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Research Paper

Pharmacokinetics, Biodistribution and Contrast Enhanced MR Blood Pool Imaging of Gd-DTPA Cystine Copolymers and Gd-DTPA Cystine Diethyl Ester Copolymers in a Rat Model

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Purpose. To investigate plasma pharmacokinetics and biodistribution of biodegradable polydisulfide Gd(III) complexes, Gd-DTPA cystine copolymers (GDCP) and Gd-DTPA cystine diethyl ester copolymers (GDCEP) and their efficacy as blood pool MRI contrast agents in comparison with a nondegradable macromolecular agent, Gd-DTPA 1,6-hexanediamine copolymers (GDHC).
Methods. The pharmacokinetics and biodistribution of GDCP and GDCEP with molecular weight of 35 KDa were investigated in Sprague-Dawley rats after intravenous administration at a dose of 0.1 mmol Gd/kg. GDHC with the same molecular weight was used as a control. The Gd content in the plasma and various tissues and organs were determined by the ICP-OES. Plasma pharmacokinetic parameters were calculated by using a two-compartment model. The contrast enhanced blood pool MR imaging of the agents was evaluated in Sprague-Dawley rats on a Siemens Trio 3T MR scanner.
Results. The biodegradable macromolecular agents, GDCP and GDCEP, had faster blood pool clearance than the nondegradable GDHC. The long-term Gd(III) tissue retention of the biodegradable polydisulfide agents was substantially lower than the nondegradable macromolecular agent. Both GDCP and GDCEP resulted in significant blood pool enhancement for the first 2 min post-injection and more rapid disappearance of the enhancement over time than GDHC. The negatively charged GDCP had prolonged enhancement duration as compared to GDCEP. The structure and biodegradability of the macromolecular contrast agents significantly affected their pharmacokinetics and blood pool contrast enhancement.
Conclusion. Both GDCP and GDCEP provided effective contrast enhancement for MR imaging of the blood pool. The accumulation of toxic Gd(III) ions in the body was greatly reduced with GDCP and GDCEP as compared to the nondegradable control.

KEY WORDS: biodegradable; Gd tissue accumulation; MRI contrast agent; pharmacokinetics; polydisulfide Gd(III) complexes.

INTRODUCTION

The clinically approved gadolinium based magnetic resonance imaging (MRI) contrast agents are small molecular weight chelates and have a short blood and tissue retention (1). They are not effective in contrast enhanced MR imaging of the vasculature and cancer (2,3). Macromolecular Gd(III) complexes have a prolonged blood circulation time and are effective contrast agents in cardiovascular and cancer MR imaging. As shown in the preclinical studies, these agents can provide a pronounced contrast enhancement window with excellent spatial resolution for cardiac and vascular examinations with MRI angiography (4–8). Macromolecular contrast agents can also preferentially accumulate in tumor tissue because of the hyperpermeability of neoplastic blood vessels, resulting in effective tumor enhancement for accurate cancer detection (3,9–12). The long blood circulation of macromolecular agents can also allow the characterization of physiology of individual tumors with dynamic contrast enhanced MRI, which is shown to be efficacious for tumor differentiation and non-invasive evaluation of tumor response to anti-cancer treatment (13,14). Polymeric Gd(III) chelate conjugate can also accumulate in rat arthritic joints resulting in contrast enhanced MR imaging of rheumatoid arthritis (15). Although macromolecular Gd(III) complexes have demonstrated superior contrast enhancement in animal models for MR angiography and cancer imaging, they cannot proceed into further clinical development because of their slow excretion, hence in-

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creased risks of cellular uptake and metabolism, resulting in long-term tissue accumulation of toxic Gd(III) ions. So far, only macromolecular Gd(III) complexes with small sizes and rapid excretion, such as Gadomer-17 (17 KDa) (16) and P792 (6,470 Da) (17), have proceeded into clinical development. However, it has been reported that macromolecular Gd(III) complexes with higher molecular weights are more effective for blood pool imaging (18).

