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RESEARCH PAPER

Dextran Gadolinium Complexes as Contrast Agents for Magnetic Resonance Imaging to Sentinel Lymph Nodes

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ABSTRACT

Purpose The aim was to investigate three dextran gadolinium complexes Dextran-DTPA-Gd as the potential MRI contrast agents in lymphatic system.

Methods Three dextran gadolinium complexes Dextran-DTPA-Gd containing differing amounts of Gd-DTPA were synthesized by the incorporation of Gd-DTPA to the hydroxyl groups of dextran. These dextran ligands and gadolinium complexes were characterized, and their properties *in vitro* and *in vivo* were also evaluated.

Results Dextran-DTPA-Gd demonstrated obviously higher relaxation effectiveness than that of Gd-DTPA. The result of *in vitro* cytotoxicity assay showed that these macromolecular ligands and their corresponding gadolinium complexes had low cytotoxicity to HeLa cells. Dextran-DTPA-Gd greatly enhanced the contrast of MR images of normal politeal lymph nodes and reactive hyperplasia of politeal lymph nodes in rabbits and provided prolonged duration in lymphatic system with lower injection doses than that of Gd-DTPA. However, Dextran-DTPA-Gd displayed low signal enhancements in MR images of politeal lymph nodes with VX2 carcinoma in rabbits during the detection time.

Conclusions These dextran gadolinium complexes Dextran-DTPA-Gd can be taken up selectively by lymphatic system and showed the potential as MRI contrast agents in lymphatic system.

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INTRODUCTION

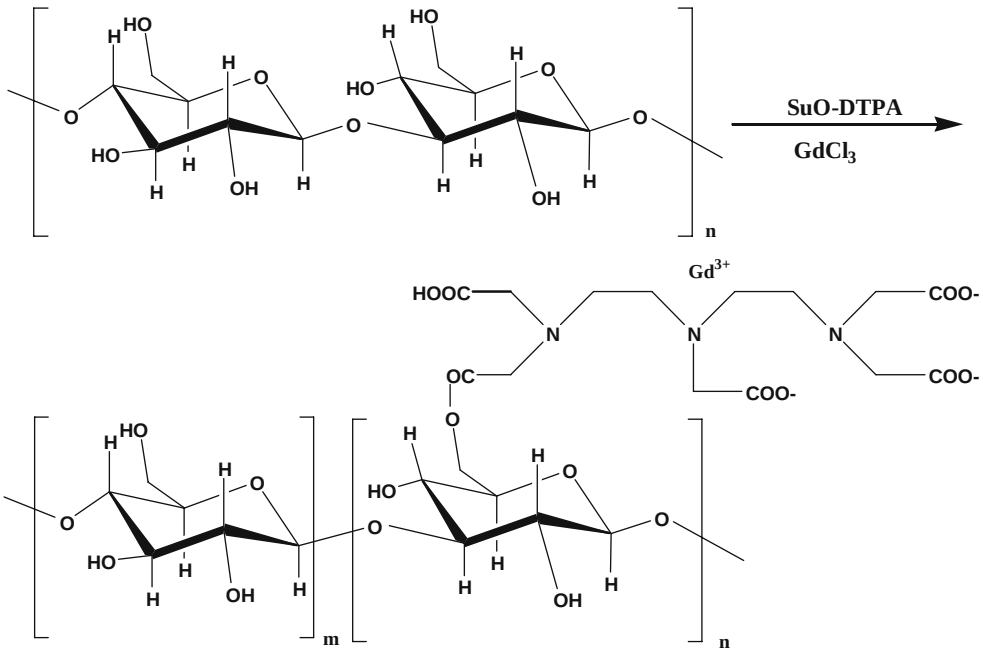
Magnetic resonance imaging (MRI) is a non-invasive clinical imaging modality, which relies on the detection of NMR signals emitted by hydrogen protons in the body placed in a magnetic field. MRI contrast agents are a unique class of pharmaceuticals that enhance the image contrast between normal and diseased tissue and indicate the status of organ function or blood flow after administration by increasing the relaxation rates of water protons in tissue in which the agent accumulates (1–6).

Recently, micro-magnetic resonance lymphangiography (MRL) imaging has been developed using gadolinium diethylenetriaminepentaacetic acid (Gd-DTPA), liposomes, Gd(III) macromolecular chelates, and iron oxide particles as MRI contrast agents to clearly visualize the lymphatic system including lymphatics and lymph nodes. This method can detect and distinguish among dilation of lymphatic vessels in a lymphangitis model, proliferative or neoplastic lymph node swellings in a lymphoproliferative model, inflammatory lymph node swellings in an infection/inflammation model, *etc.* (7–17).

However, the clinically used MRI contrast agents, for example, Gd-DTPA, are small ionic molecules that can diffuse freely through the extracellular space and are excreted rapidly by the kidney. Then, their biodistribution is nonspecific, although Gd-DTPA works well in organs such as the brain and spinal cord, where the normal brain parenchyma has a barrier to permeability of the contrast agent, and pathologic conditions such as cancer do not (18, 19).

The binding of small gadolinium complexes to a macromolecule has been shown to provide a mechanism

Scheme 1 Synthetic route to dextran gadolinium complexes.



of proton relaxation enhancement through longer rotational correlation times. The macromolecular MRI contrast agents thus obtained may show prolonged intravascular retention due to their bulky molecular volume, resulting in their clinical use as blood pool contrast agents. In addition, when a tumor-targeting group is attached to this macromolecular metal chelate, it can be endowed with tumor-specific properties (20–30).

