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Development of Nanoparticle-Based Magnetic Resonance Colonography

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Abstract

This study was to develop a novel method of nanoparticle-based MR colonography. Two types of solid lipid nanoparticles (SLNs) were synthesized with loading of (a) gadolinium-diethylenetriaminepenta-acetic-acid (Gd-DTPA) to construct Gd-SLNs as an MR T1 contrast agent; and (b) otcadecylamine-fluorescein-isothiocyanate (ODA-FITC) to construct Gd-FITC-SLNs for histologic confirmation of MR findings. Through an *in vitro* experiment, we first evaluated the size distribution and Gd-DTPA entrapment efficiency of these SLNs. The SLNs displayed a size distribution of 50–300 nm and a Gd-DTPA entrapment efficiency of 56%. For *in vivo* validation, thirty mice were divided into five groups, each of which was administered a transrectal enema using: (i) Gd-SLNs (n=6); (ii) Gd-FITC-SLNs (n=6); (iii) blank SLNs (n=6); (iv) Gd-DTPA (n=6); and (v) water (n=6). T1-weighted FLAIR MRI was then performed on mice after transrectal infusion of Gd-SLNs or Gd-FITC-SLNs, which demonstrated bright enhancement of the colonic walls, with decrease in T1 relaxation time. When Gd-FITC-SLNs were delivered, green fluorescent spots were visualized in both the extracellular space and the cytoplasm through colonic walls under confocal microscopy and fluorescence microscopy. This study establishes the “proof-of-principle” of a new imaging technique, called “nanoparticle-based MR colonography,” which may provide a useful imaging tool for the diagnosis of colorectal diseases.

Keywords

Magnetic resonance (MR); Colonography; Solid lipid nanoparticles (SLNs); Gadolinium diethylenetriaminepenta acetic acid (Gd-DTPA)

INTRODUCTION

Modern imaging technologies play an important role in the management of colonic diseases, including colorectal cancers. To date, different colonic imaging techniques, including double-contrast barium enema, CT colonography, and MR colonography, have been used to diagnose and monitor therapies for colonic diseases (1–3). These colonic imaging techniques primarily depend on the use of contrast media, such as barium for double-contrast enema, iodinated contrast agent for CT colonography, and gadolinium diethylenetriaminepenta acetic acid (Gd-DTPA) for MR colonography (1,4,5). Contrast media usually are liquid agents that function to distend the colon and thereby increase imaging contrast between the colonic lumen and pathologic lesions on the colonic wall (6–8). However, since contrast agents are not readily absorbed by the gastrointestinal tract, colonic lesions cannot be detected based on the colonic absorption of contrast agents. This weakness could potentially be overcome by incorporating nanotechnology with MR colonography.

Nanotechnology has been successfully used in many aspects of modern medicine, including laboratory diagnostics, medical imaging, and therapeutics (9,10). A recent advance in MR technology is the establishment of innovative molecular MR imaging techniques, which are based on the development of target-specific nanoparticles (11–13). One such nanoparticle is a solid lipid nanoparticle (SLN). SLNs were originally designed as potential carriers of agents during oral administration of drugs (14,15). SLNs possess several advantages, including good tolerability, high oral bioavailability, low toxicity, and relatively easy large-scale production. In addition, it has been shown that the SLN-encapsulation enhances the bioactivity of several therapeutic agents, such as chemotherapeutics and DNAs. All these factors make SLNs excellent vehicles for the targeted delivery of diagnostic and therapeutic agents to the gastrointestinal tract (16–18).

