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Demonstration of a Sucrose-derived Contrast Agent for Magnetic Resonance Imaging of the GI Tract

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Abstract

A scaffold bearing eight terminal alkyne groups was synthesized from sucrose, and copies of an azide-terminated Gd-DOTA complex were attached via copper(I)-catalyzed azide-alkyne cycloaddition. The resulting contrast agent (CA) was administered by gavage to C3H mice. Passage of the CA through the gastrointestinal (GI) tract was followed by T₁-weighted magnetic resonance imaging (MRI) over a period of 47 hours, by which time the CA had exited the GI tract. No evidence for leakage of the CA from the GI tract was observed. Thus, a new, orally administered CA for MRI of the GI tract has been developed and successfully demonstrated.

Keywords

Magnetic resonance imaging; Contrast agent; Gadolinium; DOTA; Gastrointestinal tract

Gastrointestinal (GI)[†] radiography using barium contrast media has been widely used as a first-choice diagnostic imaging modality for detection of GI pathologies.¹ However, this approach is limited by radiation dosage² and the lack of lesion specificity. Colonoscopy is the standard of care for colorectal cancer (CRC) screening. However, colonoscopy is an

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Supplementary Material

Experimental details for the synthesis of compounds **4**, **7–9**, and the mixture of Gd-DOTA-sucrose contrast agents that includes **10**; ¹H and ¹³C NMR spectra of **4**, **5**, **7**, **8**, HRMS of **9**, MALDI-TOF of the mixture of Gd-DOTA-sucrose contrast agents that includes **10**, and additional details of *in vivo* MRI experiments.

[†]Abbreviations: CA, contrast agent; CRC, colorectal cancer; CT-C, X-ray computed tomography colonography; CuAAC, copper(I)-catalyzed azidealkyne cycloaddition; DTPA, diethylenetriaminepentaacetic acid; DOTA, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid; FOV, field of view; FSEMS, fast spin echo multi-slice; GE3D, 3D spoiled gradient echo images; GI, gastrointestinal; MR-C, magnetic resonance imaging colonography; PS, progressive saturation; SEMS, spin echo multi-slice; TE, echo time; TR, repetition time.

invasive procedure that requires intravenous sedation and suffers from patient noncompliance.^{3,4} CT colonography (CT-C) has high sensitivity to identify large polyps, but sensitivity decreases with a decrease in polyp size,⁵ and radiation dosage is a concern. MRI colonography (MR-C) uses non-ionizing radiation and provides CRC lesion detection with high specificity, but modest sensitivity.^{6,7} MR-C has been indicated in cases of incomplete colonoscopy due to obstruction, and is increasingly being applied as a non-invasive screening tool without the need for sedation.⁵ MR-C has been performed with bright lumen,⁸ and more recently in rodent models using dark lumen distended intestines in combination with intravenous contrast administration, typically Gd-DTPA or Gd-DOTA.^{9–11} Dark lumen contrast has shown somewhat better performance, yet it is beneficial to the patient to undergo screening procedures that do not require enema or bowel distension.

Contrast agents (CAs) that have multiple Gd-chelates per molecule exhibit increased molar relaxivities (r_1),^{12–15} making them detectable at lower concentrations than MR-C agents such as Gd-DTPA and Gd-DOTA. Targeted contrast agents¹⁶ bind with high specificity and affinity, and for extended periods of time, to cell-surface markers that are expressed by aberrant cells, reducing the amount of CA needed and lengthening the time available for observation. If orally administered targeted CAs with multiple Gd-chelates per molecule were available for MR-C detection of lesions and pathologies of the GI tract, the drawbacks detailed in the previous paragraph would be overcome. This would result in a significantly lower limit of detectability for tumors in T_1 -weighted images, without the need to use bright or dark lumen methods.

To be useful for construction of a CA for use in the GI tract, a molecular scaffold must possess multiple attachment points for Gd-chelates and targeting ligands. Unbound CA must be nontoxic and pass through the GI tract intact, without being degraded by stomach acid or digestive enzymes or absorbed by the intestines. With these considerations in mind, our attention was drawn by Olestra (**1**), a non-digestible fat substitute derived from sucrose and mixtures of fatty acids.^{17,18} We reasoned that this molecule might serve as a useful scaffold if appropriate reporter groups and targeting ligands could be attached to the termini of the fatty acid chains. Olestra contains acid- or base-labile ester links and is a complex mixture of compounds. To make our construct more stable and less complex, we elected to employ ether links to sucrose involving chains of the same or similar constitution. We recently demonstrated that small peptide ligands¹⁹ and prodrugs²⁰ could be attached to a sucrose-derived scaffold by means of the copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC). As the next step toward development of targeted CAs, we report herein the synthesis of an untargeted, sucrose-derived CA and its use in magnetic resonance imaging of the GI tract.