Dextran, as a polysaccharide of D-glucose monomers linked by glycosidic bonds, has attracted great interest for its use as biodegradable material in drug delivery and tissue engineering because of its good medicine permeability, low immunogenity, good biocompatibility and biodegradability (31–33). In this work, dextran was chosen as the polymer carrier for MRI contrast agents, and DTPA was incorporated to dextran in order to synthesize the dextran ligands containing differing amounts of DTPA linked to polymeric repeat units. These ligands obtained were further reacted with gadolinium chloride to give the corresponding dextran gadolinium complexes (Scheme 1). These dextran ligands and gadolinium complexes were characterized, and their properties *in vitro* and *in vivo* were also evaluated.

MATERIALS AND METHODS

Instrumentation and Materials

The compounds prepared were characterized using a Spectrum One infrared spectrophotometer, a Lambda Bio40 UV/Vis spectrophotometer, a Varian Mercury-VX300 NMR spectrometer and a Carlo Erba 1106 analyzer. The concentration of the paramagnetic species [Gd^{3+}] was measured by an ICP Atomscan-2000 spectrometer. The particle size of dextran gadolinium complex Dextran-DTPA-Gd in water solution (0.02 g/mL) was detected by a zeta potential and laser diffraction particle size analyzer (Zetasizer Nano ZS, Malvern Instruments Ltd. United Kingdom). The solvent longitudinal relaxation time (T_1) for gadolinium complexes in distilled water was determined by a Varian Mercury-VX300 NMR spectrometer. MR image was performed on a 3.0 Tesla Magnetom Tiro Tim MR Scanner (Siemens, Germany). The normal New Zealand white rabbits (weight: 2.0–2.5 kg), rabbits with VX2 carcinoma in the politeal lymph nodes in both lower limbs (weight: 2.0–2.5 kg), and rabbits with the reactive hyperplasia of the politeal lymph nodes (diameter: 1 cm) in both lower limbs (weight: 2.0–2.5 kg) were provided by the School of Pharmacy (Tongji Medical College, Huazhong University of Science and Technology, China) and were cultured according to the method described in the literature (34). HeLa cells and VX2 carcinoma cells were provided by the China Center for Type Culture Collection of Wuhan University, China. The ethical approval was obtained for the *in vivo* experiments in rabbits from the Department of Science and Technology of Hubei Province, China and the Animal Center of Tongji Medical College, Huazhong University of Science and Technology, China.

All the chemicals and solvents were of analytical grade. The DTPA active ester DTPA mono(hydroxysuccinimidyl ester) (SuO-DTPA) was prepared according to the literature

(28). Dextran (Mn 4×10^4) was purchased from Sigma-Aldrich (USA). The injection solution of Gd-DTPA (Magnevist, Gadopentetic acid dimeglumine salt injection) was purchased from the Bayer Schering Pharma Division, Bayer HealthCare Company Limited, Germany.

Synthesis of Dextran-DTPA

The solution of DTPA mono(hydroxysuccinimidyl) ester (SuO-DTPA, 1.24 g, 2.54 mmol) in DMSO (20 mL) was added dropwise to a solution of dextran (2.05 g, 2.54 mmol, 1 equiv.) in 150 mL of DMSO and 8 mL of triethylamine with rapid stirring at room temperature. The reaction was continually stirred for 2 h at room temperature and a further 7 days at 60°C. The resultant mixture was filtered and precipitated with ethanol and ethyl ether. The precipitate was reprecipitated from distilled water using ethanol and ethyl ether, filtered and dried under vacuum to yield a white dextran ligand containing DTPA groups. After dialysis, the dialyzed solution was evaporated, and the solid residue was dried under vacuum to yield Dextran-DTPA (1:1) (L_1, 1.58 g, 48.15%). ^{1}H NMR (D_2O, δppm): 4.9 (d, OCH_2O), 3.8, 3.7 (d, OCH), 3.6 (m, CCH), 3.5-3.3 (s, NCH_2CO), 3.3-3.2 (m, CHC), 3.1 (t, NCH_2CH_2N); IR (KBr, cm^{-1}): 3421 (OH), 2924 (C-H), 1750, 1645 (COO), 1322 (C-N), 1010 (C-O).

The dextran ligands containing differing amounts of DTPA linked to polymeric repeat units were synthesized by the same method with differing feed mole ratios of SuO-DTPA to the repeat units of dextran as 1:2 and 2:1 to give the following results: Dextran-DTPA (1:2) (L_2, 39.2%) and Dextran-DTPA (2:1) (L_3, 60.5%), respectively.

Dextran-DTPA (L_2): ^{1}H NMR (D_2O, δppm): 4.9 (d, OCH_2O), 3.8, 3.7 (d, OCH), 3.6 (m, CCH), 3.5-3.3 (s, NCH_2CO), 3.3-3.2 (m, CHC), 3.1 (t, NCH_2CH_2N); IR (KBr, cm^{-1}): 3425 (OH), 2926 (C-H), 1749, 1648 (COO), 1325.8(C-N), 1010 (C-O).

Dextran-DTPA (L_3): ^{1}H NMR (D_2O, δppm): 4.9 (d, OCH_2O), 3.8, 3.7 (d, OCH), 3.6 (m, CCH), 3.5-3.3 (s, NCH_2CO), 3.3-3.2 (m, CHC), 3.1 (t, NCH_2CH_2N); IR (KBr, cm^{-1}): 3432 (OH), 2925 (C-H), 1752, 1646 (COO), 1261.8(C-N), 1010 (C-O).