The colon can absorb different types of therapeutics via mucosal transport. Given that the majority of colonic diseases, such as colorectal cancer and inflammatory bowel disease, originate in the mucosa, SLN-mediated MR colonography could become a natural solution for the problems in colorectal imaging. If proven successful, SLN-mediated MR colonography may achieve the following: (i) obtain contrast enhancement of the colorectal walls via a transrectal enema of Gd-DTPA-carrying SLNs (Gd-SLNs), without the intravenous administration of Gd-DTPA; (ii) develop a functional MRI examination to assess colorectal absorptive function; and (iii) provide a useful imaging tool for the evaluation of the delivery to the colon of SLNs loaded with Gd-DTPA and other therapeutics, such as DNAs and chemotherapeutics. We report our recent development of a new colonic imaging technique — Gd-SLN-based MR colonography.

MATERIALS AND METHODS

Study design

This study was divided into three phases. Phase I: synthesizing Gd-SLNs and *in vitro* evaluation of their size distribution and Gd-entrapment efficiency; Phase II: *in vivo* validation of the feasibility of the novel concept (Gd-SLN-based MR colonography) in a mouse model; Phase III: histologic confirmation of the ability of transrectally-infused SLNs to infiltrate the colonic walls in mice. The animal protocol for this study was approved by the Institutional Animal Use and Care Committee.

Synthesis and Measurement of SLNs

Three SLNs were synthesized by a previously described solvent diffusion method (19), and included blank SLNs, Gd-SLNs, and Gd-SLNs conjugated with fluorescein isothiocyanate

(FITC) (Gd-FITC-SLNs). Briefly, to prepare blank SLNs, 200 mg surfactant of Span 80 (Shanghai Chemical Reagent Co. Ltd., Shanghai, China) was dissolved in 10 ml n-hexane to form the oily phase. Eighteen mg surfactant of Tween 80 (Shanghai Chemical Reagent Co. Ltd.) was dissolved in 1 ml distilled water to form an aqueous phase. The aqueous phase was then added into the oily phase, with magnetic stirring at 400 rpm for five minutes to form a pre-emulsion. From the pre-emulsion, a water/oil (W/O) mini-emulsion was subsequently produced by ultrasonic treatment of the pre-emulsion in an ice-bath, and applying ultrasonic energy at 400 watts (W) and 20 cycles, with 1-s activation for a 2-s duration (JY92-II, Scientz Biotechnology Co., Ltd., China). Fifty mg monostearin was dissolved in 1 ml ethanol in a 60 °C water bath. The mixture was then quickly dispersed into an 11 ml mini-emulsion under mechanical agitates (DC-40, Hangzhou Electrical Engineering Instruments, China) at 400 rpm for five minutes at room temperature to obtain an original SLN dispersion. The original SLN dispersion was subsequently centrifuged for 15 minutes at 20,000 rpm (3K30, SIGMA Laborzentrifugen GmbH, Germany) to precipitate the SLNs. The SLNs were collected and then washed with 2 ml n-hexane twice, and then re-dispersed in 50 ml Poloxamer 188 solution (0.1 %, w/v).

A similar method was used to prepare Gd-SLNs and Gd-FITC-SLNs. For production of Gd-SLNs, 25 mg Gd-DTPA was dissolved in the aqueous phase with 50 mg monostearin. For production of Gd-FITC-SLNs, 25 mg Gd-DTPA was dissolved in a mixture of 45 mg monostearin and 5 mg ODA-FITC, as well as 1 ml ethanol. FITC was supplied by Zhancheng Bioscientific Co. Ltd. (Guangzhou, China), and Gd-DTPA was a gift from Beilu Pharmaceutical Co. Ltd. (Beijing, China).

The physicochemical properties of the SLNs were evaluated using different laboratory tests, including the volume average diameter, the polydispersity index, the zeta potential, and the agent entrapment efficiency. The volume average diameter was measured using an environmental scanning electron microscope (ESEM, XL-30, Philips, Netherlands) and a Zetasizer (3000HS, Malvern Instruments Ltd, UK). The polydispersity index and the zeta potential were characterized using a Zetasizer. The agent entrapment efficiency was measured by a fluorescence spectrophotometer (F-2500, Hitachi Co., Japan).

