Water population changes in glioma gene therapy as measured by $T_{1\rho}$ and $T_{2\rho}$

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INTRODUCTION Magnetic Resonance Imaging (MRI) is likely to provide a non-invasive and early detection method for gene therapy treatment response in malignant gliomas [1]. The longitudinal relaxation rate (R_1) is sensitive to the molecular fluctuations of dipolar interactions at high frequencies near Larmour frequency (ω_0) in the MHz range. Most of the dipolar fluctuations in tissue occur at much lower frequencies, in the kHz range. It has long been recognized that the rotating frame longitudinal relaxation rate constant ($R_{1\rho}$) is driven by dipolar fluctuations at frequencies near the effective precession frequency (ω_{eff}), which can provide experimental access to the relevant lower frequency spectral densities. It has been shown that increased rotating frame longitudinal ($T_{1\rho}$) is one of the earliest markers of positive treatment response in a gene therapy rat model [2]. Recently, we have developed $T_{1\rho}$ and $T_{2\rho}$ relaxation MRI methods [3, 4], which are based on the relaxation occurring during adiabatic full passage (AFP) pulses. We have demonstrated that the differences in the modulation functions of the AFP pulses used for the $T_{2\rho}$ and $T_{1\rho}$ measurements provide a tool to generate tissue contrasts *in vivo* and to determine intrinsic tissue parameters that govern relaxation (e.g., rotational and exchange correlation times as well as populations of the exchanging sites P_A and P_B at sites A and B). In the present work, we use the conventional spin-lock $T_{1\rho}$ with different spin-lock field strengths and adiabatic $T_{2\rho}$ measurements with different pulse modulation functions of the AFP pulses to study the alternations of intrinsic parameters in tumor tissue during gene therapy treatment.

MATERIALS AND METHODS

Animals BT4C gliomas were induced by implanting 10^4 HSV-tk positive cells into the corpus callosum of female BDIX rats (n=14) [2]. Rats in the treatment group (n=9) were injected with Ganciclovir (25 mg/kg, i.p., twice daily; Saline-treated animals (n=5) served as controls) for 8 days.

Magnetic Resonance Imaging MRI experiments were carried out in a 4.7 T horizontal magnet equipped with actively shielded gradients interfaced to a Varian console. A quadrature half-volume coil was used. On-resonance $T_{1\rho}$ data were acquired using a spin-lock pulse consisting of 4 ms AHP pulse, a variable length spin-lock period (four spin-lock times, SLT = 12, 24, 48 and 96 ms) and 4 ms reverse AHP before fast spin-echo acquisition (TR = 2.5 s, field of view = 25.6x25.6 mm, matrix size = 64x128, slice thickness = 1.5 mm, 16 echoes with echo-spacing of 10 ms). The maximum B_1 field of AHP was 0.4 G while the amplitude of spin-lock pulse varied (B_{1SL} = 0.2, 0.8 and 1.4 G) [4]. For $T_{2\rho}$ measurements, adiabatic half passage (AHP) pulse was followed by the train of AFP pulses of the HSn family [5] (number of HS pulses =1,4 and 8; Tp = 3 ms; nHS=4, 8, 16, 32, B_{1max} =0.8 G) with different amplitude and frequency modulation functions before the same fast spin echo sequence as used for $T_{1\rho}$. No time intervals between pulses were used.

Data Analysis All MR signal intensities (SI) measured with the $T_{1\rho}$ and $T_{2\rho}$ techniques clearly exhibited mono-exponential decay, suggesting that the system is in the fast exchange regime (FXR). Thus, for the interpretation of NMR data the dynamic processes were modeled with an equilibrium two-site-exchange (2SX) system approximation, comprising two water populations coupled by an equilibrium exchange. For spin-lock $T_{1\rho}$ analysis we assumed simultaneous rotating frame dipolar relaxation under steady state spin-lock condition [6] and isochronous equilibrium exchange (e.g., exchange between spins with identical chemical shifts, $\delta\omega = 0$, and different relaxation time constants at sites A and B) in the FXR maintained during the spin-lock pulses: $R_{1\rho} = P_A R_{1\rho A} + P_B R_{1\rho B}$ [7]. Similarly, for the $T_{2\rho}$ data analysis we used isochronous exchange model in the FXR combined with the time-dependent dipolar relaxation during the course of adiabatic rotation [3].