Reaction of sucrose (**2**) with 24 equivalents of sodium hydride in DMSO and 16 equivalents of 10-bromo-1-decyne (**3**)²¹ afforded octaalkyne **4** in 68% yield after purification by silica gel column chromatography (Scheme 1). Reaction of ester **5**²² with bromide **6**²³ in the presence of potassium carbonate in acetonitrile gave DOTA derivative **7** in 80% yield (Scheme 2). Following cleavage of the *t*-butyl esters with TFA, reaction of **8** with gadolinium (III) acetate produced azide **9** in 35% yield over the two steps. CuAAC reaction²⁴ of octaalkyne **4** with sixteen equivalents of azide **9** using CuSO₄ and sodium ascorbate in 9/1 THF/water at rt for 4 days produced, after reversed phase column chromatography, a mixture of triazole-containing compounds that differed in the number of unreacted alkynes remaining in the molecule. The principal component of this mixture, hereafter referred to as the Gd-DOTA-sucrose CA, determined by MALDI-TOF MS, was the octatriazole **10**. The average number of Gd-DOTA chelates attached per sucrose was 7.1 (Figure 1).

Initial MRI characterization of the Gd-DOTA-sucrose CA was accomplished in a phantom. An initial high concentration stock solution was prepared, and two separate phantoms were created by serial dilution. These phantoms were studied using progressive saturation experiments (PS), with 11 TR values exponentially spaced from 30 s to 60 ms. Nonlinear least squares regression allowed for the determination of the relaxation time constant (T_1) leading to the relaxation rate constant ($R_1 = 1/T_1$). The two highest concentration mixtures exhibited T_1 values that were shorter than the minimum TR used in the relaxation series, which led to large variances in the fitted R_1 values. Figure 2 shows the relationship between R_1 and [Gd-DOTA-sucrose CA], where weighted linear regression was used to determine the r_1 value, $29.5 \pm 0.1 \text{ mM s}^{-1}$, for the average multimer (r_1 per Gd = 4.1 mM s^{-1}). The weights used in the fit were inversely proportional to the variance in R_1 for each concentration, $(1/\sigma^2_{R1,i})$.

In vivo MRI studies were performed in C3H mice. Animals were anesthetized and prepared for MR imaging by induction with isoflurane. They were placed in a mouse-specific holder within an Agilent 72 mm birdcage coil (Agilent Technologies Inc., Santa Clara, CA) and inserted into an Agilent ASR 310 7T MRI system for scanning, which typically required one hour.

Imaging parameters and conditions were identical throughout the course of the experiment. The VnmrJ 3.1 pulse-sequences included scout (FLASH) scans with three-plane geometry, axial planning scans with T_2 -weighted Fast Spin Echo (FSEMS) with TR/TE = 2800/48 ms, and 25 slices. Coronal T_2 -weighted FSEMS (TR/TE = 1600/48 ms) images were acquired for anatomical reference. Coronal planes were oriented using the kidneys as a frame of reference, while placing the read dimension along the x-axis to minimize breathing artifacts. 3D spoiled gradient echo images (GE3D) were acquired (TR/TE of 25/2.4 ms) with a field of view (FOV) of $40 \times 90 \times 30 \text{ mm}^3$, an in-plane resolution of $156 \times 352 \mu\text{m}^2$, a 0.94 mm slice thickness, and a flip angle of 90° . A total of four averages were acquired with a scan time of seven minutes. To protect from acidity in the stomach, the Gd-DOTA-sucrose CA was suspended in 0.1 M phosphate buffer, pH 7.4, and administered orally via gavage for contrast enhanced MRI experiments. Post-contrast experiments were initiated immediately following gavage to include the time-points 0.5, 2.5, 3.3, 6.5, 7.7, 8.5, 16.7, 24.5, 27.5, and 47 hours. Spin-lattice relaxation experiments were performed using PS, with multiple TR values of 5, 1.87, 0.7, 0.26, and 0.098 seconds. A 2D spin echo experiment was used (SEMS) with a FOV of $45 \times 90 \text{ mm}^2$, an in-plane resolution of $176 \times 352 \mu\text{m}^2$, and a slice thickness of 1.2 mm.