Animal study groups

Healthy, male, Institute for Cancer Research (ICR) mice, weighing 22–25 grams, were obtained from our institute and housed according to institutional guidelines. Thirty mice were randomly allocated into five study groups based on the transrectal enema they received, as follows: (i) Gd-SLNs (40 mg/ml, n=6); (ii) Gd-FITC-SLNs (40 mg/ml, n=6); (iii) blank SLNs (40 mg/ml, n=6); (iv) Gd-DTPA (10 mg/ml, n=6); and (v) water (1 ml, n=6). The mice received general anesthesia with an intraperitoneal injection of urethane at 1g/kg body weight (Shanghai Chemical Reagent Co. Ltd., Shanghai, China). A cleaning enema with water was given before the administration of SLNs and control agents. One ml SLN or controlled agent was then infused into the colorectum through a 1 ml syringe and a 24-gauge cannula (Xindeyi Medical Instrument Co. Ltd. Hangzhou, China) at a speed of 2ml/min.

Twenty minutes after enema retention of SLNs and agents, the animals in each group underwent two different examinations. For the study phase II, all mice were imaged with MRI *in vivo* to validate the feasibility of nanoparticle-based MR colonography. For study phase III, all mice were sacrificed for histologic examination to evaluate the distribution of SLNs in colonic walls.

MR colonography

MR colonography images were obtained using a 3-Tesla superconducting magnet unit (SIGNA HD, General Electric Healthcare, Milwaukee, WI) with a specialized one-inch quadrature mouse coil. The mice were placed in a supine position at the center of the mouse coil. MRI of the sigmoid colon and rectum was subsequently obtained. A series of MR colonographies, using T1-weighted fluid-attenuated inversion-recovery (FLAIR) or inversion recovery sequences (20,21), were performed before and 20 minutes after the intracolonic enema. Briefly, after obtaining base-line MR colonographies, each mouse was removed from the coil for an intracolonic enema and then placed back into the coil for the second MR colonography 20 minutes after the intracolonic enema. The position of the mouse anus was marked on the inner surface of the coil, which ensured that the mouse was placed at the same imaging position between the MR colonographies, before and after the intracolonic enema.

To measure the T1 relaxation times of the colorectal walls of the mice, a T1-weighted FLAIR sequence was performed with different inversion times (500, 650, 800, and 950 ms), with identical parameters of repetition time/echo time (TR/TE)=3000/20 ms, number of excitations=2, slice thickness=1.5 mm, section number=7, spacing=0.5 mm, field of view=6×4.5 cm, matrix size=288 ×256, and spatial resolution=208 μm×176 μm. The T1 mapping technique was used based on an inversion recovery, spin echo sequence with four FLAIR series performed at different TIs of 500, 650, 800, and 950 ms, with identical TR. A custom-programmed Functool Plugin software package was used to calculate the T1 map to measure colorectal T1 relaxation times by using regions of interest at 240 pixels within the uniform areas of the colorectal walls on three images.

Histologic confirmation of SLN infiltration into colonic walls

Colorectal segments of mice were harvested and opened along the longitudinal axis. A portion of each fresh tissue sample was embedded in Optimal Cutting Temperature (OCT) and snap frozen in liquid nitrogen. Frozen tissue blocks were then brought to −20°C and sectioned at 5 μm thickness. The tissue sections were fixed for 20 min in 4% formaldehyde, treated for 10 minutes with 0.1% Triton X-100 in PBS, and then washed with PBS. Subsequently, they were incubated at room temperature for 10 minutes with 0.1 μg/mL 4',6-diamidino-2-phenylindole (DAPI; Sigma, St Louis, MO, USA). DAPI is a dye that fluoresces blue (455 nm) when bound to double-stranded DNA and is excited by exposure to 345 nm light. These histologic sections were then examined under a confocal laser-scanning microscope (Leica Microsystems, Mannheim, Germany) and a fluorescence microscope (Zeiss Axioskop 2, Carl Zeiss, Marburg, Germany). The confocal laser-scanning microscope was operated using a Leica Application Suite Advanced Fluorescence program. FITC of the colonic tissues was excited at the 488 nm wavelength of an Argon-ion laser, while FITC emission was detected and recorded at a 500–535 nm wavelength and displayed in green false color.

