Enhanced relaxivity of hydroxyapatite-targeted gadolinium contrast agents

J. Marmurek[1,2], K. Nasr[3], E. Vinogradov[2], A. J. Madhuranthakam[4], J. V. Frangioni[3], and R. E. Lenkinski[2]

[1]Harvard-MIT Division of Health Sciences and Technology, Cambridge, MA, United States, [2]Radiology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, United States, [3]Hematology and Oncology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, United States, [4]Global Applied Science Laboratory, GE Healthcare, Boston, MA, United States

Introduction: Hydroxyapatite microcalcifications are a hallmark of malignant breast cancer but cannot be detected by current clinical imaging. We have developed a high-relaxivity gadolinium-bisphosphonate contrast agent with a short linker that exhibits specific adsorption to hydroxyapatite. The longitudinal relaxivity of Gd-chelate contrast agents is dependant on both the exchange rate of coordinated water molecules and on the tumbling rate of the system . Slow-rigid molecules with long correlation times are expected to have high relaxivities, and previous work has demonstrated that shorter linkers between a Gd-chelate and targeting-ligand reduce rotational freedom when bound [1]. T_1 measurements of incremental concentrations of contrast agent incubated with hydroxyapatite crystals *in-vitro* demonstrated that adsorption followed a Langmuir isotherm. Moreover, the rigidly adsorbed contrast agent was detectable by ultra-short echo time (UTE) imaging at concentrations as low as 1 μM. The apparent relaxivity of the bound agent was approximately 10^2-fold higher than that of the free agent in solution or of conventional small molecule T_1 contrast agents.

Methods: *Contrast agent synthesis*- Gd-DOTA was conjugated to pamidronate (PAM), a hydroxyapatite binding bisphosphonate, by a short serine (Ser) linker as previously reported [2]. The contrast agent, Gd-DOTA-Ser-PAM (GDSP), shown in Figure 1, was verified by liquid chromatography and mass spectrometry. *Phantom preparation*- To test for binding specificity, 1 mL of 10 μM GDSP in water was incubated (1 hr, room temperature) with 25 mg each of hydroxyapatite (HA), calcium oxalate (CO), calcium carbonate (CC), calcium pyrophosphate (CPP), and calcium phosphate (CP). The reaction mixtures were washed (by repeated vortexing, centrifugation, decanting and re-solvation) to remove unbound contrast agent. To test the sensitivity of GDSP adsorption to HA, increasing concentrations (0, 0.1, 1, 5, 10, 20, 50, 100, and 200 μM) of GDSP in 1 mL of water were each incubated with 25 mg HA, and the reactions were washed as above. *UTE imaging measurements*- MR imaging was performed on a 1.5 T whole-body scanner (GE Healthcare) with a custom-built birdcage transmit/receive coil. Images were acquired using a UTE sequence (slice thickness = 5 mm, FOV = 9 cm, matrix = 256×299, NEX = 2, α = 90°, TE = 100 μs) at varied TR (50, 100, 200, 500, 2000 ms) in order to measure the T_1 of the samples. First, specificity was tested by comparing image-based T_1 measurements of the 10 μM GDSP reacted with the varied calcium salts to control relaxation times of each of the crystals alone (with no contrast agent). Second, the free contrast agent was dissolved at incremental concentrations (equal to above) to measure the unbound agent's relaxivity. Last, sensitivity was tested by T_1 measurements made from imaging the phantom of increasing GDSP concentrations reacted with HA. *Data analysis*- Longitudinal relaxation times were estimated (ImageJ, MATLAB) by fitting the mean signal intensities of a manual ROI (drawn over the crystals) to the signal equation: $M_z(t)=M_0(1-e^{-t/T1})$. The T_1s of increasing concentrations of free contrast agent were used to estimate the free (unbound) GDSP relaxivity, r_1^u, by linear regression of $R_1 = R_1^0 + r_1^u [GDSP]$, where $R_1=1/T_1$, and R_1^0 is the zero concentration baseline value of water.

