Approximating Water Exchange in vivo in a Rat Model

C. Bailey[1,2], F. Moosvi[1,2], and G. J. Stanisz[1,2]
[1]Sunnybrook Health Sciences Centre, Toronto, ON, Canada, [2]Medical Biophysics, University of Toronto, Toronto, ON, Canada

INTRODUCTION: Intracellular-extracellular water exchange is an important indicator of apoptotic cell death and is measureable with MRI contrast agent methods in vitro (1). In vivo studies have used DCE-MRI to examine exchange rate in tissue (2,3), but these methods measure the concentration of contrast agent in the blood using arterial catheters or an arterial input function (AIF) and require high temporal resolution. Determination of an AIF in small animals is further complicated by the need for high spatial resolution to avoid partial volume effects. In this study, T1 measurements were performed at multiple steady-state contrast agent concentrations in rats to approximate water exchange. The approximation is made in the absence of an AIF by using dosage information and T1 data following two separate injections.

METHODS: Two female nude rats were scanned at 3 T (GE Signa, Milwaukee) using a home-built surface coil (receive only), FOV=6 x 6 cm^2, 128 x 256 matrices, 2 mm slice thickness. Scans and injections were conducted as shown in Figure 1. 3D SPGR scans (TR=11.5-13 ms, TE=3.6-4 ms, 4 NEX) were acquired for four flip angles: 5, 7, 9 and 15-17 degrees. Pre-contrast T1s, were also determined with 2D Inversion Recovery (IR) scans (TR=2500 ms, TE=14 ms) at five inversion times: TI=50, 300, 900, 1200 and 1800 ms. Contrast agent was injected as a bolus via tail vein catheter and monitored for stable contrast agent concentration using a 2D SPGR sequence (TR=20 ms, flip angle=15°). Injection 1: 0.04 mL gadodiamide (Omniscan, GE Healthcare) diluted to 0.4 mL with saline. Injection 2: 0.16 mL gadodiamide (Gd) diluted to 0.4 mL using saline. Following each injection, the four 3D SPGR scans described above were reacquired.
Three ROIs of 42-86 voxels were selected in thigh muscle of each rat. Monoexponential T1s were determined by fitting SPGR data for each Gd concentration separately, but a single equilibrium signal value, S_0, was used for all fits. The difference in relaxation rates was calculated as $\Delta R1_{InjX}=1/T1_X-1/T1_0$ where X={1, 2} for the two separate injections and $T1_0$ is the pre-contrast T1. The ratio $\Delta R1_{Inj2}/\Delta R1_{Inj1}$ was also calculated. Global fits to SPGR data from all four flip angles at all three Gd concentrations were performed in two ways: under the fast exchange assumption, T1s were assumed to be monoexponential and the steady-state Gd concentration from the first injection, Gd_{eq1}, was allowed to vary, while the second concentration was constrained by $Gd_{eq2}=5*Gd_{eq1}$. There were then three free parameters to fit: S_0, $T1_0$ and Gd_{eq1}. In the second fitting method (shown in Fig. 2), the water exchange rate, k_{IE}, was not assumed to be fast and the equilibrium Gd concentration was calculated from the injected amount (0.04 mL * 500 mM), weight of the rat (350 g) and assuming a 20% distribution volume in the body: $Gd_{eq1}=(0.04*500)/(0.2*350)$. This fit also has three free parameters: S_0, intracellular relaxation time $T1_I$ and water exchange rate k_{IE}. Errors in the fit of k_{IE} were calculated using the F distribution for a 68% confidence interval.

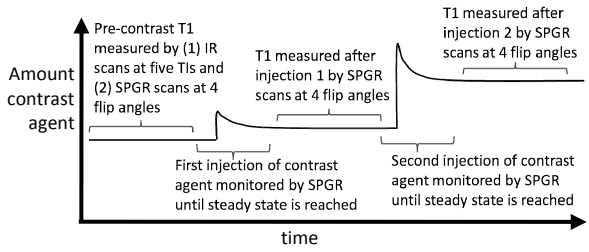

Figure 1 *Experimental design. Pre-contrast T1s were measured by both IR and SPGR methods. Two injections were performed and SPGR scans were reacquired during the steady-state portion of contrast passage.*