Preparation of Dextran Gadolinium Chelates

The dextran gadolinium complexes with differing amounts of Gd-DTPA linked to polymeric repeat units were synthesized according to the following standard procedure. Dextran-DTPA (1:1) (L_1, 1.5 g, 2.26 mmol) was dissolved in 20 mL of distilled water, and gadolinium chloride ($GdCl_3$, 0.89 g, 3.39 mmol) was added. The mixture was stirred for 1 h, adjusted with 2 M NaOH solution to pH 5, and continually to stirred for 12 h at room temperature. After dialysis, the dialyzed solution was evaporated, and the solid residue was dried under vacuum to yield dextran gadolinium complex Dextran-DTPA-Gd (M_1, 1.61 g, 67.52%). Dextran-DTPA-Gd (M_2, 65%) and Dextran-DTPA-Gd (M_3, 74%) were similarly synthesized.

Dextran-DTPA-Gd (M_1): IR(KBr, cm^{-1}): 3421 (OH), 2924 (C-H), 1640, 1450 (COO), 1322 (C-N), 1010 (C-O). The average mole ratio of attached Gd-DTPA to all polymeric repeat units (mol%): Gd-DTPA 4.78 (as determined by an ICP Atomscan-2000 spectrometer). The average particle size of Dextran-DTPA-Gd (M_1) in water solution (0.02 g/mL): 235 nm.

Dextran-DTPA-Gd (M_2): IR(KBr, cm^{-1}): 3425 (OH), 2926 (C-H), 1635,1448 (COO), 1261.8(C-N), 1010 (C-O). The average mole ratio of attached Gd-DTPA to all polymeric repeat units (mol%): Gd-DTPA 8.01 (as determined by an ICP Atomscan-2000 spectrometer). The average particle size of Dextran-DTPA-Gd (M_2) in water solution (0.02 g/mL): 212.5 nm.

Dextran-DTPA-Gd (M_3): IR(KBr, cm^{-1}): 3432 (OH), 2925 (C-H), 1632,1446 (COO), 1261.8(C-N), 1010 (C-O). The average mole ratio of attached Gd-DTPA to all polymeric repeat units (mol%): Gd-DTPA 3.89 (as determined by an ICP Atomscan-2000 spectrometer). The average particle size of Dextran-DTPA-Gd (M_3) in water solution (0.02 g/mL): 241.4 nm.

Relaxivity

In the absence of solute-solute interactions, the solvent relaxation rates are linearly dependent on the concentration of the paramagnetic species ([M]); relaxivity, r_1, is defined as the slope of this dependence (2):

$$(1/T_1)_{obsd} = (1/T_1)_d + r_1[M]$$

where $(1/T_1)_{obsd}$ is the observed solvent relaxation rate in the presence of a paramagnetic species, $(1/T_1)_d$ is the solvent relaxation rate in the absence of a paramagnatic species. In this experiment, the concentrations of the paramagnetic species [Gd^{3+}] were measured by an ICP Atomscan-2000 spectrometer. The solvent longitudinal relaxation time (T_1) for gadolinium complexes was carried out on a 10^{-3} M solution of gadolinium complexes in distilled water. Thus, r_1 for gadolinium complexes in distilled water could be calculated.

In Vitro Cytotoxicity Assay

HeLa cells (2×10^5/mL) were plated in 96-well plates in the growth medium (the RPMI-1640 media: 10% fetal bovine serum (Gibco.Co., USA), 100 units/mL penicillium, 100 μg/mL streptomycin), and the number of cells in each well was 2×10^4. The cells were incubated for 24 h in

incubator (37°C, 5% CO_2), and the growth medium was then removed and replaced with 100 μL of the growth medium containing the gadolinium complex Dextran-DTPA-Gd (M_1) or macromolecular ligand Dextran-DTPA (L_1). After a 48-h incubation, 20 μL of a 5.0 mg/mL MTT (thiazolyl blue (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) solution in PBS (phosphate buffered saline) were added to each well. The cells were incubated for 3 h again, and 100 μL of DMSO was then added and shaken for 30 min at room temperature, after which the growth medium was removed. The optical densities (OD_{570}) were measured at 570 nm with a DG-3022A ELISA-Reader and expressed as a percentage relative to control (no macromolecular ligand or its gadolinium complex) cells.

MR Imaging

MR imaging of lymph nodes was carried out on a 3.0 Tesla Magnetom Tiro Tim MR Scanner (Siemens, Germany). The six normal New Zealand white rabbits (weight: 2.0–2.5 kg), six rabbits with VX2 carcinoma in the politeal lymph nodes in both lower limbs and six rabbits with the reactive hyperplasia of the politeal lymph nodes (diameter: 1 cm) in both lower limbs (weight: 2.0–2.5 kg) were anaesthetized with Ketamine (30 mg/kg) injected by intramuscular injection and then 4-hydroxy-butyric acid (100 mg/kg) injected via the auricular vein, positioned lateral and fixed to a polystyrene cradle with adhesive tape to minimize respiratory motion. After performing non-enhanced MR imaging, a solution of Dextran-DTPA-Gd (M_1, 0.2 mL, 0.02 molGd/L, 0.004 mmolGd/kg) or Gd-DTPA (0.2 mL, 0.4 molGd/L, 0.08 mmolGd/kg) in 0.9% sodium chloride was injected into interstitial space of popliteal fossa. Coronal images of the politeal lymph nodes were obtained with a T_1-weighted spin-echo sequence [Repetition time (TR) 539 msec, echo time (TE) 14 msec, the field of view is 40 mm, with an image matrix of 256× 256 and FOV of 180 mm. Six slices were taken and slice thickness was 3 mm, with an 0.1 mm interslice gap.].

