D} = 0.005$; and (2) a homogenous distribution of PSRs of 0.0638 everywhere inside tumor with $M_{0A} = 0.705$, $M_{0B} = 0.235$, $M_{0C} = 0.045$ and $M_{0D} = 0.015$. Only the results of the first type of simulation are shown here, and the results of the second type are shown in the Supplementary Materials. All simulated results were shown with observed R_1 of the whole system. Note that Gd-DTPA also introduces magnetic susceptibility gradients that cross cell membranes (35), which may alter intracellular R_{2A} . The simulations suggest the influence of R_{2A} on our qMT measurements is negligible (data not shown), so R_{2A} was assumed constant in the simulations.

Numerical simulations of qMT signals—Numerical simulations were performed by solving modified Bloch equations (see Supporting Materials) with either off-resonance saturation pulses to simulate pulsed MT data or on-resonance inversion pulses to simulate SIR MT data. For the pulsed MT, Gaussian-shaped saturation pulses were used with pulse width = 14.6 ms and time of repetition (TR) = 28 ms. When the M_{z0} of the intracellular free water component varied < 0.01% in two adjacent repetitions, the system was considered in a steady state. Spoiler gradients were simulated by setting the transverse magnetization to zero. The sampling scheme consisted of two irradiation powers with nominal flip angles of 220° and 820° and ten frequency offsets (34). The R_1 values obtained using the SIR method were used to fit pulsed qMT parameters.

The SIR data were simulated by four steps according to the model described previously (33). Briefly, the initial transverse magnetization of all pools was first set to zero, simulating our recent method in which an additional saturation pulse train was applied to fully saturate the magnetization of all proton pools (19); a pre-delay (t_d) allowed the z magnetization to recover followed by an on-resonance 1-ms 180° inversion hard pulse to invert the water longitudinal magnetization; finally, an inversion recovery (t_i) time allowed the system to recover before the integrated z magnetization was measured.

Irrespective of the number of pools used in the simulations, all simulated signals were fit using the two-pool model to evaluate whether two pools are sufficient to describe the qMT data. The simulated pulsed data were fit to the two-pool Ramani's model (14). Also, a subset of the simulated SIR data ($t_i > 100\text{ms}$) were fit as described in (33) to estimate qMT parameters and R_1 the apparent relaxation rate of whole tissues. Note that such a R_1 was also used in the pulsed MT data fitting. The true PSR values were calculated = $(M_{0C} + M_{0D}) / (M_{0A} + M_{0B})$. To mimic the injections of Gd-DTPA, 14 R_{1B} 's were used evenly distributed from 0.3 to 2.9 s.

Animal preparation

All animal procedures were approved by the Institutional Animal Care and Use Committee at Vanderbilt University. 9L glioblastoma cells were injected into the right brains of six male Fischer 344 rats (250 – 300 grams). Imaging was performed after tumors grew to 30 – 40 mm^3 , about 14 – 18 days after the tumor cell injections. Details of the surgical procedures have been reported previously (19,36).

Animals were anesthetized with 3% isoflurane for induction and 2% during MRI scanning. The position of each rat head was secured using an MRI compatible cradle with bite and head bars. A warm-air feedback system was used to ensure rat rectal temperature was maintained at $\sim 37^\circ\text{C}$ throughout the studies.

MRI experiments in vivo

All images were acquired on a 9.4T Agilent horizontal MRI scanner. A diagram of the experimental design is shown in Figure 2. In the baseline period, high-resolution anatomical images, B_1 , B_0 , R_2 , pulsed MT, R_1 and SIR MT data were acquired. All MR parameters were acquired with a single axial slice crossing the center of the tumor with a slice thickness of 2 mm, field of view of $32 \times 32 \text{ mm}^2$ and matrix size of 64×64 , yielding an isotropic in-plane resolution of 500 μm . B_1 maps were obtained using a double-angle method, in which pre-pulses with flip angles of 33° and 66° were applied before a spin-echo (SE) EPI readout sequence (34). B_0 maps were obtained using a spoiled gradient recalled echo (SPGR) sequence, with four echo times (TE) ranging from 3 to 9 ms, stepped by 2 ms. The acquisitions of R_2 , pulsed, and SIR were interleaved and repeated 11 times with a scanning time of 4 min for each repetition. R_2 maps were obtained using single-shot spin-echo EPI acquisitions with two TEs of 30 and 80 ms. Pulsed MT data were acquired using a MT-weighted SPGR sequence using the same sampling scheme as the simulations, with $\text{TR}/\text{TE} = 28/4 \text{ ms}$, MT pulse width = 14.6 ms and excitation flip angle 7° . Note that with such a small flip angle and correspondingly low saturation power, the potential bias in using Ramani's model has been minimized (34). SIR MT data were acquired using a single-shot SE-EPI sequence with a pre-inversion pulse and a saturation pulse train with varying TR's and a fixed TE = 30 ms as described in (19). The optimized 7-point sampling scheme (see Table 1) was used. After the acquisition of a baseline, a bolus of Gd-DTPA (0.2 mmol kg^{-1}) was intravenously delivered via a tail vein. During and after the injection of Gd-GTPA, a T_1 -weighted SPGR sequence ($\text{TR}/\text{TE} = 100/2.1 \text{ ms}$) was performed to monitor R_1 changes, as indicated in Figure 2. For each study, 11 serial acquisitions (10 after each Gd-DTPA injection) of T_2 , pulsed, and SIR MT data were obtained. Note that R_1 values changed