We have recently developed a novel class of polydisulfide-based macromolecular Gd(III) complexes as biodegradable MRI contrast agents for blood pool and cancer imaging (3,19–22). The polydisulfide Gd(III) complexes result in superior contrast enhancement as compared to the clinically available low molecular weight contrast agents in the blood pool and cancer imaging (2,3), and readily degrade into small Gd(III) complexes *in vivo* by the endogenous thiols, including glutathione (reduced form), cysteine and homocysteine, via disulfide–thiol exchange reaction (3,20). These agents initially behave as macromolecular agents for blood pool imaging and then degrade and rapidly excrete as low molecular weight Gd(III) complexes. For example, (Gd-DTPA)-cystamine copolymers (GDCC) result in a prolonged blood pool retention time and then rapidly excrete with minimal accumulation of Gd(III) ions comparable to the clinical agent Gd(DTPA-BMA) (2,20). Since the agent is biodegradable, the size of GDCC does not significantly affect its pharmacokinetics and excretion from the body (2). The structure of GDCC has been modified to slow down the degradation rate (19) and to develop biodegradable macromolecular blood pool agents with different blood half-lives to satisfy various clinical demands for contrast enhanced MRI.

We have previously shown that Gd-DTPA cystine copolymers (GDCP) and Gd-DTPA cystine diethyl ester copolymers (GDCEP) had slower degradation rates and resulted in more significant contrast enhancement in tumor than GDCC (3). In this study, the plasma pharmacokinetics, biodistribution and long-term tissue accumulation, and efficacy for MR blood pool imaging of GDCP and GDCEP were investigated in Sprague-Dawley rats in comparison with a nondegradable macromolecular agent, Gd-DTPA 1,6-hexanediamine copolymers (GDHC), to evaluate their potential as effective MR blood pool contrast agents for cardiovascular imaging. The structure and degradability of the macromolecular contrast agents on pharmacokinetics, biodistribution and blood pool imaging were also investigated.

MATERIALS AND METHODS

Preparation of Contrast Agents

GDCP and GDCEP were prepared as previously described (3). GDHC was similarly synthesized by copolymerization of DTPA dianhydride with 1,6-hexanediamine, followed by the complexation with $Gd(OAc)_3$. Briefly, DTPA dianhydride with 1,6-hexanediamine at a molar ratio of 1:1 were stirred in triethylamine and DMSO for 48 h at room temperature. The polymeric ligand, DTPA 1,6-hexanediamine copolymers, was precipitated from the reaction mixture by adding acetone and dialyzed against de-ionized water. The ligand was then reacted with a slight excess of $Gd(OAc)_3$ in aqueous solution at pH 5.5. The excess of $Gd(OAc)_3$ was removed by dialysis and size exclusion chromatography (SEC) with Sephadex G-25 medium (Pharmacia).

All three macromolecular agents were fractionated by SEC using a Sephacryl S-300 column on a Pharmacia FPLC system (Gaithersburg, MD) to prepare macromolecular agents with narrow molecular distributions. The average molecular weights of the fractions were determined by SEC with poly[*N*-(2-hydroxypropyl)methacrylamide] as standard on an AKTA FPLC system (Amersham Biosciences Corp., Piscataway, NJ). The Gd(III) content in the agents was determined by inductively coupled plasma optical emission spectroscopy (ICP-OES, Perkin Elmer Optima 3100XL).

