

A Novel Gadolinium-Based Contrast Agent Targeted to Cathepsin-D

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Introduction: Cathepsin-D (Cat-D) is a lysosomal protease found in high abundance in β -amyloid plaques, neuronal cells and cerebrospinal fluid of Alzheimer's disease patients and in some cancers (1, 2). We have designed a novel magnetic resonance imaging (MRI) contrast agent, Gd³⁺-DOTA-CAT, that is targeted to Cat-D for detection of Alzheimer's disease (AD). This contrast agent is T₁-based and is coupled to a Cat-D recognition site and a cell-penetrating peptide that allows it to cross the blood-brain-barrier (3). The targeting ability of a similar compound has been previously demonstrated in cultured cells (4). The purpose of this study was to investigate the magnetic resonance sensitivity and T₁ relaxivity of Gd³⁺-DOTA-CAT and evaluate the potential for Cat-D detection *in-vivo*.

Methods: Gd³⁺-DOTA-CAT was dissolved in 10% bovine serum albumin (BSA) and heated to 80°C to create an *in-vitro* tissue phantom (5). Various concentrations (50, 100, 250, 500 μ M) of Gd³⁺-DOTA-CAT and Gd³⁺-DTPA (Magnevist®) were added to 10% BSA (w/v) to compare T₁ relaxivity. All magnetic resonance images were acquired on a 9.4 Tesla small animal MRI scanner (Varian, Palo Alto, Ca.) at 37°C. The T₁ time constants were measured using an inversion prepared spin-echo multi slice pulse sequence (TE = 14 ms, TR = 50 ms, TI = 0, 10, 20, 40, 80, 160, 320, 640, 1280, 2500 ms, 2 averages, 2 dummy scans). The T₁ time constant of each solution was measured by fitting an exponential curve to the signal intensity as a function of the inversion times (TI). The inverse of the longitudinal relaxation time (T₁) was plotted against concentration and fit to a straight line to measure the relaxivities of each contrast agent. To evaluate the potential for *in-vivo* detection, this agent (100 μ L of 10 mM Gd³⁺-DOTA-CAT) was injected through a tail-vein into an Amyloid Precursor Protein (APP) transgenic Alzheimer's disease mouse (Jackson Laboratories), which develops many of the histopathological changes of AD (6). Mice were anaesthetized with isofluorane and sacrificed after each experiment. A T₁-weighted fast spin-echo (FSE) pulse sequence (TE = 8.5 ms, TR = 550 ms, FOV = 19.2 x 19.2, Matrix = 128 x 128, Echo Train Length = 4, Acquisition Time = 17.5 s per volume) was used to image the mouse brain at 9.4 Tesla. Following imaging, the mouse brain was extracted and stored in 10% neutral buffered formalin for histological analysis.

Results and Discussion: Phantoms containing Gd³⁺-DOTA-CAT had a greater signal intensity in the T₁-weighted images (Figure 1) at each concentration compared to Gd³⁺-DTPA (Magnevist®) as Gd³⁺-DOTA-CAT shortened T₁ compared to Gd³⁺-DTPA (Magnevist®). Gd³⁺-DOTA-CAT had a relaxivity of 6.9 (mM s^{-1}) compared to clinically used Gd³⁺-DTPA (Magnevist®) which had a relaxivity of 3.2 (mM s^{-1}) shown in Figure 2. Transgenic APP mice injected with 10 mM Gd³⁺-DOTA-CAT produced up to a 3% increase in signal intensity in the brain, 45% increased signal intensity in non-cerebral vasculature, and 14% increased signal intensity in brain vasculature (Figure 3). Signal intensity was normalized to 100% at baseline. The APP mouse's vital signs significantly declined at approximately 16 minutes resulting in termination of the experiment at 20 minutes.

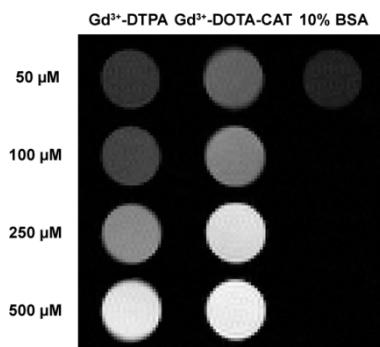


Figure 1. T₁-weighted spin-echo multi slice images of Gd³⁺-DTPA, Gd³⁺-DOTA-CAT and 10% BSA at 37°C at 9.4T.

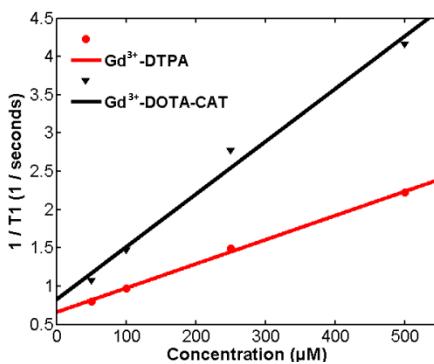


Figure 2. Relaxation rate ($R_1 = 1/T_1$) as a function of concentration. Gd³⁺-DOTA-CAT has a greater relaxivity 6.9 (mM s^{-1}) than clinically used Gd³⁺-DTPA (Magnevist®) of 3.2 (mM s^{-1}).

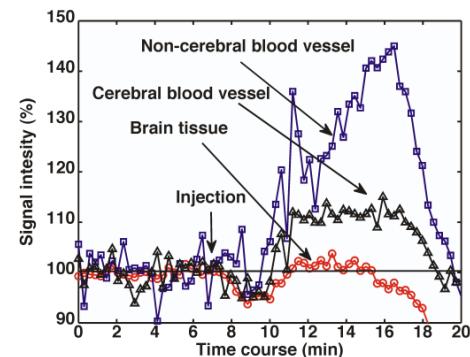


Figure 3. Signal intensity plots from 3 regions of interest: brain tissue, cerebral and non-cerebral blood vessels. There was an increase in signal intensity within all regions of interest post-injection of Gd³⁺-DOTA-CAT.

Conclusions: Gd³⁺-DOTA-CAT demonstrated a greater relaxivity than Gd³⁺-DTPA (Magnevist®) at 9.4T at 37°C. *In-vivo*, in a transgenic APP mouse, Gd³⁺-DOTA-CAT increased the signal intensity within the brain vasculature by 14% and the whole brain by 3% within minutes of injection. Therefore, the novel contrast agent, Gd³⁺-DOTA-CAT shows significant potential as a magnetic resonance imaging agent for Cathepsin-D activity *in-vivo*.

References: (1). Benes, et al., Crit Rev Oncol/Hematol 2008. 68(1):12-28. (2). Zaidi, et al. Biochem Biophys Res Commun 2008. 376(1):5-9. (3). Kersemans, V et al., Pharm Rev 2010. 3:600-620. (4). Suchy, et al., Org Biomol Chem 2010. 8(11):2560-6. (5). Koenig, et al., Magn Reson Med 1993. 29:311-316. (6). Sturchler-Pierrat, et al., Proc Natl Acad Sci USA. 1997. 94(24):13287-92.