The remaining portion of each fresh tissue was fixed in 10% buffered formalin for 24 hours, embedded in paraffin, and then sectioned at 5 μm thickness. The sections were deparaffinized in xylene, rehydrated, and dehydrated using a series of ethanol solutions, and then stained with a standard hematoxylin and eosin (H&E) method for histologic examination of the morphology of the colonic tissue.

Statistical Analysis

Statistical analysis was performed using SPSS 13.0 for Windows (SPSS Inc., Chicago, IL). A paired samples test was used to assess the significance of differences in T1 relaxation times of the colorectal walls before and after the intracolonic enema in different animal

groups with various treatments. A *P* value of less than 0.05 was considered statistically significant.

RESULTS

Characterization of SLNs

Table 1 displays the physicochemical properties of SLNs, including the volume average diameter, the polydispersity index, the zeta potential, and the agent entrapment efficiency. All three types of SLNs prepared in W/O mini-emulsion exhibited bimodal particle size distribution at a range from 50 to 300 nm (Fig. 1), with their sizes slightly increased when ODA-FITC was added to SLNs. The Gd-DTPA entrapment efficiencies of the Gd-SLNs and the Gd-FITC-SLNs were 55.8% and 55.0%, respectively. There was no significant difference in zeta potentials and agent entrapment efficiencies between Gd-SLNs and Gd-FITC-SLNs.

SLN-based MR colonography

The colorectal walls of all mice were clearly visualized with T1-weighted FLAIR MR imaging, especially with an 800-ms inversion time to generate high-quality images. Significant homogeneous enhancement of the colonic walls was seen in all mice treated with either Gd-SLNs (*n*=6) or Gd-FITC-SLNs (*n*=6), which was not visualized in the three control animal groups treated with Gd-DTPA-only, blank SLNs, or water (Fig. 2). T1 map showed that T1 relaxation times on colorectal walls in both Gd-FITC-SLN and Gd-SLN groups significantly decreased from 549.3±29.6 ms to 439.1±45.2 ms (*P*<.001), and from 539.3±25.1 ms to 432.4±63.3 ms (*P*<.005), respectively (Fig. 3). However, there were no such changes on T1 relaxation times between before and after intracolonic enemas in each of the control groups with Gd-DTPA, blank SLNs, or water. T1 relaxation time in the blank SLN group was slightly higher than that in other groups, but there was no statistically significant difference between them.

Histologic Confirmation

Fluorescence microscopic examination of the mouse colorectums, harvested 20 minutes after a Gd-FITC-SLN enema, showed a high concentration of green fluorescence (due to FITC emission) within the entire colorectal wall, indicating Gd-FITC-SLN infiltration from the mucosa to the serosa (Fig. 4). There was no such fluorescence observed in the colorectal walls of control mice treated with Gd-SLNs, Gd-DTPA-only, blank SLNs, or water. Confocal microscopy revealed that fluorescence was homogeneously distributed in both the extracellular space and the cytoplasm at various layers of the colorectal walls, including the mucosa, the submucosa, the tunica muscularis, and the serosa, 20 minutes after a Gd-FITC-SLN enema (Fig. 5).

DISCUSSION

Nanotechnology has shown great potential to advance modern medicine. Nanoparticle-based molecular imaging is opening new avenues for the early diagnosis and target-specific treatment of different diseases (22). In the gastrointestinal tract, nanoparticles have been shown to facilitate the absorption of diagnostic and therapeutic agents administered orally (23–25). This enhanced absorption should also be expected to occur in the colorectum due to the powerful absorptive ability of the colorectum.