quickly during the acquisition of the 2nd ($+135 \pm 10.2\%$) and 3rd ($+7.9 \pm 7.6\%$) dynamic scans (See Figure 5), resulting in significant biased estimates of qMT parameters. Hence, the data of #2 and #3 dynamic scans were excluded from the following analyses.

Experimental data analysis

All MR imaging data were processed on pixel-wise basis. The pulsed-MT data were fitted to Ramani's model, with B_1 maps for irradiation power correction, B_0 maps for RF frequency offset correction. R_2 values were calculated by fitting two echoes to an exponential decay. The observed R_1 was obtained by fitting the SIR data to a mono-exponential function, as described in (33). A super-Lorentzian lineshape was adopted in pulsed MT data fitting (37). The SIR data were fit to the bi-exponential model (33) to determine qMT parameters. In the SIR data fitting, the effect of the inversion pulse on the macromolecular pool was set at 0.83, according to numerical simulations from our previous work (18). A Gaussian lineshape was adopted in simulating the inversion pulse effects in the SIR sequence, since the super-Lorentzian is not defined at zero offset. This change of lineshape is unlikely to cause significant effects, as the inversion pulse effects on the macromolecular pool have only a very weak dependence on lineshape (17). Both qMT models yielded five qMT parameters each, and the PSR values and k_{ex} values were of interest in the current study. Regions-of-interest (ROIs) were visually identified and manually defined for each rat. The mean and standard deviation (SD) of parameter values within each ROI were used for comparisons.

Statistical analysis

Most statistical analyses were performed using Matlab. For both pulsed and SIR qMT methods, the PSR and k_{ex} obtained from all dynamic scans (excluding #2 and #3) were compared to investigate if fitted qMT parameters varied with different relaxation rates using one-way ANOVA. Bland-Altman figures were used to evaluate the agreement between the estimates of PSR and k_{ex} obtained using pulsed and SIR methods. For the correlations of multiple MR parameters with R_1 , linear mixed-effects models with compound symmetry structure of the correlation were employed to account for animal-specific variations. This last analysis was performed with R statistical software version 3.0.2 (<http://www.r-project.org/>). For each model, histogram and scatter plot of residuals were visually inspected to assure the normality of each parameter and randomness of the error, respectively.

Results

Numerical Simulations

Simulated PSR and k_{ex} values as a function of R_1 are shown in Figure 3. Four different values of k_{AB} (transcytolemmal water exchange rate) spanned the range from 0 to 6 s^{-1} . For the pulsed qMT method, PSR's were all overestimated, while k_{ex} 's depended on the choices of k_{AB} and R_1 . In the R_1 range acquired *in vivo* (indicated as gray regions in Figure 3), the fitted PSR's were $< 1.40\%$ higher than the true value of 6.38% . When $R_1 > 0.8 \text{ s}^{-1}$, the fitted PSR's were overestimated by at least a factor of two when $k_{AB} = 0$ (no transcytolemmal water exchange). The fitted k_{ex} 's decreased monotonically with R_1 for all k_{AB} values. For the SIR method, the fitted PSR was less than 0.70% higher than the true value 6.38% in the *in vivo* R_1 range, while the fitted k_{ex} deviated from the true k_{ex} values by a slight decrease of

1.3% with any choice of k_{AB} in the *in vivo* R_1 range. This indicates that the influence of transcytolemmal water exchange rate on SIR qMT measurements is minor in tumors at high fields *in vivo*. However, when $R_1 > 0.8 \text{ s}^{-1}$, both PSR and k_{ex} change with k_{AB} significantly. Note that the same range of R_{1B} 's were used (0.3 to 2.9 s) in the simulations, while the corresponding R_1 values were different for different k_{AB} values.