Pharmacokinetic Study

Male Sprague-Dawley rats (250–350 g; Charles River Laboratories, Wilmington, MA) were used in the pharmacokinetic study. The animals were cared for under an approved protocol and the guidelines of the University of Utah Institutional Animal Care and Use Committee. A group of six rats were used for each contrast agent. The rats were anesthetized with an intraperitoneal injection of a mixture of ketamine (Bedford, OH, 90 mg/kg) and xylazine (St. Joseph, MO, 10 mg/kg). A heparinized catheter was inserted into the jugular vein for administration of the contrast agent and blood sampling. The contrast agent was injected at a typical clinical dose of 0.1 mmol Gd/kg. Blood samples (250 μl) were collected from the catheter before injection and at 2, 4, 10, 20, 30, 60, 120, 180, 300, and 420 min after injection for a total of ten time points. The sampling catheter was flushed with heparinized saline into the vein after the injection of contrast agents or blood sampling to avoid the contamination and to compensate for the lost body fluid. The blood samples were centrifuged at 10,000 rpm at 4°C for 10 min to obtain plasma. The plasma was diluted with sterile water (Baxter) and the Gd content was determined by ICP-OES. A two-compartment pharmacokinetic model was used to analyze the data and calculate the pharmacokinetic parameters with WinNonLin software (Pharsight Corporation).

Biodistribution of Macromolecular Agents

Biodistribution or the long-term tissue accumulation of macromolecular Gd(III) complexes is a critical parameter to determine their safety properties. The time for determining long-term tissue accumulation varies from 7 to 14 days in the literatures. The median value (10 days) was chosen for the biodistribution study in our previous study (2) and this study. A group of six male Sprague-Dawley rats were used in the study of biodistribution or long-term Gd(III) retention in major organs and tissues for each agent. The rats were injected at a dose of 0.1 mmol Gd/kg via a tail vain. The rats were sacrificed with CO_2 10 days post-injection and the organ and tissue samples (femur including bone and marrow, heart, lung, liver, muscle, spleen and kidney) were collected and weighed. Ultra-pure nitric acid (1.00 ml, 70%, EMD, Gibbstown, NJ) was added to each sample and the tissue samples were liquefied within 2 days. The solutions were centrifuged at 13,000 rpm for 12 min and the supernatant was diluted ten times with ultra-pure water to determine Gd(III) content with ICP-OES. Percentage of injected dose (ID) per organ/

tissue was calculated to express biodistribution of the agents in the organs and tissues (2).

Contrast Enhanced MR Blood Pool Imaging in Rats

A group of three male Sprague-Dawley rats weighing approximately 150 grams were used in contrast enhanced MR blood pool imaging for each agent. The rats were anesthetized by the intraperitoneal administration of a mixture of ketamine (90 mg/kg) and xylazine (10 mg/kg). The contrast agents were injected via a tail vein at a dose of 0.1 mmol Gd/kg. MR images were acquired before and at 2, 5, 10, 15 and 30 min post-injection of the contrast agents on a clinical 3T MRI system (Trio, Siemens Medical Solutions, Erlangen, Germany). The body RF coil was used for RF excitation and a human wrist coil was used for MR signal reception. A 3D FLASH (FL3D) pulse sequence with 1.64 ms echo time (TE), 4.3 ms repetition time (TR), 19° RF flip angle, 0.5 mm coronal slice thickness, 80 slices was used for image acquisition. Three dimensional maximum intensity projection (MIP) images were reconstructed using the IDL (Interactive Data Language, Boulder, CO) program. MR images were analyzed using Osirix (http://homepage.mac.com/rossetantoine/osirix/). Signal intensity (SI) of regions of interest (the left ventricle of the heart, liver, iliac vein and muscle around the iliac vein) was measured in source images. The ratios of the SI of the heart, liver and small blood vessel to that of the muscle at various time points were calculated for each animal and averaged among the animals administered with same contrast agent.

Statistical Analysis

Statistical analysis was performed using the *t*-test (GraphPad Prism; GraphPad Software, San Diego, CA). *P* values were two-tailed with a confidence interval of 95%.