MR colonography has shown several advantages, including high-resolution imaging, the ability to provide detailed soft tissue background, and no need for ionized radiation (3,26). In addition, MR colonography provides an option for virtual colonoscopy, which has shown

promise in replacing the invasive endoscopic procedure in colon cancer screening. To date, dark-lumen MR colonography is the most commonly used technique, which is based on a water or air enema for colorectal luminal distention and an intravenous injection of Gd-DTPA to enhance the colorectal walls (3). However, intravenously-delivered Gd-DTPA is limited to the extracellular space and distributes non-selectively throughout the entire body (27–29). In addition, the intravenous delivery of Gd-DTPA into the colorectal wall is not related to the bowel absorptive function, but rather, to the local blood supply and vascular densities. Our approach in this study was to develop an SLN-based MR colonography by combining the advantages of MR technology, nanotechnology, and the natural colorectal absorptive function.

In a previous study, we had successfully synthesized ODA-FITC-loaded stearic acid SLNs by a solvent diffusion method (14). Via oral administration, we were able to demonstrate the efficient absorption of SLNs by the gastrointestinal tract. The transport efficiency of SLNs was estimated at approximately 30%, first into the gastrointestinal wall, and then, the blood circulation or the lymphatic duct (30). In the present study, we also produced Gd-DTPA-loaded SLNs with sizes as small as 50–300 nm, and observed high Gd-DTPA entrapment efficiency at approximately 56%. The results of the present study have confirmed that these Gd-SLNs can be efficiently absorbed into colorectal tissues to generate high MR T1 signals in the colorectal walls.

The use of Gd-SLNs enabled us to have a sufficient time window to achieve contrast-enhanced MRI of colonic walls. In a previous pilot study, we performed serial MR colonographies at a time period from 5 to 60 minutes after intracolonic Gd-SLN administration. We found that MR images at 20 minutes presented the strongest contrast enhancement of colorectal walls. Thus, MR colonography at 20 minutes after an intracolonic Gd-SLNs enema was adopted in the present study, where the colonic walls appeared as a high signal intensity ring, with a decreased MR T1 relaxation time. In addition, the intracolonic infusion of 1 ml Gd-SLNs was at a relatively slow speed of 2ml/min, which avoided potential damage to the mucous membranes of the colorectal wall due to over-distention.

In the present study, we also achieved a consistent correlation between SLN-mediated MR colonography and histology. Of the animal group treated with Gd-FITC-SLNs, fluorescence microscopy revealed a large quantity of FITC fluorescent spots that were distributed throughout the entire colorectal wall, while confocal microscopy further confirmed SLNs to be localized in both the extracellular space and the cytoplasm. These results indicate that the Gd-carrying, lipid-based nanoparticles can be efficiently and extensively absorbed into the colorectal walls. The potential mechanisms for the enhanced absorption of Gd-DTPA by the colorectal walls include (i) SLN can specifically adhere to the mucosa (31); and (ii) SLN can be efficiently absorbed by the epithelium, followed by subsequent diffusion into extracellular spaces (10). Similar to the currently-available MR colonography techniques, SLN-based MR colonography requires a careful and full bowel cleansing before MR imaging, because bowel contents, such as feces, gas, and water, may affect not only the colorectal absorption of SLNs, but also create susceptibility artifacts or partial volume effects during MR imaging.

This novel SLN-mediated MR colonography method has several significant advantages, including: (i) achieving contrast-enhanced MRI of the colorectal walls via intracolonic delivery of highly-concentrated Gd-DTPA-carrying SLNs, which might avoid the current intravenous administration of Gd-DTPA, as used for dark lumen MR colonography; (ii) laying the groundwork for the further development of a functional MRI technique to assess the colorectal absorption efficiency of Gd-DTPA or drugs/agents that are encapsulated in

Gd-SLNs; and (iii) providing a useful nanoparticle-based molecular imaging technique for basic science to precisely establish MRI and histologic correlations using Gd-FITC-SLNs. Further efforts are warranted to produce targeted Gd-SLNs by conjugating target-specific ligands onto the SLNs. When the targeted Gd-SLNs are co-loaded with therapeutics, such as antitumor drugs or genes, molecular MRI could be used to non-invasively monitor the delivery and biodistribution of these therapeutic Gd-SLNs in the colorectal targets, and thus, guide the therapies of various colonic diseases via nanoparticle-based MR colonography.