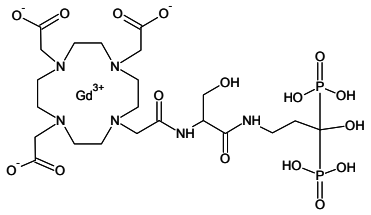

Figure 1- Gd-DOTA-Ser-PAM structure

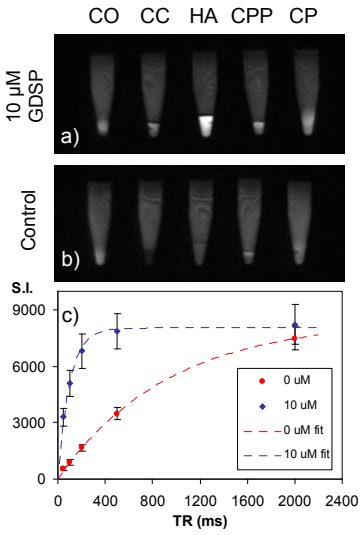

Figure 2 – Hyrdoxyapatite specificity

Results: The UTE image (TR = 100 ms) of the varied calcium salts reacted with GDSP shows specific binding to HA (Figure 2a). The image of the control salts is shown in Figure 2b. Mean ROI signal intensity (S.I.) for the control HA and GDSP bound to HA are plotted versus TR in Figure 2c along with the fits used to estimate relaxation times. T_1 of the control HA was 932 ms: reacting with 10 μM GDSP reduced the T_1 to 100 ms. Slight T_1 reduction was also noticed in CC and CP. The HA-adsorbed contrast agent could be detected at concentrations as low as 1 μM (Figure 3a). The free unbound contrast agent, in comparison, required concentrations > 20 μM for detection. The relaxivity of the free unbound contrast agent was estimated to be 5.4 s^{-1}/mM. ROI analysis showed reduced T_1 in the HA crystals with increasing bound GDSP concentration. Since there is a finite amount of binding sites on the HA, the effect of increasing R_1 is saturated at high GDSP concentrations (Figure 3b). This is consistent with adsorption according to a Langmuir isotherm, previously shown by radiolabeled HA-targeted Tb-DOTA-PAM conjugates [3]. The T_1 of the HA reacted with 1 μM GDSP was 475 ms (~50 % reduction), hence, the apparent relaxivity of the bound contrast agent, calculated as $r_1 = \Delta R_1/[GDSP]$, is 1030 s^{-1}/mM.

Discussion: The relaxivities of conventional small molecule gadolinium contrast agents are typically around 5 s^{-1}/mM. Strategies which raise contrast agent relaxivity upon binding (e.g. to human serum albumin) with optimal exchange rate and rotational correlation times have displayed relaxivities near 80 s^{-1}/mM [4]. We demonstrated that the apparent relaxivity of GDSP when bound to HA is greater than 1000 s^{-1}/mM. This relaxivity enhancement occurs only when the contrast agent is bound: unbound GDSP maintains a much lower relaxivity of 5.4 s^{-1}/mM. The observed relaxivity enhancement is likely caused by restricted rotational freedom (low correlation times) of the GDSP when adsorbed to HA. The bound contrast agent rigidity is due to a combination of high-affinity binding of pamidronate to HA [5] and the short ligand-to-agent linker length. In the Langmuir binding sensitivity experiments, it is evident that a proportion of the incubated GDSP does not bind. Future experiments will be aimed to quantify and account for the amount of unbound agent and may reveal that the true relaxivity of HA-bound GDSP is higher than estimated here. The specific binding to HA motivates our interest to evaluate the contrast agent in malignant breast cancer microcalcifications. We are preparing for experiments in a rat model of breast cancer expressing HA-laden microcalcifications.

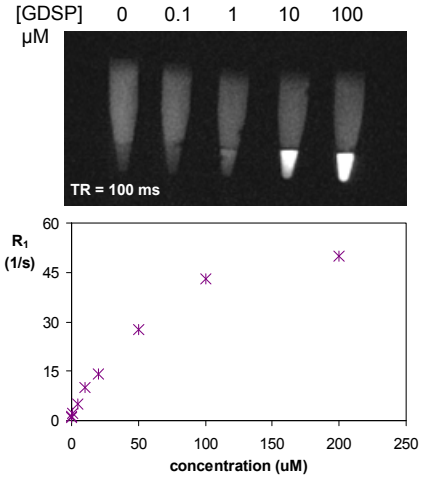

Figure 3 – GDSP sensitivity

References:

1. P. Caravan. *Chem. Soc. Rev*. 35:512 (2006)
2. K.S. Bhushan et al. *Angew. Chem*. 46(42):7969 (2007)
3. T. Vitha et al. *Langmuir*. 24(5):1952 (2008)
4. S. Avedano et al. *Chem Commun*. 4726 (2007)
5. S. Mukherjee et al. *JACS*. 131: 8374 (2009)