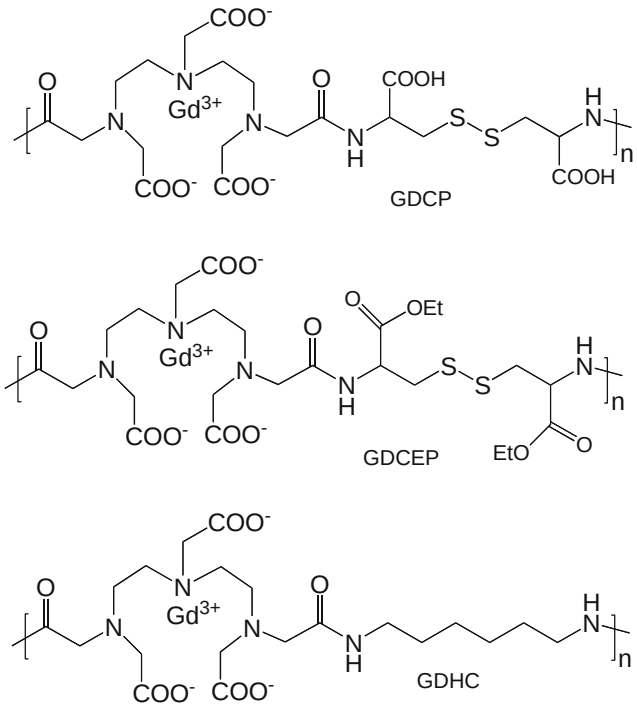

Fig. 1. Chemical structures of GDCP, GDCEP and GDHC.

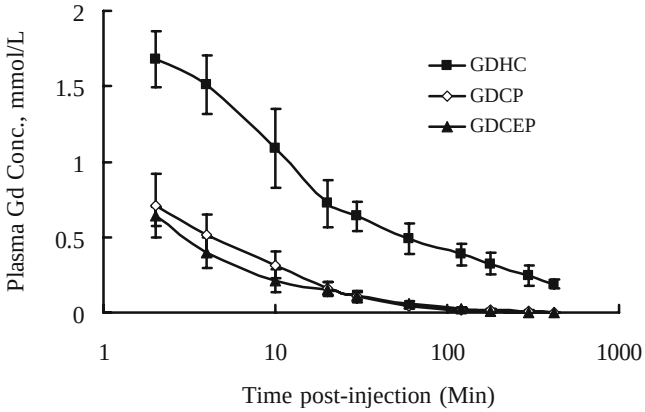

Fig. 2. Blood clearance of Gd(III) complexes in rats after intravenous injection of GDHC, GDCP and GDCEP at a dose of 0.1 mmol Gd/kg. Values are shown as means ± SD (n = 6).

RESULTS

Contrast Agents

The chemical structures of GDCP, GDCEP and GDHC are shown in Fig. 1. GDCP and GDCEP are modified polydisulfide Gd(III) complexes with different degradation rates toward disulfide–thiol exchange reaction (3). GDHC is a nondegradable macromolecular contrast agent and is used as a control. The fractionation of the macromolecular Gd(III) complexes with size exclusion chromatography resulted in macromolecular MRI contrast agents with narrow molecular weight distribution. The number average and weight average molecular weights of the GDCP, GDCEP and GDHC used in the *in vivo* studies including pharmacokinetics, biodistribution and MRI were 25 and 35 KDa, respectively. The macromolecular agents with the same narrow molecular weight distribution allowed accurate comparison of *in vivo* properties of the agents with different structures. The T_1 relaxivity of the contrast agents was 6.79, 5.17 and 6.76 $mM^{-1}s^{-1}$ per complexed Gd(III) ion at 3 Tesla for GDCP, GDCEP and GDHC, respectively.

PHARMACOKINETICS

Figure 2 shows the blood plasma concentration time profiles of GDCP, GDCEP and GDHC in Spague–Dawley rats over a period of 7 h after intravenous bolus injection at a Gd(III) equivalent dose of 0.1 mmol Gd/kg. The average plasma Gd(III) concentration of the nondegradable agent GDHC is much higher than that of the biodegradable agents, GDCP and GDCEP, over the entire period of experiment. The average plasma Gd concentration of GDHC at 2 min post-injection was 1.68 mmol/l, while that of GDCP and

Table I. Pharmacokinetic Parameters of GDHC, GDCP and GDCEP after i.v. Injection at a Dose of 0.1 mmol Gd/kg in Rats

	GDHC	GDCP	GDCEP
$t_{1/2}$, α (min)	5.92 ± 2.02	3.15 ± 1.26	1.60 ± 0.73
$t_{1/2}$, β (min)	222.6 ± 63.9	33.1 ± 19.3	29.3 ± 15.9

Values are shown as means ± SD; n = 6.

