Characterization of a $\alpha_v\beta_3$-Integrin Targeted Gadolinium Chelate-Based Contrast Agent

A. G. Reish[1,2], D. A. Herzka[3], D. Thomasson[2], N. S. Danthi[2], L. Potti[2,4], M. J. Bur[2], C. A. Burnett[2], A. Gharib[2], M. Bernardo[5], G. Metzger[6], K. C. Li[2]

[1]University of Virginia Medical School, Charlottesville, VA, United States, [2]G Magnuson Clinical Center, NIH, DHHS, Bethesda, MD, United States, [3]Clinical Sites Research Program, Philips Research USA, Briarcliff Manor, NY, United States, [4]University of Maryland, College Park, MD, United States, [5]National Cancer Institute, NIH, DHHS, Bethesda, MD, United States, [6]Philips Medical Systems, Bethesda, MD, United States

Introduction: Tumor vasculature is typically composed of a permeable or leaky network of capillaries that are created through angiogenesis to sustain the increasing nutrient requirements of the growing tumor. This higher degree of permeability permits more diffusion than in healthy, non-cancerous tissue, leading to enhancement upon administration of contrast media. Furthermore, tumor neovasculature uniquely expresses the receptor endothelial Integrin $\alpha_v\beta_3$, during angiogenesis [1], providing a site for selective targeting. A contrast agent was previously designed by combining an $\alpha_v\beta_3$-complexing agent, Integrin Antagonist (IA), with a paramagnetic nanoparticle (Gadolinium) for in-vivo visualization of tumors with MRI (Fig 1a). In this preliminary study, we characterize the relaxivity and washout properties of the novel, $\alpha_v\beta_3$ Integrin-targeting Gd-chelate agent {Gd(IA_2DTPA)} and compare it to a non-specific contrast-enhancing Gd-chelate marketed as Magnevist ® (Schering, Berlin) {Gd-DTPA}[2].

Methods: The 50% inhibition concentration, a measure of the binding affinity of the agent, was previously determined to be IC_{50} = 12.7±1.1nm. To determine the novel agent's *in vitro* relaxivity, T_1 values at various concentrations of Gd(IA_2DTPA) and Magnevist were measured on a 3.0 T Intera system (Philips Medical Systems, Best, NL) using the two angle method proposed in [3,4]. Data was acquired using an inductively coupled, locally-built coil. Contrast enhancement (CE) curves were acquired for both Gd(IA_2DTPA) and Gd-DTPA to observe CA washout behavior. These initial characterization experiments were performed on Balb/c nu/nu mice without tumors. For both agents, 0.1mmol/kg was injected into the tail vein of the mice. Four consecutive spoiled gradient echo (fast field echo) images were acquired with the following parameters: TR = 6.3ms, TE = 2.2ms, FA = 10º, 256^2 matrix, 100mm^2 matrix, 0.5mm slice thickness. Images were acquired approximately one minute apart. To assess the agent washout rate, kidney signal intensity values were measured by ROI analysis to determine relative signal time courses.

Results: Fig 1b demonstrates the relaxivity data obtained *in vitro*. Magnevist yielded a relaxivity (r_1) value of 4.7 $mmol^{-1}sec^{-1}$ while Gd(IA_2DTPA) yielded a value of 10.1 $mmol^{-1}sec^{-1}$. Fig 1c demonstrates the CE curves demonstrating washout properties determined from CA injections in mice. Though the curves are undersampled due to time constraints, the results show that the Gd(IA_2DTPA) has similar washout characteristics and accumulation patterns (images not shown).

Discussion: In the era of molecular imaging, targeted CAs are becoming critically important tools to optimize diagnostic methods and improve the treatment of disease, in particular cancer. Methods are sought for differentiation between benign and malignant tumors to lessen the number of invasive biopsies performed, to better diagnose the stage of tumor development and better define appropriate treatment. By coupling a paramagnetic CA's chelate molecule, in this case DTPA, with the Integrin Antagonist, the molecule of Gd(IA_2DTPA) shows potential as a method for targeted contrast enhancement and tumor characterization.

In our initial in-vitro and in-vivo baseline (tumor-less) examination of Gd(IA_2DTPA), the $\alpha_v\beta_3$ Integrin-targeting CA showed increased relaxivity relative to Magnevist, as expected from a decreased tumbling rate. ROI-specific washout measurements were similar between the non-selective Magnevist and the selective Gd(IA_2DTPA) when performed on a Balb/c nu/nu Mouse after injection of equivalent 0.1mmol/Kg doses. Future experiments will characterize the first-pass behavior of the CA with greater temporal resolution in both normal mice and mice injected with melanoma cells. The targeting capabilities of the Integrin antagonist have been shown to be good, in the nanomolar range, and when combined with the contrast-enhancing properties of the Gd-chelate, may yield increased sensitivity in the presence of tumors. Diagnostic molecular imaging shows tremendous promise in catching cancer's early stages and quantifying the very extent of the tumor's growth to optimize the treatment regiment.

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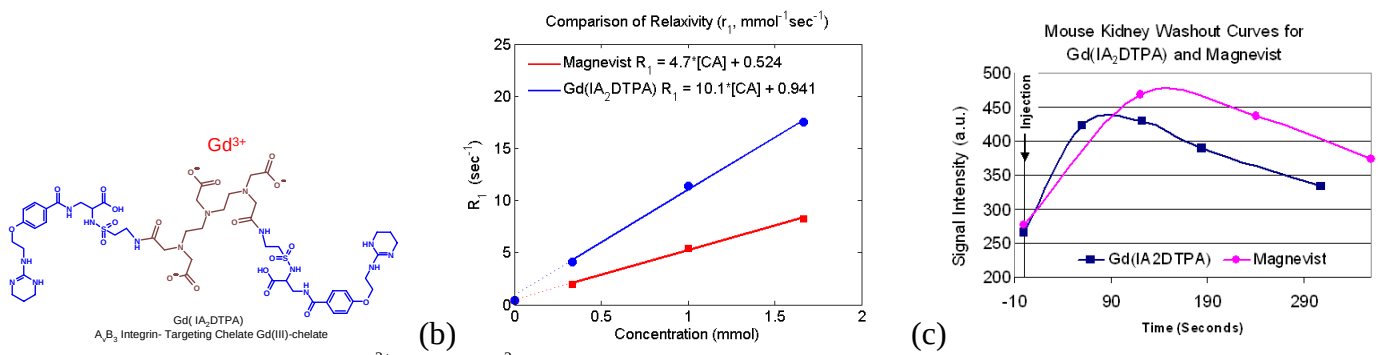

Figure 1: (a) Chemical structure of the $Gd^{3+}(IA_2DTPA)^{3-}$ $\alpha_v\beta_3$ Integrin-targeting Gd-chelate characterized in this work. Two IA sites are used to optimize in-vivo efficacy. (b) Relaxivity curves for Magnevist (red squares) and Gd-IA_2DTPA(blue circles) obtained in vitro. The relaxivity (r_1, slope) of our contrast agent is approximately twice that of Magnevist (10.1 $mmol^{-1}sec^{-1}$ vs 4.7$mmol^{-1}sec^{-1}$). Note $R_1=1/T_1$. (c) Washout curves for both Magnevist and Gd-IA_2DTPA obtained in vivo in Balb/c nu/nu mice kidneys. Due to imaging constraints, the curves were temporally undersampled. However, the passage of the contrast agent closely resembles that of the standard Gadolinium chelate.