

Imaging hypoxia using a nitroimidazole based T₁ MR contrast agent

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Introduction:

Hypoxic regions in tumors are known to affect radiation sensitivity and promote development of metastases [1]. Noninvasive imaging based methods such as MRI are particularly suitable for longitudinal measurements and generation of three-dimensional spatial maps of tumor hypoxia [2]. An MRI method that could differentiate hypoxic *versus* normoxic tissues without relying on washout modeling would be clinically useful. Previous research demonstrated that 2-nitroimidazole accumulated in hypoxic tissues due to an enzyme mediated reduction of the nitro group under hypoxic conditions [3]. In this work, we report both *in vitro* and *in vivo* evidence for accumulation of a T₁ shortening agent, a GdDOTA monoamide conjugate of 2-nitroimidazole abbreviated as GdDO3NI (Fig 1b), in hypoxic tumor tissue.

Materials and Methods:

MR experiments were performed on a Varian 4.7T MR scanner. *In vitro* measurement of T₁ relaxivity (r_1) of GdDO3ABA (control agent, Fig 1a) and GdDO3NI (hypoxia targeting agent, Fig 1b) was performed at 37°C in a tissue - simulating 1% agarose phantom. For the r_1 measurements, a spin-echo sequence was used with several TR values (0.1-6s). Relaxivity was extracted as slope of linear fit to relaxation rates (R_1) vs. concentration. *In vivo* imaging studies were performed on 10 Copenhagen rats bearing subcutaneous syngeneic R3327 - AT1 prostate tumors (volume ~3cc), since they are known to have hypoxic regions in the core [4]. Following baseline T₁ mapping (TR: 0.1-6 s and TE: 12 ms), T₁-weighted images (TR/TE = 200/10 ms, FOV = 5 cm X 5 cm, matrix = 128 X 128, slice thk = 1 mm) were obtained pre and post injection of 0.1 mmole/kg body wt contrast agent (GdDO3ABA or GdDO3NI, n = 5 each) for 150 min. Data analysis was performed by segmenting the voxels in the tumor region into periphery and center, based on a criterion of 50% enhancement at 90 s post injection. Gd concentration in the tumor slices was calculated using the relation $[CA] = (R_{1,post} - R_{1,pre}) / r_1$. For validation, *ex vivo* inductively coupled plasma mass spectroscopy (ICP-MS) analysis was performed to quantify the Gd concentration in the periphery and core regions of tumors (n=3).

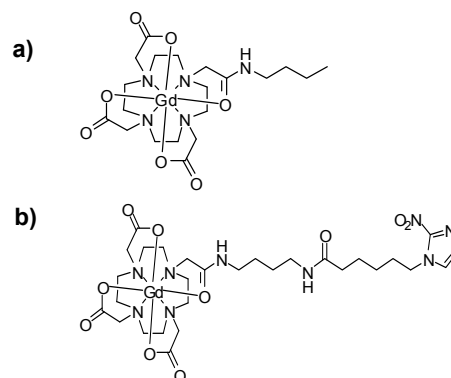


Figure 1: Structure of a) GdDO3ABA (control) and b) GdDO3NI (hypoxia targeted).

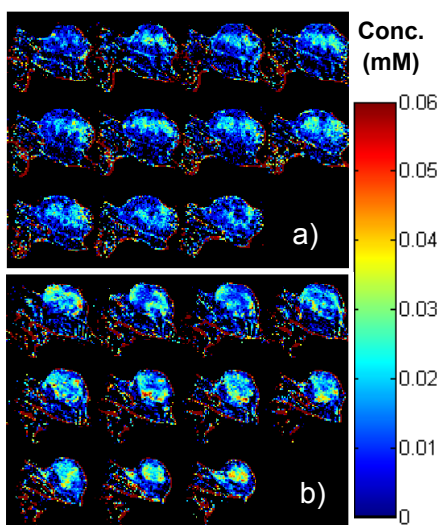
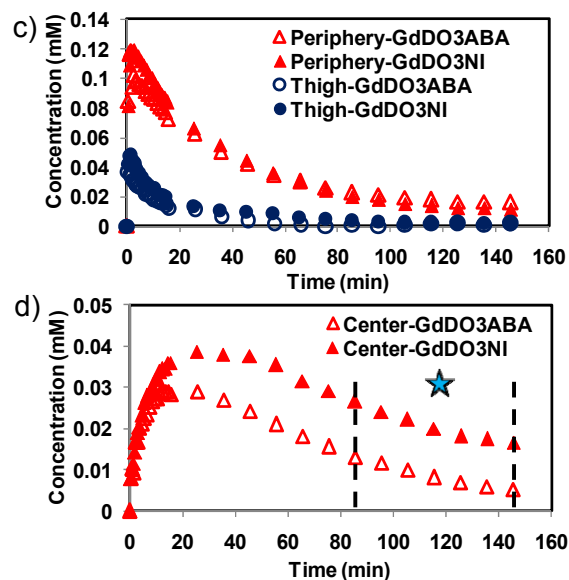


Figure 2: Gd concentration maps of multiple slices of representative tumors in animals injected with a) GdDO3ABA and b) GdDO3NI. The image panels show gadolinium concentration at 150 minutes post injection.

Kinetics of mean Gd concentration (n=5) c) for well perfused (peripheral) region & thighs and d) for poorly perfused (central) regions of AT1 tumors in animals injected with GdDO3ABA and GdDO3NI. Region enclosed within (★) represents $p < 0.05$



Results and Discussion:

A linear fit to the relaxation rates (R_1) vs. concentration data yielded relaxivity values of $4.76 \pm 0.27 \text{ mM}^{-1} \text{ s}^{-1}$ and $5.45 \pm 0.294 \text{ mM}^{-1} \text{ s}^{-1}$ for GdDO3ABA and GdDO3NI, respectively. Contrast agent kinetics were compared following intravenous injections of GdDO3NI and GdDO3ABA in AT1 rat prostate tumor bearing animals. The time course signal intensities (qualitative) and the calculated Gd concentrations (quantitative) showed a clear difference in the enhancement patterns (Fig 2 a & b) for the two (GdDO3ABA and GdDO3NI) and between well perfused and poorly perfused regions. A statistically significant difference in contrast enhancement / concentration was observed in poorly perfused (and potentially hypoxic) regions at late time points (85-150 min) between GdDO3ABA and GdDO3NI injected animals (Fig 2d). Gd concentrations obtained from ICP-MS analysis of tumor samples correlate well with the concentration profiles observed. Thus, GdDO3NI shows promise as a hypoxia targeting small molecular contrast agent.

Acknowledgements: Small Animal Imaging Resource U24 CA - 126608, NIH CA - 115531 and BTRP P41- RR02584.

References: 1) Tatum *et al.*, *Int J Radiat Biol* 2006;82(10):699-757. 2) Cho *et al.*, *Neoplasia* 2009; 11(3): 247-259. 3) Krohn *et al.*, *J Nucl Med* 2008;49 Suppl 2:129S-148S. 4) Zhao *et al.*, *Neoplasia* 2003; 5(4): 308 -318.