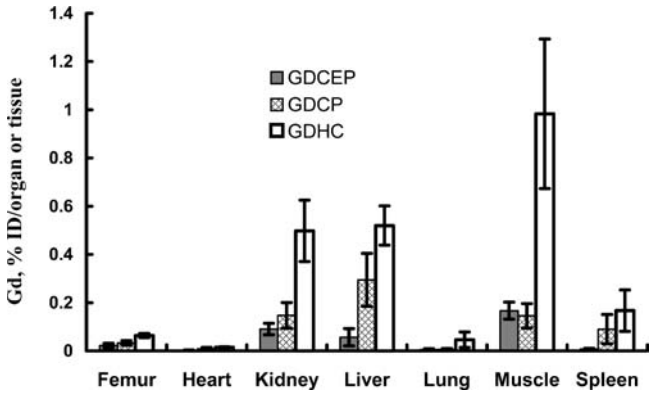

Fig. 3. Biodistribution of Gd(III) in rats 10 days after intravenous injection of GDHC, GDCP and GDCEP at a dose of 0.1 mmol Gd/kg. Values are shown as means ± SD (n = 6).

GDCEP was 0.708 and 0.640 mmol/l, respectively. The blood concentration gradually decreased for all agents and at 7 h post-injection the plasma Gd concentration was 189 μmol/l for GDHC, 2.29 μmol/l for GDCP and 0.890 μmol/l for GDCEP. The average plasma Gd concentration of GDCP was higher than that of GDCEP, but the difference was not significant ($p > 0.05$).

The Gd(III) plasma concentration profiles of the agents were analyzed with a two-compartment pharmacokinetic model. The pharmacokinetic parameters including half-life ($t_{1/2}$) of blood distribution (α) and elimination phases (β) for GDHC, GDCP and GDCEP are listed in Table I. The α phase $t_{1/2}$ of the nondegradable GDHC was longer than those of the biodegradable GDCP ($p < 0.05$) and GDCEP ($p < 0.05$). The α phase $t_{1/2}$ of GDCP was significantly longer than that of GDCEP ($p < 0.05$). The β phase $t_{1/2}$ for GDCP and GDCEP was similar, approximately 30 min ($p = 0.72$), while that of GDHC was about six times longer ($p < 0.001$ for both). The results indicated that the *in vivo* degradation resulted in more rapid clearance of GDCP and GDCEP from the blood than the nondegradable agent GDHC.

Biodistribution

Figure 3 shows the biodistribution of Gd(III) in the femur, heart, kidneys, liver, lung, muscle and spleen of rats 10 days after a single injection of GDHC, or GDCP and GDCEP at a dose of 0.1 mmol Gd/kg. The accumulation of Gd(III) in the organs and tissues for nondegradable GDHC is significantly higher than those for GDCP and GDCEP ($p < 0.05$) except in the spleen for GDCP ($p = 0.19$). The accumulation of GDHC in the kidneys, liver and muscle was much higher than that in other organs or tissues. The negatively charged GDCP had significantly higher accumulation in the kidneys, liver and spleen than GDCEP ($p < 0.05$). Both GDCP and GDCEP had low accumulation in the heart and lung and similar accumulation in muscle ($p = 0.400$) and femur ($p = 0.053$). The biodegradable GDCP and GDCEP exhibited much lower overall long-term Gd(III) body accumulation than the nondegradable GDHC.