The levels of detection are quite promising, in that the r_1 is relatively large compared to Gd-DOTA and in keeping with other reported multimeric Gd contrast agents.^{12–15} After orally introducing 0.5 mL of a 2.5 mM solution of the Gd-DOTA-sucrose CA mixture by gavage, high contrast in vivo [~ 11 relative enhancement (Enh–Bckgrnd)/Bckgrnd] was observed, and the untargeted agent passed through the GI tract within 47 h (Figure 3).²⁵ Based on T_1 values at the 8.5 hour time-point, concentrations of CA reached up to 0.25 mM in the most concentrated pixels, with a lower limit of detection estimated to be 5–10 μM at the edges of the CA enhanced region.

It is notable that these images were acquired on a pre-clinical scanner at 7 T, and that the sensitivity of larger voxel dimensions on a human scanner at 3 T is expected to increase dramatically. An initial calculation suggests that the sensitivity should increase by 100 (by 800 relative to a single Gd-DOTA). This calculation assumed that there are 45,000 receptors per cell and that cellular density is 260,000 cells/ mm^3 . To determine cellular density in adenomas, an experienced GI pathologist (D.C.) selected a set of representative histology sections stained with hematoxylin and eosin (H&E) from patient colon adenoma samples.

Slides were scanned using the Aperio™ ScanScope XT (Vista, CA) with a 200x/0.8NA objective lens via Basler tri-linear array. Image analysis was performed using an Aperio Nuclear® v1 customized histology algorithm to count the cells in regions of known area (mm²) and calculate the cells by volume (mm³). The pathologist's observation that adenoma cells comprised 70% of the cells in the sample was included in the estimate of adenoma cellular density.

It is expected that binding of targeted Gd-DOTA-sucrose CAs to receptors should increase their relaxivity by some non-negligible amount and provide greater contrast.²⁶ However, it is not possible to know how much of an increase in relaxivity will occur without measurements.

In summary, we have developed a Gd-DOTA-sucrose CA that can be delivered orally for three-dimensional MRI imaging of the gut which remains in the GI tract throughout its passage. The agent has the potential for use in diagnostic imaging comparable to barium agents, but without the associated exposure to ionizing radiation. This CA has superior relaxometric properties, relative to Gd-DOTA, in its lower limit of detectability. In addition, it may be possible to target this scaffold to colon lesions by conjugating this sucrose-derived CA with binding ligands for GI cancer-specific cell-surface markers, allowing for directed molecular imaging of GI lesions. The sensitivity of such a targeted molecular imaging agent is expected to be sufficient to detect polyps or lesions of 1–2 mm in diameter, and could greatly improve the specificity and sensitivity of MRI colonography. We are currently in the process of validating cell-surface markers for colon adenomas and adenocarcinomas and developing high-affinity marker-specific binding ligands that can be attached to this scaffold.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. MALDI-TOF MS analysis of the Gd-DOTA-sucrose contrast agent mixture containing **10**. Labeled peak cluster identities: 7507.3 $[\text{Gd}_8]^+$, 6749.4 $[\text{Gd}_7]^+$, 5988.6 $[\text{Gd}_6]^+$, 5228.6 $[\text{Gd}_5]^+$, 4469.7 $[\text{Gd}_4]^+$, 3754.6 $[\text{Gd}_8]^{2+}$, and 2994.4 $[\text{Gd}_6]^{2+}$. The average number of Gd-DOTA chelates attached per sucrose was 7.1.

Figure 2.

Weighted linear regression of experimental phantom data (R_1) as a function of concentration. The fitted value for molar relaxivity (r_1) is $29.5 \pm 0.1 \text{ mM s}^{-1}$, with $R^2=0.992$. Relaxation experiments were accomplished with multiple TR spin echo experiments on two separate Eppendorf tube phantoms prepared with identical concentrations of Gd-DOTA-sucrose CA. Error bars denote standard deviations.

Figure 3.

T₁-weighted GE3D images of the Gd-DOTA/sucrose construct mixture passing through the GI tract of a C3H mouse. Arrows indicate points of bright CA-related contrast. At 0.5 h post-gavage, bright contrast is observed in the esophagus and stomach; at 8.5 h contrast is observed in the small intestine; at 24.5 h contrast is observed in the large intestine; and by 47 h all contrast has cleared the GI tract.

Scheme 1.
Synthesis of Octaalkyne **4**.

Scheme 2.
Synthesis of Azide **9**.