MRI experiments in vivo

Figure 4 shows parametric maps of R_2 , R_1 , PSR(SIR), and PSR(pulsed) obtained from the dynamic scans (excluding #2 and #3) from one representative rat. The ROI of the tumor is shown in the first PSR(SIR) map. As expected, after the injection of Gd-DTPA, R_2 and R_1 increased rapidly, and then decreased gradually during the washout. However, the values of both PSR(SIR) and PSR(pulsed) were found to be approximately similar across all of these scans, during which the relaxation rates in tumors were significantly different from time to time. Note that these parameters did not change with time in the normal tissue regions because of the absence of Gd-DTPA extravasation. Interestingly, k_{ex} (pulsed) was found changed between #1 and #4 in the normal tissue region of the representative rat shown in Figure 4, while such a variation was not found in the group analyses of all animals. A possible explanation is that the k_{ex} fitting was more prone to physiological noise.

The ROI data for R_2 , R_1 , PSR(SIR), k_{ex} (SIR), PSR(pulsed), and k_{ex} (pulsed) for all rat tumors are plotted in Figure 5. Before the Gd-DTPA injection, R_2 values were $28.7 \pm 1.8 \text{ s}^{-1}$ across all rats and R_1 values were $0.47 \pm 0.03 \text{ s}^{-1}$, which were consistent with previous reports of the same tumor type at this magnetic field strength 9.4T (36). After the injection of Gd-DTPA, R_2 increased rapidly to $45 - 60 \text{ s}^{-1}$, and then decreased gradually to $\sim 35 \text{ s}^{-1}$ at the last dynamic scan. Meanwhile, R_1 values first increased up to $1.0 - 1.2 \text{ s}^{-1}$, and then gradually decreased to $\sim 0.75 \text{ s}^{-1}$ for the last dynamic scan. For dynamic scans before and following contrast injection (#1 and #4–11), one-way ANOVA provided $p = 0.99$ and $p = 0.69$ for PSR(pulsed) and PSR(SIR), indicating there are no significant differences between different dynamic scans. This indicates that PSR values of tumors obtained using the two-pool model for both qMT acquisitions were constant even in the presence of real water compartmentation and with significant variations in relaxation rates. In other words, a two-pool qMT model may be sufficient for imaging PSR in tumors to provide a macromolecular metric that is distinct from the conventionally measured relaxation rates. Although k_{ex} (SIR) kept constant across all dynamic scans ($p = 0.76$), k_{ex} (pulsed) showed significant variations between different scans ($p = 0.02$), which means the estimates of k_{ex} (pulsed) were influenced by different relaxation rates. Note that a significant inter-subject variation (up to $\sim 30\%$) was observed for all fitted qMT parameters at baseline, indicating heterogeneity among different tumors.

Figure 6 shows the correlations between R_1 and R_2 , PSR(pulsed), PSR(SIR), k_{ex} (pulsed), and k_{ex} (SIR) for all dynamic scans except #2–3. A linear mixed-effects model was used to evaluate correlations. As expected, R_2 showed a linear correlation with R_1 , as longitudinal and transverse relaxation rates both change in proportional to the concentration of Gd-DTPA. PSR(pulsed) and PSR(SIR) were not correlated with R_1 (with $p = 0.26$ and 0.51 , respectively), indicating that measurements of PSR were not influenced by the variations of

relaxation caused by the injection of Gd-DTPA. $k_{ex}(\text{pulsed})$ showed a weak correlation with R_1 ($p = 0.05$). By contrast, $k_{ex}(\text{SIR})$ did not correlate with R_1 ($p = 0.16$). This independence of PSR(pulsed), PSR(SIR), and $k_{ex}(\text{SIR})$ from R_1 and dependence of $k_{ex}(\text{pulsed})$ on R_1 are in general agreement with the simulations in figure 3. However the dependence of $k_{ex}(\text{pulsed})$ on R_1 goes in opposite directions in the experimental results and simulations, suggesting that our choice of 4 pool parameters may not fully match *in vivo* conditions.

Agreement of pulsed-qMT and SIR-qMT methods in tumors

Figure 7 shows Bland-Altman plots of PSR and k_{ex} obtained using pulsed and SIR qMT methods, respectively. Except for one outlier in the k_{ex} data, the PSR or k_{ex} values from two qMT approaches were in good agreement, i.e. within the range of ± 1.96 standard deviations. Note that the mean PSR difference of the two qMT methods was close to zero (0.03%), indicating that they are equivalent for measuring the macromolecular pool size ratio. However, $k_{ex}(\text{SIR})$ values were apparently higher than $k_{ex}(\text{pulsed})$ values, and the mean k_{ex} difference was -4.8 s^{-1} , indicating that the influence of water relaxation on measurements of k_{ex} is different in pulsed and SIR qMT methods in tumors.