Blood Pool MR Imaging in Rats

Figure 4 shows the three-dimensional maximum intensity projection (MIP) images of rats contrast enhanced by

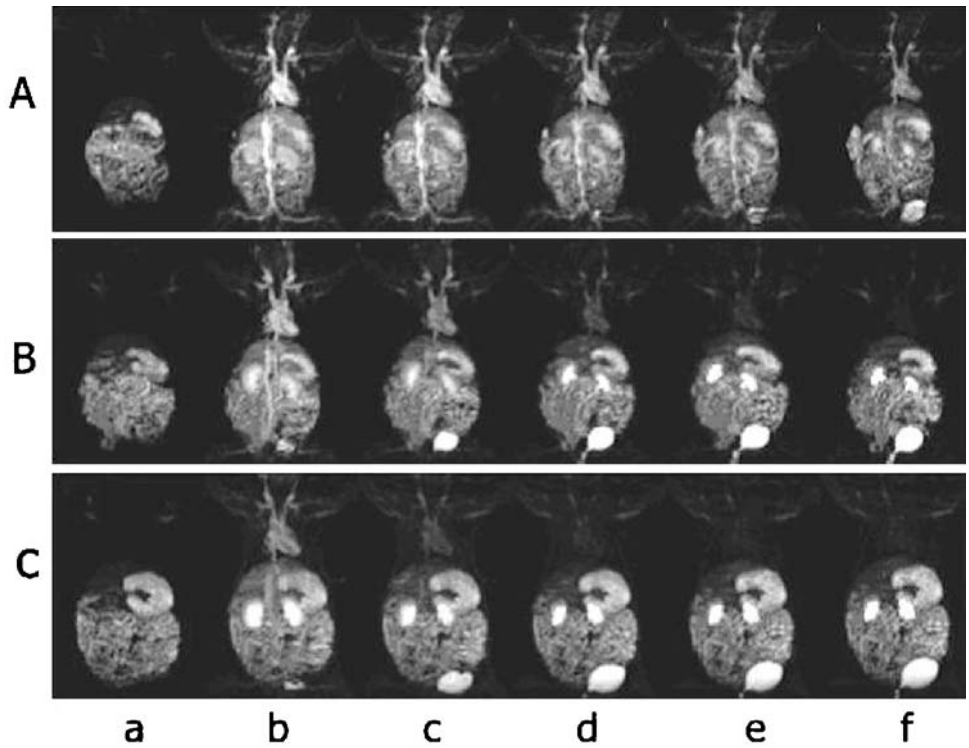

Fig. 4. 3D maximum intensity projection MR images of rats before (a), at 2 min (b), 5 min (c), 10 min (d), 15 min (e) and 30 min (f) after intravenous injection of GDHC (A), GDCP (B) and GDCEP (C) at a dose of 0.1 mmol Gd/kg.