In conclusion, this study establishes the “proof-of-principle” of a novel imaging technique, nanoparticle-based MR colonography, by combining MR technology, nanotechnology, and the natural absorptive ability of the colorectum. We have demonstrated that Gd-SLNs can be efficiently absorbed into colorectal walls via a transrectal approach. This novel MR colonography technique may provide a useful imaging tool for the early diagnosis of colorectal diseases, such as colorectal cancers.

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References

1. Kung JW, Levine MS, Glick SN, Lakhani P, Rubesin SE, Laufer I. Colorectal cancer: screening double-contrast barium enema examination in average-risk adults older than 50 years. *Radiology*. 2006; 240:725–735. [PubMed: 16837671]
2. Gluecker TM, Johnson CD, Harmsen WS, Offord KP, Harris AM, Wilson LA, Ahlquist DA. Colorectal cancer screening with CT colonography, colonoscopy, and double-contrast barium enema examination: prospective assessment of patient perceptions and preferences. *Radiology*. 2003; 227:378–384. [PubMed: 12732696]
3. Hartmann D, Bassler B, Schilling D, Adamek HE, Jakobs R, Pfeifer B, Eickhoff A, Zindel C, Riemann JF, Layer G. Colorectal polyps: detection with dark-lumen MR colonography versus conventional colonoscopy. *Radiology*. 2006; 238:143–149. [PubMed: 16304088]
4. Runge VM, Stewart RG, Clanton JA, James AE, Partain CL. Work in progress: potential oral and intravenous paramagnetic NMR contrast agents. *Radiology*. 1983; 147:789–791. [PubMed: 6844614]
5. Nagata K, Singh AK, Sangwaiya MJ, Näppi J, Zalis ME, Cai W, Yoshida H. Comparative evaluation of the fecal-tagging quality in CT colonography: barium vs. iodinated oral contrast agent. *Acad Radiol*. 2009; 16:1393–1399. [PubMed: 19596591]
6. Ott DJ, Gelfand DW, Wu WC, Ablin DS. Colon polyp morphology on double-contrast barium enema: its pathologic predictive value. *Am J Roentgenol*. 1983; 141(5):965–970. [PubMed: 6605070]
7. Johansen JG. Assessment of a non-ionic contrast medium (Amipaque) in the gastrointestinal tract. *Invest Radiol*. 1978; 13:523–527. [PubMed: 755030]
8. Laniado M, Kornmesser W, Hamm B, Clauss W, Weimann HJ, Felix R. MR imaging of the gastrointestinal tract: value of Gd-DTPA. *Am J Roentgenol*. 1988; 150:817–821. [PubMed: 3126628]
9. Ferrari M. Cancer nanotechnology: opportunities and challenges. *Nat Rev Cancer*. 2005; 5:161–171. [PubMed: 15738981]

