The Utility of Gd-DTPA Labeled Red Blood Cells as a Contrast Agent for DSC-MRI Studies

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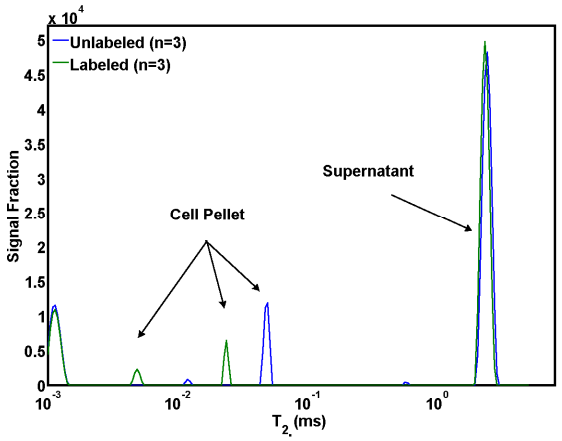
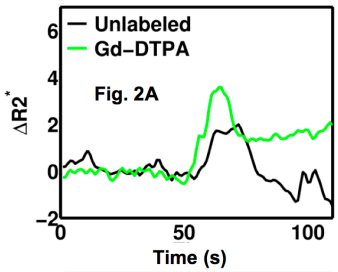
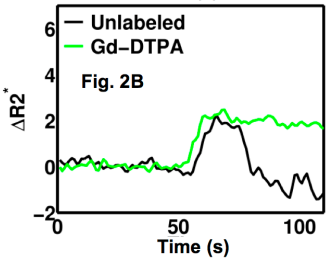
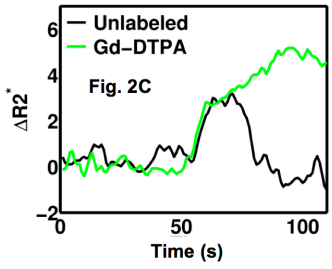
Introduction: Intravascular MRI contrast agents for have several potential uses for clinical and research applications such as the assessment of tissue blood volume and blood flow. To mimic the behavior of blood in tissues, it is important that the contrast agent distributes in a manner similar to red blood cells (RBCs). Previously, RBCs have been labeled with Gd-DTPA and Dy-DTPA using an osmotic pulse technique (1, 2). The goal of our current study is to investigate the potential of Gd-DTPA labeled RBCs as a first pass contrast agent for Dynamic Susceptibility Contrast (DSC)-MRI studies of tumor angiogenesis and hemodynamics *in vivo.*



Materials and Methods: Whole blood from male Sprague Dawley rats (Bioreclamation, East Meadow, NY) was centrifuged and washed with phosphate buffer saline solution (PBSS) to obtain the RBCs. The RBCs were labeled with Gd-DTPA (Magnevist®; Bayer Healthcare Pharmaceuticals, Cambridge, MA) using the osmotic pulse technique (1, 2). Briefly, the cells were exposed to a hypoosmolar solution (108 mosm/kg to 170 mosm/kg) containing Gd-DTPA, incubated in a hyperosmolar solution (2740 mosm/kg), washed with a dextran solution (300 mosm/kg) and then washed with PBSS (285 mosm/kg). To verify cell labeling, cell solutions were first centrifuged and *in vitro* multi-spectral T_2 data (3) were acquired on a 4.7T Varian MRI system using a 10mm diameter RF coil with a CPMG sequence (TR/TE = 10000 ms/1 ms, FOV = 120 mm, slice thickness = 4mm). *In vivo* DSC-MRI images of male Wistar rats (n = 3) were acquired using an EPI pulse sequence (TR/TE = 1000 ms/20 ms, 1 shot, 4 slices, slice thickness = 2 mm, FOV 40 x 40 mm, matrix = 64 X 64). For a control DSC-MRI study, 0.5 mL of saline was injected over 8 seconds into a jugular catheter of a normal rat using an infusion pump (Harvard Apparatus). The scan was repeated for a solution of unlabeled RBCs (50% hematocrit) followed by a solution of Gd-DTPA labeled RBCs (50% hematocrit). The catheter was flushed with saline between each scan.







Results: The *in vitro* T_2 average spectra for unlabeled and labeled cell solutions are shown in Figure 1. The average T_2 value of the supernatant (Snt) was the same for both the unlabeled and labeled cell solutions, while the average Gd-DTPA labeled cell pellet T_2 value was 50% lower than the unlabeled cell pellet (20 ms vs 40ms). In general, we found that cells could be successfully labeled with Gd-DTPA, without damaging cell integrity, using hypoosmotic solutions ranging from 175 to 85 mosm/kg. Figure 2.A-C shows example DSC-MRI ΔR2* time courses for the *in vivo* rat brain studies. The shapes of the ΔR2* time courses were highly variable across the brain and across animals for both unlabeled and labeled cell solutions. Bolus injections of saline alone did not alter the DSC-MRI signal. Thus far, the kinetics of the unlabeled and labeled RBCs are unlike those typically measured using plasma distributed contrast agents. The duration of the ΔR2* enhancement for the unlabeled and labeled cells is markedly prolonged (duration ~25 sec) and prevented the calculation of RBC flow using standard first pass tracer kinetic methods. The maximum change in the ΔR2* was typically found to be similar between unlabeled and labeled cell solutions. Increasing the cell solution hematocrit yielded larger signal changes but also yielded time courses that were more prolonged. The largest difference between the unlabeled and labeled cell solution ΔR2* time courses occured approximately 1 minute after injection where the labeled cell ΔR2* values remained elevated while the unlabeled ΔR2* values returned to baseline or slightly lower.

Discussion: These preliminary studies have demonstrated that unlabeled and labeled cell solutions can generate susceptibility-induced changes in DSC-MRI signal time courses but, to date, these time courses are unlike those measured using plasma distributed contrast agents. The prolonged transit times of the labeled and unlabeled cells were unexpected since RBC flow is equivalent to plasma flow in most vessels and slightly less than plasma flow in the microcirculation due to the Fahraeus effect. One possibility is that the RBCs are coagulating prior to injection thus hindering their passage through the microcirculation. The aggregation of the cells could cause them to lodge in the microcirculation and slowly separate over time by shear stress and plasma dissolution. In the future, our studies will utilize the labeled cells to measure RBC distribution volume within animal rat brain tumor models. DSC-MRI of labeled RBCs could reveal new insights into the development of abnormal tumor vasculature and potentially indicate regions within tumor tissue with inadequate oxygen delivery despite substantial plasma flow.

References: 1. K.M., Johnson *et al*., Magnetic Resonance in Medicine 45:920-923, 2001. 2. K.M., Johnson *et al*., Magnetic Resonance in Medicine 40:133-142, 1998. 3. Horch, R.A., *et al*., Magma 20:51-6, 2007.

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