

A Novel Gd-Sucrose Scaffold for Oral Administration in MR-colonography at 7 T

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Introduction: Colorectal cancer (CRC) is the third most common cancer in both males and females, and the second leading cause of death in the United States¹. CRC treatment often includes surgical resection followed by chemo- and radiation therapy and in spite of the progress in targeted chemotherapeutic agents, CRC prognosis still relies on early-stage-disease detection thus emphasizing the need for an efficient screening method. The current standard, colonoscopy, is invasive, requires sedation, a cathartic bowel preparation, and often suffers from patient non-compliance. As a non-invasive alternative, magnetic resonance colonography (MR-C) provides high spatial resolution three-dimensional images that encompass the entire colon². Although computed tomography colonography is the alternative typically used in the US, the lack of ionizing radiation, analgesics and excellent soft-tissue contrast suggests the potential for use of MR for detecting and monitoring colorectal polyps. To increase the sensitivity of MR-C, Gd-based contrast agents (e.g. Magnevist) are commonly used via intravenous delivery. However, these agents are not specifically targeted to CRC. Herein, we propose the development of targeted contrast agents for pre-screening of CRC by MRI, i.e. Molecular Colonography. To initiate this process, we have developed and evaluated a non-toxic Gd-DOTA sucrose based scaffold³ that can be used for delivery of contrast via oral administration and can be conjugated to targeting moieties specific for CRC.

Methods: A Gd-DOTA sucrose-derived agent was synthesized using an argon-degassed solution of azide, octaalkyne in 9/1 THF/water, CuSO₄ and sodium ascorbate (Fig 1A). MRI was acquired using a 7-T horizontal magnet (Agilent Technologies, Inc.) with 205/120/HDS gradients and 310 mm bore. Phantom experiments were performed using progressive saturation with 11 TR values exponentially spaced from 30 s to 60 ms. The relaxation time (T₁) and rate (R₁ = 1/ T₁) constants were obtained by nonlinear least squares regression (Fig 1B). 6 female SCID/beige mice were injected intra-rectally with 1x10⁶ HCT 116/luc cells (i.e. human colorectal cancer cells expressing luciferase). The contrast agent was dissolved in 100mM sodium phosphate buffer, pH 7.4, at a concentration of 2.5 mM for gavage administration (10μl/gram body weight). Mice were anesthetized with 2% isoflurane and restrained in a specific holder. Using a 35-mm quadrature coil (Doty Scientific), coronal T₁-weighted gradient echo (GE 3D) and T₂-weighted multislice spin-echo (SEMS) sequences were acquired with TE/TR=7.6/25ms and 50/2750 ms, respectively. For both scans, slice thickness was 1 mm and spatial resolution kept at 350x350x100 μm to be acquired in 6 min. Scans were acquired at baseline and 0.5, 5, 24 and 48 hours following gavage administration. To evaluate potential intestinal absorption of the agent, the compound was dissolved in PBS (25 μM/kg body weight), and administered in 150 μl volume via a tail vein catheter during imaging, with acquisitions prior to, during and post injection. Tumor signal intensity was calculated using manually drawn regions of interest in VnmrJ.

Results & Discussion: Previously, we introduced the first generation of this Gd-DOTA-Sucrose contrast agent for MR-C of the mouse gastro-intestinal (GI) tract³. Our compound had superior relaxometric properties compared to Gd-DOTA in its lower limit of detectability and is designed to remain in the GI tract throughout the passage. In this current study, we improved our agent by increasing the average number of Gd-DOTA chelates per sucrose molecule to 8. To improve relaxivity, the length of each chelate also was shortened to yield a stiffer compound thus increasing the rotational correlation time. These modifications yielded an 8-fold increase in Spin-Lattice-relaxivity (Fig 2) which also was observed in the *in vivo* experiments as tumor signal intensity enhancements of 40% and 93% following gavage and tail-vein injection, respectively (Fig 2). Kidney clearance also was evaluated and showed maximum uptake by 3 min following i.v. injection and was cleared by 10 min which is significantly faster than reported Magnevist renal clearance. Future efforts will evaluate this agent targeted to a CRC marker using tumors with endogenous expression.

References: [1]. Siegel R *et al.*, *CA CJC.*, 2008;15(1). [2]. Luboldt W *et al.*, *Rad.*, 2000; 216. [3]. Martinez GV *et al.*, *BMC*, 2011; 19.

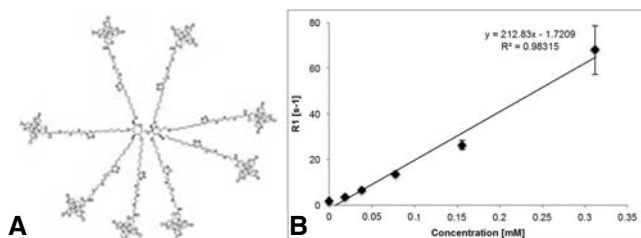


Fig 1. (A) Molecular structure of the 8-Gd sucrose scaffold employed herein. (B) Weighted linear regression of experimental phantom data (R₁) as a function of concentration. The fitted value for molar relaxivity (r₁) is 213 mM s⁻¹ (26.6 mM s⁻¹ per Gd) with R²=0.983.

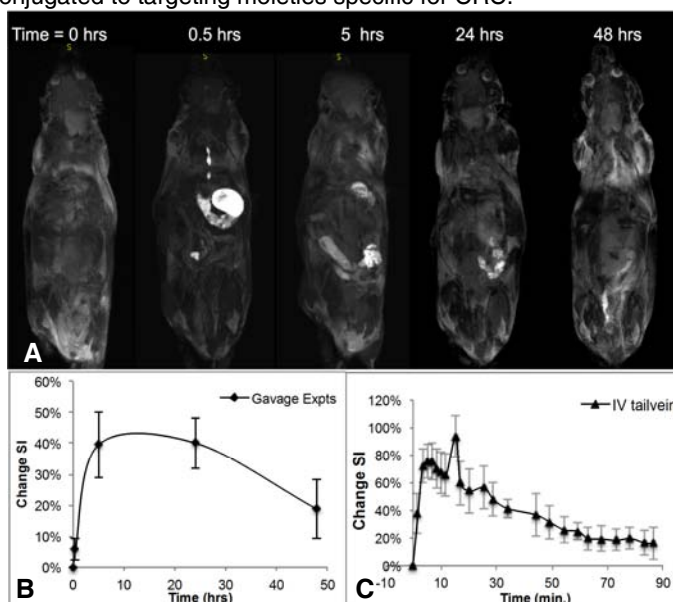


Fig 2. (A) Maximum intensity projections (MIP) obtained from T₁-weighted GE 3D images showing the passage of the contrast agent throughout the mouse gastro-intestinal tract at different time points. (B) Percent change in signal intensity within the tumor with time following gavage administrations versus (C) tailvein i.v. injections of the agent.