Table 1 Relaxivity of Gadolinium Complex in Water Solution

Gadolinium complex	Graft ratio of Gd-DTPA unit in gadolinium complex (molar %)	$[Gd^{3+}]$ ($mmol \cdot L^{-1}$)	$T_{1\ obsd}$(s)	R_1 ($mmol^{-1} \cdot L \cdot s^{-1}$)
Gd-DTPA		0.8364	0.1764	5.67
Dextran-DTPA-Gd				
M_1 (1:1)	4.78	0.8021	0.03173	38.06
M_2 (1:2)	8.01	0.8025	0.03353	35.94
M_3 (2:1)	3.89	0.8132	0.03556	33.37

Temp: 18°C NMR Frequency: 300 MHZ T_{1d}=1.014 s

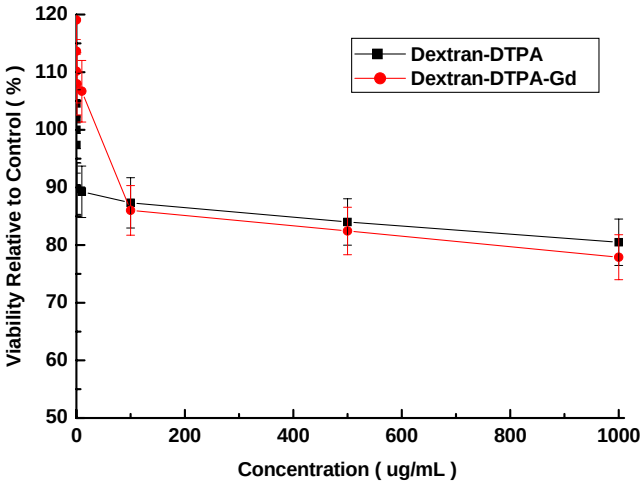


Fig. 1 *In vitro* cytotoxicity assay of macromolecular ligand Dextran-DTPA (L_1) and its gadolinium complex Dextran-DTPA-Gd (M_1) to HeLa cells.

Six T_1-weighted image slices were taken for the politeal lymph nodes of each rabbit. The top and bottom slices were not included in the data analysis to prevent confounding partial volume effects at the edges of the politeal lymph nodes. The average relative signal intensities (pre-injection and post-injection at different times) within the region of interest were normalized to the water label background ($RI_{background}$) at each of the imaging conditions.

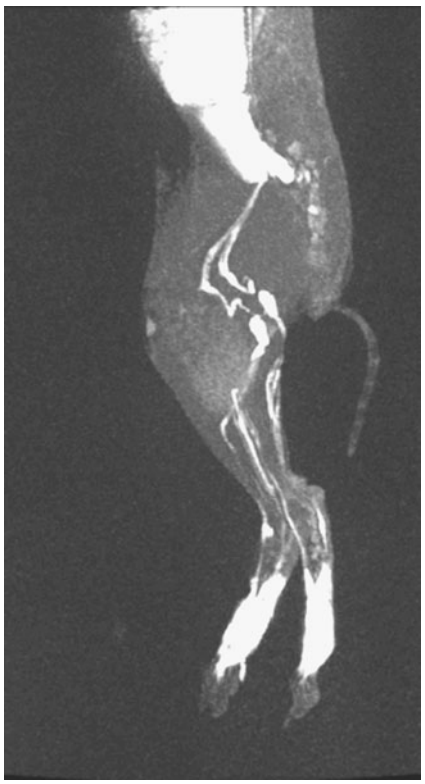


Fig. 2 *In vivo* MR images of lymphatic system in New Zealand white rabbits.

The percentage of contrast enhancement in the signal from the politeal lymph nodes was calculated according to the following:

$$\text{Enhancement}(\%) = 100\%\left(\text{RI}_{\text{post}} - \text{RI}_{\text{pre}}\right)/\left(\text{RI}_{\text{pre}} - \text{RI}_{\text{background}}\right)$$

Statistical Analysis

All results were expressed as mean differences and were tested for significance by a t test, $P<0.05$ being considered a significant difference.

RESULTS AND DISCUSSION

Synthesis and Characterization

A series of dextran ligands Dextran-DTPA and their corresponding gadolinium complexes Dextran-DTPA-Gd containing differing amounts of Gd-DTPA linked to polymeric repeat units were synthesized. The ^{1}H NMR spectra showed the characteristic peaks of NCH_2COOH groups, indicating that DTPA was covalently bound to dextran. The IR spectra of the free dextran ligands Dextran-DTPA showed characteristic absorption peaks of carboxyl with 1755-1645 cm^{-1}, while these peaks disappeared and strong absorption peaks with 1640-1446 cm^{-1} were present in IR spectra of their gadolinium complexes. Thus, the results exhibited the formation of gadolinium complexes.