Discussion

Practical qMT imaging is usually based on a two-pool model consisting of one free water pool and one macromolecular pool. However, real tissues contain multiple water compartments and macromolecular components, so more complex models may be needed to interpret qMT data obtained *in vivo*. Despite their theoretical shortcomings, the data acquisition and analyses of a two-pool model are time efficient and hence are attractive for practical applications. The present study investigated the actual behaviors of a two-pool model for estimating qMT parameters in tumors with heterogeneous relaxation properties. This is based on an assumption that the transcytolemmal water exchange rate is high enough in tumors so that water fractions are well mixed during qMT measurements. Computer simulations suggest that for the R_1 range *in vivo* at high field (9.4T in this work), the two-pool model provides an accurate estimate of PSR even for varying transcytolemmal water exchange and relaxation rates. Our *in vivo* qMT measurements with Gd-DTPA injections confirmed that the fitted PSR's did not change significantly before and after the injections of Gd-DTPA. These results suggest that a two-pool model is sufficient to estimate the PSR in tumors as intrinsic indicator of macromolecular content independent of relaxation rates, and hence there is no need to develop more complicated three- or four-pool model to estimate PSR in brain tumors. By contrast, k_{ex} (the MT exchange rate) depends on the transcytolemmal water exchange rate (shown in simulations) and R_1 (shown in simulations and *in vivo* experiments), suggesting that k_{ex} obtained using a two-pool model is significantly affected by other tissue properties and may not reflect the intrinsic MT exchange rate *in vivo*. Under such circumstances, more complicated e.g. four-pool model may be necessary for better estimation of MT exchange rate.

A reliable imaging biomarker should have high specificity for a certain tissue property of interest with minimal influences from other tissue properties and acquisition approaches. Although more sophisticated data analysis models may provide more specific parameters,

such as the four-pool qMT model deemed necessary in white matter, the acquisition time is a practical limiting factor. Hence, the scanning protocols and accuracy of estimating parameters are usually compromised. The current study shows that a robust PSR can still be estimated from a simplified two-pool model in tumors, which meets both the requirements of scanning feasibility, parameter accuracy (at least for rapid transcytolemmal exchange in brain tumors), and independence from changes in other (relaxation) parameters. Considering most qMT studies focus on PSR, this conclusion may have significant clinical relevance. By contrast, k_{ex} was found to be influenced by the variations of R_1 and transcytolemmal water exchange rate, indicating that k_{ex} obtained using a two-pool model is not a reliable imaging metric to quantify MT exchange rate in tumors. Moreover, it has been reported that k_{ex} is more prone to noise (19), which results in a lower precision of k_{ex} *in vivo*.

Due to a lack of knowledge of the distribution and values of relaxation rates of macromolecules in tissues, our computer simulations assumed values based on previous simulations and *in vivo* results (19,34), but the chosen parameters may deviate from real values. To alleviate this bias, a broad range of R_1 and transcytolemmal water exchange rate k_{AB} were used to comprehensively investigate the influence of water compartmentation and heterogeneous relaxation on qMT imaging. The dependency of PSR and k_{ex} on R_1 and k_{AB} should be qualitatively correct, although the R_1 range with which PSR values are constant in realistic cancerous tissues may be different. Nevertheless, the simulations and experiments *in vivo* in the current work showed consistent results. For example, the computer simulations showed that PSR was independent on R_1 and k_{AB} in the R_1 range of $0.43 - 0.51 \text{ s}^{-1}$ obtained in experiments *in vivo*. and k_{AB} range of 0 to 6 s^{-1} . Recall that the k_{AB} of human solid brain tumors was reported up to $2.9 \pm 0.8 \text{ s}^{-1}$ and k_{AB} in apoptotic cancer cells was reported further enhanced to $6.8 \pm 3.9 \text{ s}^{-1}$ (32)). Therefore, both R_1 and k_{AB} of the 9L brain tumors used in the current study met the requirements predicted by the simulations, which explains there was no significant dependence of PSR values on R_1 in the experiments *in vivo*.