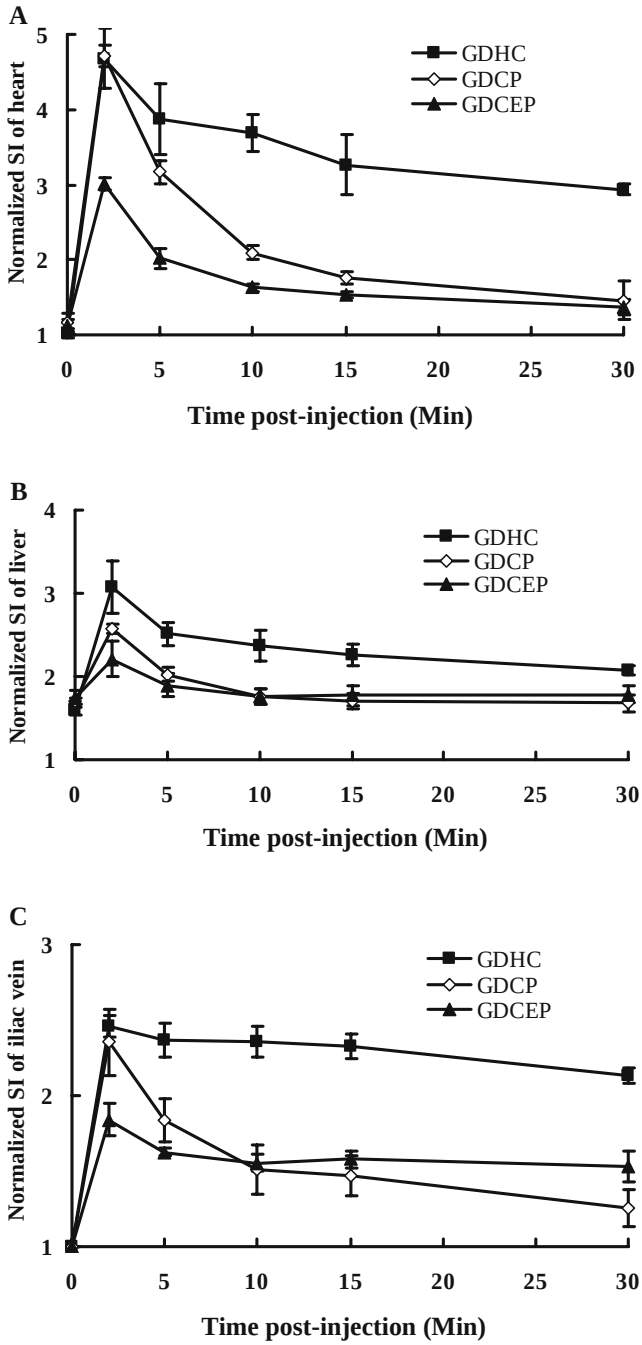

Fig. 5. Normalized SI in the heart (A), liver (B) and iliac vein (C) (SI of heart, liver or iliac vain/SI of muscle) before and at various time points post-injection of GDHC (■), GDCP (◇) and GDCEP (▲) at a dose of 0.1 mmol Gd/kg. Values are shown as means ± SD ($n = 3$).

GDHC, GDCP and GDCEP before and at 2, 5, 10, 15 and 30 min post-injection of the contrast agents. Animals injected with the same agent had similar contrast enhancement in the MR images. Contrast enhancement in the heart and vasculature varied with the agents at the same injected dose. Figure 5 shows the signal intensity (SI) ratio in the heart, liver and iliac vein to the muscle. The nondegradable agent GDHC resulted in stronger and more prolonged contrast enhancement than GDCP and GDCEP in the heart and blood vessels. GDCP and GDCEP resulted in significant blood pool enhancement at 2 min post-injection. The signal gradually faded away due to the degradation and clearance of the agents from the blood (3). The negatively charged GDCP exhibited longer enhancement duration than GDCEP. The contrast enhancement pattern of the agents in the blood pool was consistent to the plasma pharmacokinetic results.

The macromolecular contrast agents also resulted in significant enhancement in the liver and kidneys. The more significant liver enhancement was observed with GDHC than GDCP and GDCEP. The degradable agents resulted in more significant enhancement in the kidneys than GDHC (Fig. 4). The SI in the urinary bladder of the rats injected with GDCP and GDCEP gradually increased over time. This indicates that GDCP and GDCEP degraded in the plasma, resulting in rapid excretion of the Gd(III) complexes via renal filtration and accumulation in the urinary bladder. Consequently, little enhancement was observed in the heart and blood vessels as compared in the kidneys and bladder over time.

DISCUSSION

Slow excretion and long-term tissue accumulation are the main safety concern for clinical development of macromolecular Gd(III) complexes as blood pool MRI contrast agents. Polydisulfide Gd(III) complexes are biodegradable macromolecular MRI contrast agents that can degrade *in vivo*, resulting in rapid excretion of Gd(III) complexes (2,3). These biodegradable macromolecular MRI contrast agents are effective for contrast enhanced blood pool imaging including MR angiography and tumor imaging as shown in preclinical studies. Detailed evaluation of the pharmacokinetic properties and long-term tissue accumulation of the biodegradable polydisulfide Gd(III) complexes is critical for selecting a suitable agent for further development.