Moreover, the average mole ratio of attached DTPA to polymeric repeat units increased, and the amount of gadolinium ion in the dextran complexes became larger when the feed mole ratio of DTPA active ester/(polymeric repeat units) in the reaction process increased. The average particle sizes of Dextran-DTPA-Gd (M_1, M_2 and M_3) in water solution (0.02 g/mL) were 235 nm, 212.5 nm, and 241.4 nm, respectively, indicating that the average particle sizes of Dextran-DTPA-Gd in water solution increased when the average mole ratio of attached Gd-DTPA to polymeric repeat units increased.

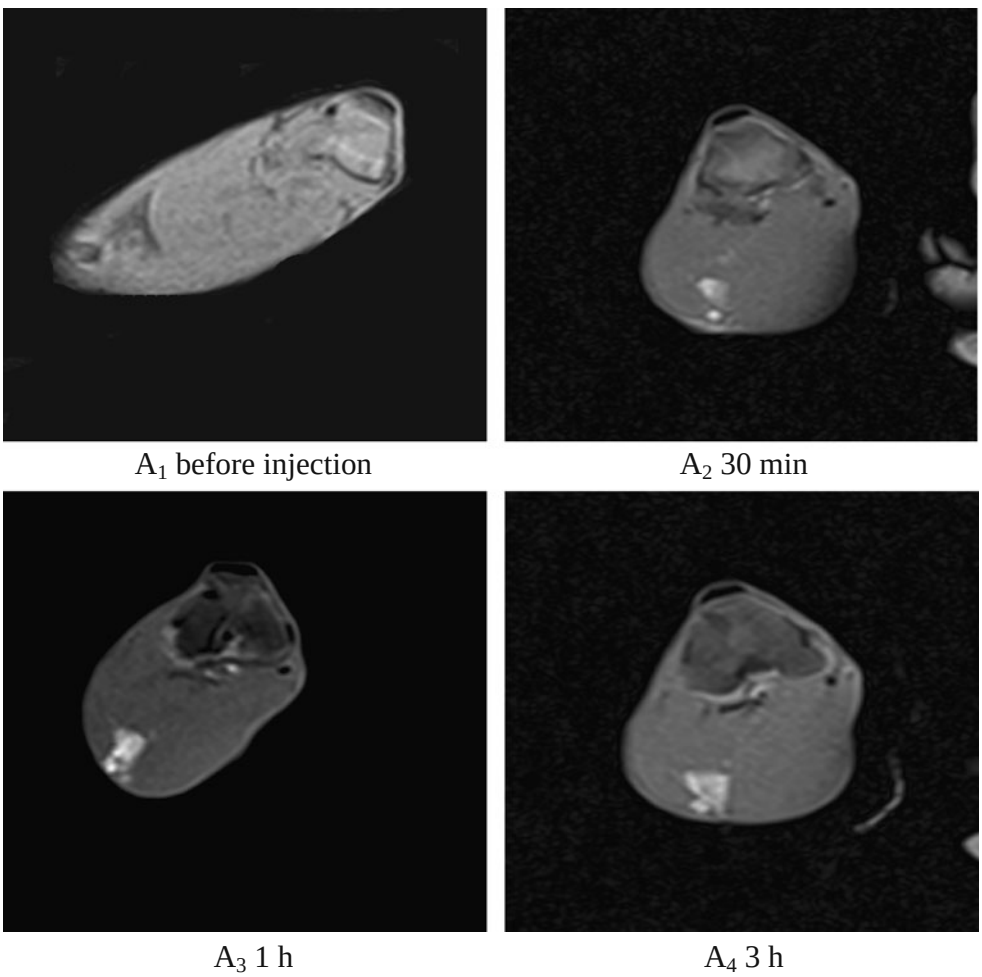


Fig. 3 *In vivo* MR images of normal politeal lymph nodes in the lower limb of New Zealand white rabbits. A_1 is the T_1-weighted image of normal politeal lymph nodes in the lower limb of New Zealand white rabbits receiving no MRI contrast agent; A_2, A_2 and A_3 are the T_1-weighted images of normal politeal lymph nodes in the lower limb of New Zealand white rabbits which received injection with Dextran-DTPA-Gd (M_1, 0.004 mmolGd/kg) after 30 min, 1 h and 3 h, respectively.

Relaxivity of Gadolinium Complex

Dextran MRI contrast agents can exhibit more effective relaxation rates than that of the low molecular weight metal complexes alone and improve the relaxivity per gadolinium atom due to a slowly tumbling systems and an increase in rotational correlation time. Table I illustrates that all of the dextran gadolinium complexes possessed obviously higher relaxation effectiveness than that of the corresponding small molecular complex Gd-DTPA. The relaxivities of dextran gadolinium chelates accordingly enhanced markedly when the average percentage of Gd-DTPA linked to polymeric repeat units increased. However, the relaxivity would reduce if the average percentage of Gd-DTPA linked to polymeric repeat units increased beyond a critical level. The relaxivities of Dextran-DTPA-Gd (M_1) were higher than that of corresponding Dextran-DTPA-Gd (M_2) and Dextran-DTPA-Gd (M_3), respectively.

In Vitro Cytotoxicity Assay

The effects of the gadolinium complex and macromolecular ligand to HeLa cell growth and metabolism were shown in Fig. 1. At the concentration (1,000 μg/mL) of Dextran-DTPA (1:1) or Dextran-DTPA-Gd (M_1, 1:1) in the growth medium, the viabilitiy of HeLa cells incubated with Dextran-DTPA (1:1) or Dextran-DTPA-Gd (M_1, 1:1) retained 80.49% and 77.91%, respectively, relative to control. It illustrated that Dextran-DTPA or Dextran-DTPA-Gd possessed lower cytotoxicity to HeLa cells than that of Gd-DTPA. Meanwhile, Dextran-DTPA had lower cytotoxicity to HeLa cells than that of Dextran-DTPA-Gd.