Although previous studies have suggested pulsed and SIR qMT methods provide similar PSR values in normal white matter tissues and rodent brain tumors, there has not been comprehensive studies to compare these two different qMT methods. Note that these two different qMT methods are based on different simplified assumptions so that each method will potentially be biased to different degrees from the effects of additional exchanging pools. However, interestingly, we have shown via *in vivo* experiments in the present study that, over a broad range of R_1 values, these two qMT methods provide equivalent PSR values in tumors at relatively high magnetic fields strength, while the pulsed qMT method provides lower k_{ex} values compared with the SIR qMT method. This may be of interest for researchers practicing qMT imaging via different qMT methods. Note that the computer simulations suggested that, when R_1 was much higher (corresponding to tissue R_1 at lower field strengths), both methods significantly overestimate PSR, and the SIR method provides even higher values than that from the pulsed method.

The 9L gliosarcoma has been the most widely used experimental rat brain tumor model, and has been extensively used e.g. to investigate blood-brain and blood-tumor barriers, image brain tumors including MRI and PET, and assess therapeutic effects of radiation and chemo treatments (38). Specifically for qMT measurements, Figure 5 indicates the PSR of 9L

tumors used in the current study was in the range of 4.5 – 6.2 %, which is very similar to the PSR range of 4 – 6% found in human gliomas (15). This indicates that the 9L gliosarcoma used in the current study is an appropriate tumor model to investigate qMT imaging in human brain tumors.

Conclusions

With the fast transcytolemmal water exchange rate and relatively low R_1 values in tumors, a two-pool model is a good approximation to estimate the pool size ratio of macromolecular protons in tumors, while estimated MT exchange rates are significantly affected by the variations in water compartmentation and relaxation changes. These results may assist better interpretation of qMT data in tumors. In addition, pulsed and SIR qMT methods are equivalent for estimating PSR in tumors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was funded by National Institutes of Health (NIH) K25CA168936, R01CA109106, R01CA173593, R01EB000214, P50CA128323, R01EB017767, R01CA184693 and R21EB017873. The authors thank Ms Zou Yue for assistance in animal surgeries.

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Figure 1.

The scheme of the four-pool MT model used in the numerical simulations. It includes intracellular free (mobile) water “A”, extracellular water “B”, intracellular macromolecules (immobile) “C”, and extracellular macromolecules (immobile) “D” pools. The model parameters and spin exchange rates are labeled.

Figure 2.

(a) The time course of spoiled-gradient echo (SPGR) signals of a tumor (red squares) and contralateral normal brain tissue (blue circles) from a representative rat. (b) Schematic diagram of the experimental protocol. The acquisitions of R_2 , SIR, and Pulsed-MT signals were interleaved and repeated 11 times. Between two repetitions, SPGR signals were acquired to monitor R_1 various. The black arrows indicate the time when Gd-DTPA was injected.

Figure 3.

The simulated dependence of PSR (top) and k_{ex} (bottom) on tumor R_1 using pulsed (left) and SIR (right) qMT methods based on a four-pool qMT model of tumors. Note that k_{AB} is the transcytolemmal water exchange rate, and dashed lines in each figure represent the true values. The gray region represents the R_1 range of 9L tumors measured *in vivo* without Gd-DTPA.

Figure 4.

The parametric maps of R_2 , R_1 , PSR(SIR), k_{ex} (SIR), PSR(Pulsed) and k_{ex} (Pulsed) obtained from all dynamic scans (excluding #2 and #3) from one representative rat. The ROI of the tumor was shown in the first PSR(SIR) map.

Figure 5.

The longitudinal changes of R_1 , R_2 , PSR(pulsed), PSR(SIR), k_{ex} (pulsed), and k_{ex} (SIR) with different dynamic scans. The numbers in the figure legend represent different animals.

Figure 6.

Correlations of MR parameters vs R_1 . The black lines represent linear fittings when there was a significant correlation, i.e. when $p < 0.05$. The numbers in the figure legend represent different animals.

Figure 7.

The Bland-Altman plots of PSR (A) and k_{ex} (B) show the agreement of pulsed and SIR qMT methods in characterizing tumors.

Table 1

Pulse sequence parameters used in both the simulations and the imaging acquisitions.

Methods	Key parameters	Parameter values
Pulsed saturation	Pulse width	14.6 ms
	TR	28 ms
	Irradiation flip angles	220°, 820°
	RF offsets	1– 100 ks ⁻¹ , logarithmically spaced
SIR	Inversion pulse	1-ms 180° hard pulse
	Pre-delay time (t_d)	4.735, 4.729, 4.531, 1.080, 3.589, 3.454, 0.01 s
	Inversion recovery time (t_i)	0.004, 0.050, 0.078, 0.252, 0.319, 0.450, 1.331 s