10. Couvreur P, Vauthier C. Nanotechnology: intelligent design to treat complex disease. *Pharm Res.* 2006; 23:1417–1450. [PubMed: 16779701]
11. Artemov D, Bhujwalla ZM, Bulte JW. Magnetic resonance imaging of cell surface receptors using targeted contrast agents. *Curr Pharm Biotechnol.* 2004; 5:485–494. [PubMed: 15579038]
12. Aime S, Cabella C, Colombatto S, Geninatti Crich S, Gianolio E, Maggioni F. Insights into the use of paramagnetic Gd(III) complexes in MR-molecular imaging investigations. *J Magn Reson Imaging.* 2002; 16:394–406. [PubMed: 12353255]
13. Weissleder R, Mahmood U. Molecular imaging. *Radiology.* 2001; 219:316–333. [PubMed: 11323453]
14. Yuan H, Chen J, Du YZ, Hu FQ, Zeng S, Zhao HL. Studies on oral absorption of stearic acid SLN by a novel fluorometric method. *Colloids Surf B Biointerfaces.* 2007; 58:157–164. [PubMed: 17446050]
15. Humberstone AJ, Charman WN. Lipid-based vehicles for the oral delivery of poorly water soluble drugs. *Adv Drug Deliver Rev.* 1997; 25:103–128.
16. Morel S, Terreno E, Ugazio E, Aime S, Gasco MR. NMR relaxometric investigations of solid lipid nanoparticles (SLN) containing gadolinium(III) complexes. *Eur J Pharm Biopharm.* 1998; 45:157–163. [PubMed: 9704912]
17. Müller RH, Mäder K, Gohla S. Solid lipid nanoparticles (SLN) for controlled drug delivery – a review of the state of the art. *Eur J Pharm Biopharm.* 2000; 50:161–177. [PubMed: 10840199]
18. Pedersen N, Hansen S, Heydenreich AV, Kristensen HG, Poulsen HS. Solid lipid nanoparticles can effectively bind DNA, streptavidin and biotinylated ligands. *Eur J Pharm Biopharm.* 2006; 62:155–162. [PubMed: 16290122]
19. Yuan H, Huang LF, Du YZ, Ying XY, You J, Hu FQ, Zeng S. Solid lipid nanoparticles prepared by solvent diffusion method in a nanoreactor system. *Colloids Surf B Biointerfaces.* 2008; 61:132–137. [PubMed: 17888636]
20. de Bazelaire CM, Duhamel GD, Rofsky NM, Alsop DC. MR imaging relaxation times of abdominal and pelvic tissues measured in vivo at 3.0 T: preliminary results. *Radiology.* 2004; 230:652–659. [PubMed: 14990831]
21. Mamisch TC, Dudda M, Hughes T, Burstein D, Kim YJ. Comparison of delayed gadolinium enhanced MRI of cartilage (dGEMRIC) using inversion recovery and fast T1 mapping sequences. *Magn Reson Med.* 2008; 60:768–773. [PubMed: 18816842]
22. Yang X. Nano- and microparticle-based imaging of cardiovascular interventions: overview. *Radiology.* 2007; 243:340–347. [PubMed: 17456865]
23. Desai MP, Labhasetwar V, Amidon GL, Levy RJ. Gastrointestinal uptake of biodegradable microparticles: effect of particle size. *Pharm Res.* 1996; 13:1838–1845. [PubMed: 8987081]
24. Damge C, Michel C, Aprahamian M, Couvreur P, Devissaguet JP. Nanocapsules as carriers for oral peptide delivery. *J Control Release.* 1990; 13:233–239.
25. Sanders E, Ashworth CT. A study of particulate intestinal absorption and hepatocellular uptake. Use of polystyrene latex particles. *Exp Cell Res.* 1961; 22:137–145. [PubMed: 13746138]
26. Rinck, PA. Magnetic resonance in medicine. Oxford: Blackwell scientific publication; 2003.
27. Runge VM. Safety of approved MR contrast media for intravenous injection. *J Magn Reson Imaging.* 2000; 12:205–213. [PubMed: 10931582]
28. Caravan P, Ellison JJ, McCurry TJ, Lauffer RB. Gadolinium (III) chelates as MRI contrast agents: Structure, Dynamics and Applications. *Chem Rev.* 1999; 99:2293–2352. [PubMed: 11749483]
29. Weinmann HJ, Brasch RC, Press WR, Wesbey GE. Characteristics of gadolinium-DTPA complex: a potential NMR contrast agent. *Am J Roentgenol.* 1984; 142:619–624. [PubMed: 6607655]
30. Bargoni A, Cavalli R, Caputo O, Fundaro A, Gasco MR, Zara GP. Solid lipid nanoparticles in lymph and plasma after duodenal administration to rats. *Pharm Res.* 1998; 15:745–750. [PubMed: 9619784]
31. Chen HH, Le Visage C, Qiu B, Du X, Ouwerkerk R, Leong KW, Yang X. MR imaging of biodegradable polymeric microparticles: a potential method of monitoring local drug delivery. *Magn Reson Med.* 2005; 53:614–620. [PubMed: 15723408]

FIG. 1.