MR Imaging

Dextran-DTPA-Gd (M_1) was selected as the sample model for *in vivo* MRI experiment in New Zealand white rabbits because of its good experimental data. The average

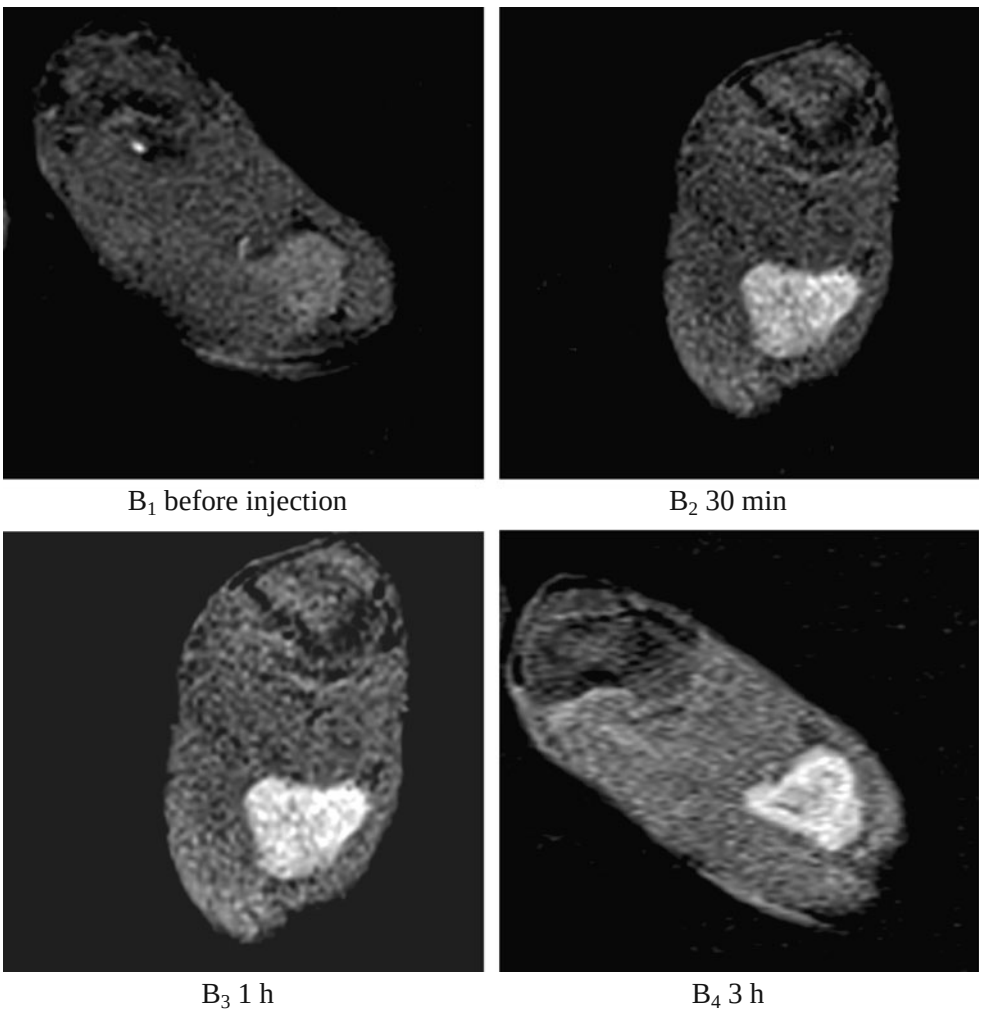


Fig. 4 *In vivo* MR images of the reactive hyperplasia of politeal lymph nodes in the lower limb of New Zealand white rabbits. B_1 is the T_1-weighted image of the reactive hyperplasia of politeal lymph nodes in the lower limb of New Zealand white rabbits receiving no MRI contrast agent; B_2, B_2 and B_3 are the T_1-weighted images of the reactive hyperplasia of politeal lymph nodes in the lower limb of New Zealand white rabbits which received injection with Dextran-DTPA-Gd (M_1, 0.004 mmolGd/kg) after 30 min, 1 h and 3 h, respectively.