Gd-DTPA cystine copolymers (GDCP) and Gd-DTPA cystine diethyl ester copolymers (GDCEP) are biodegradable macromolecular MRI contrast agents with different degradability (3). GDCP is a negatively charged ionic agent and GDCEP is a neutral agent. GDCP had a slower degradation rate via the disulfide–thiol exchange reaction than the neutral agent GDCEP and GDCC (3). The pharmacokinetic study demonstrated that GDCP and GDCEP with the same narrow molecular weight distribution cleared more rapidly from the vasculature and the body than nondegradable GDHC. Both GDCP and GDCEP had a longer α phase plasma half-life than the low molecular weight agent Gd(DTPA-BMA) $\left(t_{1/2,\alpha} = 0.48 \pm 0.16\right)$ in rats (2). The slow degradation of GDCP also resulted in a longer blood half-life than GDCEP. The α phase plasma half-life of GDCEP (35 kDa) was similar to that of GDCC with an apparent molecular weight of 60 kDa (1.74 ± 0.57 min) (2).

The pharmacokinetic study also revealed substantial difference in the initial plasma concentrations between the biodegradable agents and the nondegradable agent GDHC at the same injected dose. This suggests that the *in vivo* degradation of the polydisulfide Gd(III) complexes started immediately post-injection and the smaller Gd(III) complexes formed excreted rapidly through renal filtration, resulting in substantial decrease of the blood concentration of GDCP and GDCEP. This is consistent with results of the

contrast enhanced MR images. The *in vivo* degradation and rapid clearance of GDCP and GDCEP resulted in shorter enhancement duration in the blood pool and stronger enhancement in the kidneys and urinary bladder than that of GDHC (Fig. 4).

In vivo degradation and rapid excretion resulted in substantially low long-term tissue retention for GDCP and GDCEP as compared to the nondegradable GDHC. Minimal accumulation was detected in the bone, heart and lung for GDCP and GDCEP, and both agents had comparable accumulation in the kidneys and muscle. However, much higher accumulation in the liver and spleen was observed for the negatively charged GDCP than GDCEP. This might be attributed to non-specific interaction of the negatively charged GDCP with the liver and spleen. The overall tissue accumulation of GDCEP was similar to that of GDCC and comparable to that of Gd-(DTPA-BMA) in rats (2).

Both GDCP and GDCEP had a prolonged contrast enhancement in the blood pool as compared to the low molecular weight agent Gd-(DTPA-BMA) (2). The negatively charged GDCP with a slow degradation rate had longer enhancement duration and resulted in clearer visualization of small blood vessels than the neutral agent GDCEP. GDCP with a molecular weight of 35 KDa can provide effective contrast enhancement in the heart and vasculature for about 15 min in rats while the effective enhancement window for GDCEP with the same molecular weight is approximately 5 min. The effective enhancement window of both GDCP and GDCEP in human might be longer than in rats because the blood circulation in human is much slower than in rats. The washout from the blood of the degradable contrast agents in human might be slower.

This study demonstrated that the biodegradable polydisulfide agents had much less long-term body accumulation than the nondegradable macromolecular Gd(III) complexes. Currently, we are evaluating the safety including acute and subacute toxicity, and maximum tolerated dose of the polydisulfide Gd(III) complexes.

CONCLUSIONS

The biodegradable macromolecular contrast agents GDCP and GDCEP cleared more rapidly than the nondegradable GDHC from the vasculature. Biodistribution study showed that both GDCEP and GDCP resulted in less Gd(III) retention in major organs and tissues than GDHC. The neutral GDCEP had less Gd(III) accumulation in the liver and spleen than the negatively charged GDCP. Both GDCP and GDCEP resulted in significant and prolonged enhancement in the blood pool. The study demonstrated that the structure and biodegradability had a significant impact on the pharmacokinetics, biodistribution and *in vivo* contrast enhancement of the macromolecular MRI contrast agents. These polydisulfide based biodegradable macromolecular MRI contrast agents have a potential for use in contrast enhanced MR angiography.

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