Environmental scanning electron microscope (ESEM) analysis of Gd-SLNs, demonstrating that Gd-SLNs have a particle size distribution at a range of 50 to 300 nm.

FIG. 2.

Axial MR colonographies of the mouse rectums with a T1-weighted FLAIR sequence (TR/TE/TI, 3000/20/800) before (a-e) and 20 minutes after (f-j) intracolonic administration of Gd-FITC-SLNs (a&f), Gd-SLNs (b&g), Gd-DTPA-only (c&h), blank SLNs (d&i), and water (e&j). MR colonography shows contrast enhancement (arrows on f&g) of the rectal walls after intracolonic administration of Gd-FITC-SLNs and Gd-SLNs. This finding is not seen in the three control groups with Gd-DTPA-only (h), blank SLNs (i), or water (j). Bars =2 mm.

FIG. 3.

The distribution of mean T1 relaxation times of colorectal walls before and 20 min after an intracolonic enema with Gd-FITC-SLNs, Gd-SLNs, Gd-DTPA, blank SLNs, and water. The T1 map shows a significant decrease in the mean T1 relaxation times of the colorectal walls in the Gd-FITC-SLN group (* $P = .0004$) and the Gd-SLN group (** $P = .0023$) at 20 minutes after the intracolonic SLN enema. However, there are no significant changes of mean T1 relaxation times in Gd-DTPA, blank SLNs, or the water groups.

FIG. 4.

Comparison of representative histological images of colorectal slices among different animal groups with various treatments of Gd-FITC-SLNs (a&f), Gd-SLNs (b&g), Gd-DTPA (c&h), blank SLNs (d&i), and water (e&j). All slides were obtained after MR colonography and examined by microscopy (a-e, H&E stain, original magnification $\times 100$. Bars = 50 μm) and fluorescent microscopy (f-j, original magnification $\times 100$. Bars = 50 μm). Fluorescent microscopy revealed green fluorescent signals in Gd-FITC-SLNs-treated tissues only (due to FITC emission), which was not visualized in the other four control groups. This finding indicates the successful infiltration of SLNs to the colonic wall via the intracolonic enema approach.

FIG. 5.

Confocal microscopic images using the green channel for FITC fluorescence (a), the blue channel for DAPI staining of nuclei (b), and a combination of both the green and blue channels (c). Green fluorescence due to FITC emission is distributed throughout the entire colonic wall, from the mucosa to the serosa (arrows), at 20 minutes after the intracolonic Gd-FITC-SLNs enema, and is detected in both the extracellular space and the cytoplasm. Original magnification, $\times 200$. Bars = 25 μm . Images d-f (original magnification, $\times 400$) are magnifications of insets in a-c, respectively.

Table 1

Physicochemical properties of different SLNs

SLNs	Volume average size (nm)				PI	ξ (mV)	EE (%)
	Area (%)	Mean	Width				
Blank SLN	74.5	40.8	17.2	0.357	-39.1 $\pm$ 2.0		
	25.5	243.9	194.1				
Gd-SLN	91.7	85.4	25.4	0.296	-31.6 $\pm$ 2.5		55.8
	8.3	266.3	74.4				
Gd-FTTC-SLN	52.1	124.1	56.1	0.324	-29.3 $\pm$ 3.4		55.0
	47.9	300.8	144.8				

Note. —PI, ξ , and EE indicate the polydispersity index (the coefficient of variation/mean diameter), the zeta potential, and the percentage of agent entrapment efficiency, respectively.