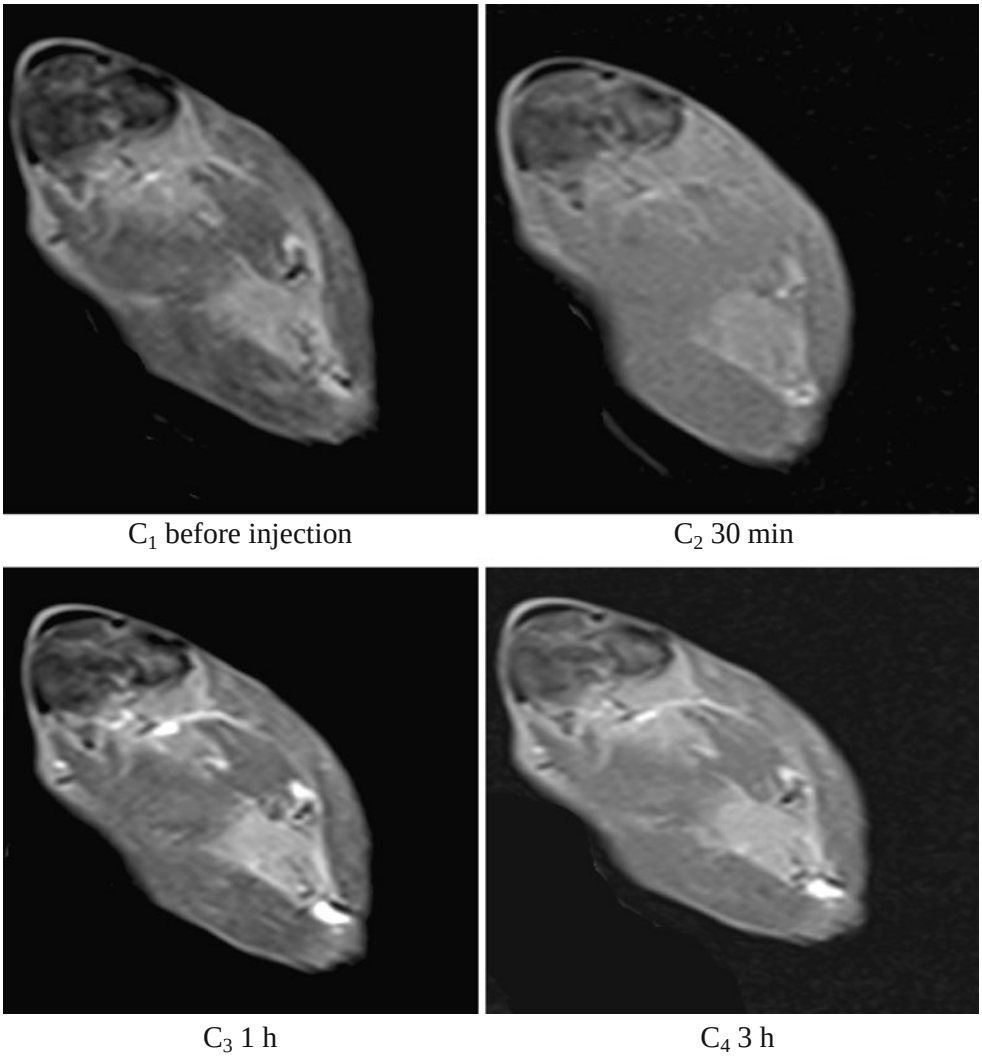


Fig. 5 *In vivo* MR images of politeal lymph nodes with VX2 carcinoma in the lower limb of New Zealand white rabbits. C_1 is the T_1-weighted image of politeal lymph nodes with VX2 carcinoma in the lower limb of New Zealand white rabbits receiving no MRI contrast agent; C_2, C_2 and C_3 are the T_1-weighted images of politeal lymph nodes with VX2 carcinoma in the lower limb of New Zealand white rabbits which received injection with Dextran-DTPA-Gd (M_1, 0.004 mmolGd/kg) after 30 min, 1 h and 3 h, respectively.

particle sizes of Dextran-DTPA-Gd (M_1) in water solution (0.02 g/mL) were 235 nm and then larger than 212.5 nm of Dextran-DTPA-Gd (M_2) and less than 241.4 nm of Dextran-DTPA-Gd (M_3). The relaxivities of Dextran-DTPA-Gd (M_1, R_1, 38.06 $mmol^{-1}\cdot L\cdot s^{-1}$) were higher than that of corresponding Dextran-DTPA-Gd (M_2, R_1, 35.94 $mmol^{-1}\cdot L\cdot s^{-1}$) and Dextran-DTPA-Gd ($M_3$, R_1, 33.37 $mmol^{-1}\cdot L\cdot s^{-1}$), respectively. Moreover, *in vitro* cytotoxicity assay indicated Dextran-DTPA-Gd (M_1) and their corresponding ligands Dextran-DTPA (1:1) possessed lower cytotoxicities to HeLa cells than that of Gd-DTPA. Thus, Dextran-DTPA-Gd (M_1) was expected to potentially obtain greatly enhanced contrast of MR images of the lymphatic system in white rabbits.

The T_1-weight MR images of lymphatic system in New Zealand white rabbit *in vivo* are shown in Fig. 2. Compared to those rabbits receiving no injection of MRI contrast agent, the signal intensities (SI) of lymphatic system in rabbits injected with Dextran-DTPA-Gd (M_1, 0.004 mmolGd/kg) were obviously enhanced; the irradiated portions of lym-

Table II Signal Intensities (SI) of Politeal Lymph Nodes in Rabbits at Different Times after Injection with Dextran-DTPA-Gd (M_1, 0.004 mmolGd/kg) *In Vivo*

Different politeal lymph nodes in the lower limb of New Zealand white rabbits	Before injection	30min	1h	3h
Normal politeal lymph nodes (A)	1.21 ± 0.21	1.83 ± 0.31	2.43 ± 0.32	1.96 ± 0.28
Reactive hyperplasia of politeal lymph nodes (B)	1.12 ± 0.18	1.65 ± 0.21	2.17 ± 0.24	1.77 ± 0.27
Politeal lymph nodes with VX2 carcinoma (C)	1.25 ± 0.26	1.37 ± 0.22	1.48 ± 0.16	1.23 ± 0.35

Table III MRI Signal Enhancement (%) of Politeal Lymph Nodes in Rabbits at Different Times after Injection with Dextran-DTPA-Gd (M_1, 0.004 mmolGd/kg) *In Vivo*

Different politeal lymph nodes in the lower limb of New Zealand white rabbits	Before injection	30min	1h	3h
Normal politeal lymph nodes (A)	0	52	100	63
Reactive hyperplasia of politeal lymph nodes (B)	0	50	98	61
Politeal lymph nodes with VX2 carcinoma (C)	0	15	23	2

phatic system, such as lymphatic vessel and lymph nodes, were brighter, and their demarcations became clearer.