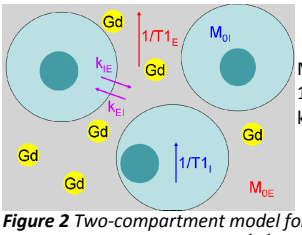

Figure 2 *Two-compartment model for global fit (1). Fixed parameters: $T1_{E0}$=1.5 s, relaxivity r_1=4 $mM^{-1}s^{-1}$ and M_{0E}=0.1. Equilibrium extracellular concentration of Gd was approximated as injected dose of 500 mM gadodiamide distributed through 20% of the volume of a 350 mL rat: $Gd_{eq1}=(0.04*500)/(0.2*350)$, $Gd_{eq2}=5Gd_{eq1}$.*

RESULTS: The data acquired during injections (not shown) demonstrated unchanging T1 approximately three minutes after injection persisting for at least five minutes. The ratio of monoexponential T1 relaxation rates $\Delta R1_{Inj2}/\Delta R1_{Inj1}$ was 2.5 ± 0.1 in Rat 1 and 3.5 ± 0.1 in Rat 2. Figure 3 shows the fits to the steady-state data at all three Gd concentrations for (a) the fast exchange limit and (b) accounting for exchange but assuming known equilibrium Gd concentration. The χ^2 was 55% lower in fits including exchange. The average value for the exchange constant, k_{IE}, was 0.24 ± 0.10 s^{-1}.

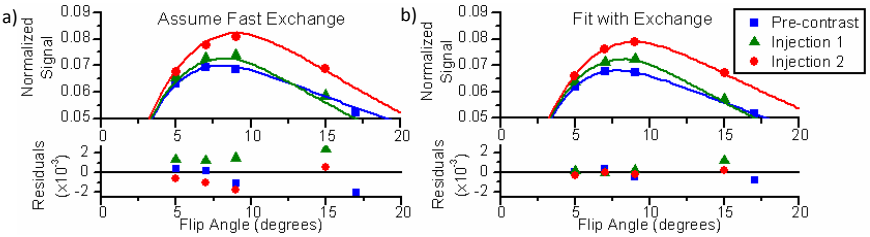

Figure 3 *A set of SPGR data for all four flip angles and all three Gd concentrations. Lines indicate fits to the experimental data and residuals are shown at the bottom. a) Fit assuming common S_0 for all three curves and fast exchange limit so $\Delta R1_{inj2}=5*\Delta R1_{inj1}$. Note the poor fit for the highest Gd concentration following the second injection. b) Fit to a 2-compartment model including exchange.*

DISCUSSION: This study develops an approximate model for intracellular-extracellular water exchange when no AIF is available. This is valuable in preclinical studies where the AIF may be unobtainable due to partial volume and/or high blood flow, but steady-state is reached rapidly due to the animal's small size. The importance of exchange is emphasized by the ratio $\Delta R1_{Inj2}/\Delta R1_{Inj1}$. For infinitely fast exchange, relaxation rate is proportional to the concentration of gadolinium, which is in turn proportional to total dose injected (if clearance via the kidneys is negligible during data acquisition). For this study, the fast exchange limit predicts $\Delta R1_{Inj2}/\Delta R1_{Inj1}=5$. The lower values calculated here indicate that exchange is too slow for this approximation to be valid. Other factors may influence the ratio of ΔR1s, including poor fitting of the SPGR data due to errors in the flip angle. However, the T1 of the ROIs agreed within 12% with those from IR data, which included a B1 error parameter. There may also be systematic errors due to underestimation of injection volume from catheter dead space or clearance of contrast agent from the body. However, similar signal from the monitoring SPGR scan following the first injection and just prior to the second injection suggest clearance amounts are small.
ROIs with exchange rates larger than 1 s^{-1} could not be fit at the Gd concentrations used in this study, as indicated by larger upper errors in the fit parameter. For the six ROIs with reliable k_{IE} values, the average exchange rate of 0.24 ± 0.10 s^{-1} is consistent with previously measured values of water exchange, including 0.9 s^{-1} in rat thigh muscle (2). This exchange value gives a permeability of ~0.12 x 10^{-3} cm/s for a 1 cm muscle fibre, 20 µm in diameter, which is lower than previous permeability measurements of (2.8 ± 0.3) x 10^{-3} cm/s for red blood cells (4) and 3.6 x 10^{-3} cm/s in HeLa cells (5). The precision of the exchange constant, k_{IE}, is low, but can be improved by making measurements at a wider range of Gd concentrations (simulations not shown) although injection volume is limited in small animals. However, in vitro studies indicate apoptosis can alter exchange by up to a factor of 5 (1), so the precision obtained may be sufficient to detect cell death.

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