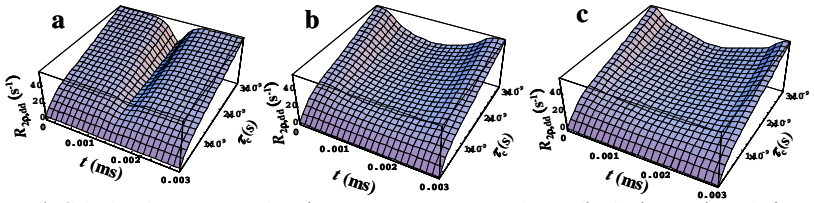

Figure 1. Calculated transverse relaxation rate constants $R_{2\rho,dd}$ due to dipolar interactions during the HS1 (a), HS4 (b) and HS8 (c) AFP pulses.

In Figure 1, we show the dependence of $R_{2\rho,dd}$ (=$1/T_{2\rho,dd}$, due to dipolar interactions) on rotational correlation times (τ_c) during HSn (n=1,4 and 8) pulses. The relaxation rate constants resemble modulation functions of AFP pulses.

Further, $T_{1\rho}$ and $T_{2\rho}$ maps were calculated by fitting the signal intensities to monoexponential relaxation formulae on a pixel-by-pixel basis. Regions of interests (ROI) covering the whole tumor were selected using T_2-weighted images.

Histology Cell counting was performed from sections stained with the Nissl method including only cells with intact, well-defined margins.

RESULTS Histology showed remarkable treatment response indicated by decreased cell density by 30-70 %. Figure 2 shows $T_{1\rho}$ and $T_{2\rho}$ maps on day 0 (before the treatment) and on day 8. The differences between control and treated animals are evident both in $T_{1\rho}$ and $T_{2\rho}$ maps. Figure 3 shows mean values for $T_{1\rho}$ (a) and $T_{2\rho}$ (b) during treatment. Both $T_{1\rho}$ and $T_{2\rho}$ values were significantly elevated already at day 4 and continued to increase for the rest of the observation period. The theoretical analysis indicates a decrease of population associated with low correlation time (site A) from day 4 onwards (Table 1).

Table1. Simulation parameter values

		τ_{cA} (ns)	τ_{cB} (ps)	P_A	P_B
$T_{1\rho}$	Day 0	3.3	40	0.25	0.75
	Day 4	3.3	40	0.22	0.78
	Day 8	3.3	40	0.18	0.82
$T_{2\rho}$	Day 0	3.3	40	0.25	0.75
	Day 4	3.3	40	0.22	0.78
	Day 8	3.3	40	0.18	0.82

τ_c is correlations time; P_A and P_B are the populations of the pools A and B.

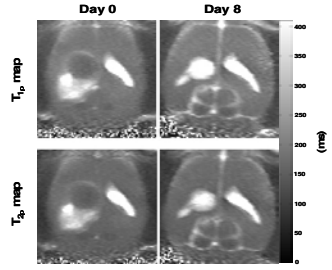

Figure 2. $T_{1\rho}$ (1.4G) and $T_{2\rho}$ (HS8) maps on day 0 and 8.

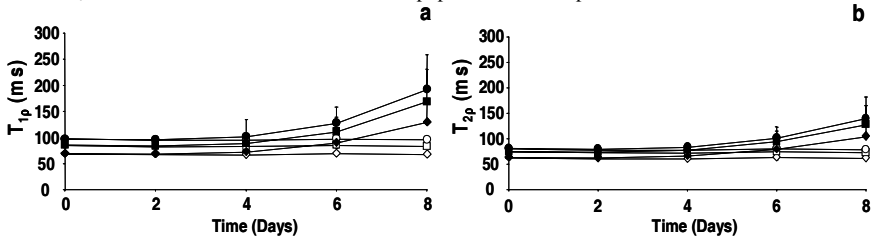

Figure 3. (a) $T_{1\rho}$ values from control (white symbols) and treated animals (black symbols) with different B_1 values (♦ 0.2G, ■ 0.8G and ● 1.4G) during the treatment. (b) $T_{2\rho}$ values from control (white symbols) and treated animals (black symbols) calculated with different AFP pulses of the hyperbolic secant family (♦ HS1, ■ HS4 and ● HS8) during the treatment. Values are given as mean ± SD. All values at treatment day 4-8 were significantly different ($p<0.05$) from day 0 (Student's paired t-test)

CONCLUSIONS The data indicate that rotating frame $T_{1\rho}$ and $T_{2\rho}$ relaxation measurements are sensitive indicators of treatment response. Populations and the correlation times of sites A and B obtained both from $T_{1\rho}$ data and $T_{2\rho}$ data were the same, indicating validity of the theoretical formalism applied. Population changes are consistent with cell shrinkage and increasing extracellular space during apoptotic cell death. Our data demonstrate how detailed analysis of the fundamental parameters governing relaxation may provide an insight into pathological changes in tumour tissue.

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