The T_1-weight MR images of normal politeal lymph nodes, reactive hyperplasia of the politeal lymph nodes and the politeal lymph nodes with VX2 carcinoma in the lower limb of New Zealand white rabbits before or after receiving injection with Dextran-DTPA-Gd (M_1, 0.004 mmolGd/kg) at various points in time *in vivo* are shown in Figs. 3, 4 and 5, respectively. Compared to those rabbits receiving no injection of MRI contrast agent, all signal intensities of normal politeal lymph nodes, reactive hyperplasia of politeal lymph nodes and the politeal lymph nodes with VX2 carcinoma in rabbits injected with Dextran-DTPA-Gd (M_1, 0.004 mmolGd/kg) were obviously enhanced; the irradiated portions of politeal lymph nodes were brighter, and their demarcations became clearer during the detection time, while those of the surrounding tissues, such as the muscle, bone and blood vascular system in the lower limb, showed little change (Table II).

Three hours after injection with Dextran-DTPA-Gd (M_1, 0.004 mmolGd/kg), the MRI signal enhancements in normal politeal lymph nodes and reactive hyperplasia of politeal lymph nodes in rabbits were still enhanced by 63% and 61%, respectively, indicating it had a prolonged duration time in normal politeal lymph nodes and reactive hyperplasia of politeal lymph nodes of approximately three hours (Table III). It displayed that Dextran-DTPA-Gd could accumulate specially and remain in normal politeal lymph nodes and reactive hyperplasia of politeal lymph nodes significantly longer.

On the other hand, compared to those rabbits receiving injection of Gd-DTPA (0.08 mmolGd/kg), the MRI signal enhancements of normal politeal lymph nodes in rabbits injected with Dextran-DTPA-Gd (M_1, 0.004 mmolGd/kg) were markedly better and lasted longer than that of Gd-DTPA (maximum value 42%) (Fig. 6). These results indicated that Dextran-DTPA-Gd can greatly enhance the contrast of conventional MR images and provide prolonged enhancement of normal politeal lymph nodes and reactive hyperplasia of politeal lymph nodes with lower detectable concentration than that for Gd-DTPA. However, the far fewer signal enhancements had been shown in MR images of politeal lymph nodes with VX2 carcinoma in rabbits injected with Dextran-DTPA-Gd (M_1, 0.004 mmolGd/kg) during the detection time. Moreover, Dextran-DTPA-Gd can distinguish normal politeal lymph nodes, reactive hyperplasia of politeal lymph nodes, and the politeal lymph nodes with VX2 carcinoma in rabbits according to MR image signal enhancements.

CONCLUSIONS

Gd-DTPA was incorporated to the hydroxyl groups of dextran to obtain the macromolecular ligands. These corresponding dextran gadolinium complexes possessed obviously higher relaxation effectiveness, lower cytotoxicity to HeLa cells, significantly higher enhanced signal intensities (SI) of politeal lymph nodes in rabbits with lower injection doses than that of Gd-DTPA. Moreover, Dextran-DTPA-Gd greatly enhanced the contrast of MR images of normal politeal lymph nodes and reactive hyperplasia of politeal lymph nodes, provided prolonged intravascular duration and produced highly contrasted visualization of lymphatic system. Thus, Dextran-DTPA-Gd can be taken up selectively by lymphatic system and show potential as MRI contrast agents in lymphatic system.

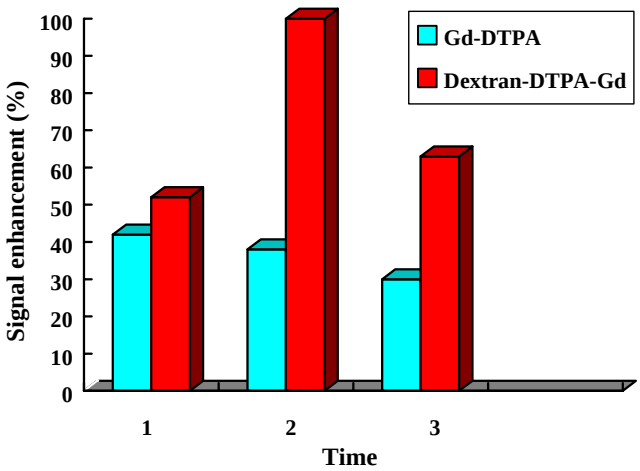


Fig. 6 *In vivo* MRI signal enhancement (%) of normal politeal lymph nodes in the lower limb of New Zealand white rabbits. Gd-DTPA represented the T_1-weighted MRI signal enhancement (%) of normal politeal lymph nodes in the lower limb of New Zealand white rabbits which received injection with Gd-DTPA (0.08 mmolGd/kg) after 1 (5 min), 2 (10 min) and 3 (20 min), respectively. Dextran-DTPA-Gd represented the T_1-weighted MRI signal enhancement (%) of normal politeal lymph nodes in the lower limb of New Zealand white rabbits which received injection with Dextran-DTPA-Gd (M_1, 0.004 mmolGd/kg) after 1 (30 min), 2 (1 h) and 3 (3 h), respectively.

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