*Pharmaceutical Research, Vol. 22, No. 4, April 2005 (© 2005)*
10.1007/s11095-005-2489-7

*Research Paper*

# Pharmacokinetics and Tissue Retention of (Gd-DTPA)-Cystamine Copolymers, a Biodegradable Macromolecular Magnetic Resonance Imaging Contrast Agent

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*Received August 3, 2004; accepted December 17, 2004*

***Purpose.*** To investigate the pharmacokinetics, long-term tissue retention of Gd(III) ions, and magnetic resonance imaging (MRI) contrast enhancement of extracellular biodegradable macromolecular Gd(III) complexes, (Gd-DTPA)-cystamine copolymers (GDCC), of different molecular weights.
***Methods.*** The pharmacokinetics of blood clearance and long-term Gd(III) retention of GDCC were investigated in Sprague-Dawley rats. Pharmacokinetic parameters were calculated by using a two-compartment model. The blood pool contrast enhancement of GDCC was evaluated in Sprague-Dawley rats on a Siemens Trio 3T MR scanner. Gd-(DTPA-BMA) was used as a control.
***Results.*** The α phase half-life of Gd-(DTPA-BMA) and GDCC with molecular weights of 18,000 (GDCC-18) and 60,000 Da (GDCC-60) was 0.48 ± 0.16 min, 1.08 ± 0.24 min, and 1.74 ± 0.57 min, and the β phase half-life was 21.2 ± 5.5 min, 26.5 ± 5.9 min, and 53.7 ± 15.9 min, respectively. GDCC had minimal long-term Gd tissue retention comparable to that of Gd-(DTPA-BMA). GDCC resulted in more significant contrast enhancement in the blood pool than Gd-(DTPA-BMA).
***Conclusions.*** GDCC provides a prolonged blood pool retention time for effective MRI contrast enhancement and then clears rapidly with minimal accumulation of Gd (III) ions. It is promising for further development as a blood pool MRI contrast agent.

**KEY WORDS:** biodegradable macromolecular contrast agent; (Gd-DTPA)-cystamine copolymers; Gd tissue accumulation; MRI; pharmacokinetics.

## INTRODUCTION

Magnetic resonance imaging (MRI) is a powerful noninvasive diagnostic imaging modality that can provide high-quality anatomic images and other physiologic data. MR image contrast is mainly generated by the relaxation differences of water protons in adjacent tissues. Gadolinium(III) has a high magnetic moment, which can alter the relaxation rate of surrounding protons to enhance image contrast (1). However, the Gd(III) ion is highly toxic and only stable Gd(III) chelates can be used as contrast agents for MRI. Currently, Gd(III) chelates with diethylenetriaminepentaacetic acid (DTPA), 1,4,7,10-tetraazacyctododecome-1,4,7,10-tetraacetic acid (DOTA), or their derivatives are mainly used in clinical practice. These agents rapidly extravasate from the vasculature and distribute in the surrounding tissues after administration. The pharmacokinetic properties of these low-molecular-weight agents limit their application in many cases including cardiovascular imaging and cancer imaging.

Macromolecules have a prolonged blood pool retention time and can preferentially accumulate in tumor tissue because of the hyperpermeability of neoplastic blood vessels (2,3). Macromolecular Gd(III) complexes have a potential for improved blood pool pharmacokinetics and MR contrast enhancement when compared to low-molecular-weight Gd(III) complexes (4–6). A number of macromolecular Gd(III) complexes have been prepared as blood pool MRI contrast agents (7–13). Macromolecular Gd(III) complexes significantly increase blood pool and tissue retention time of contrast agents, resulting in superior contrast enhancement in animal models. For example, the conjugation of Gd-DTPA to polyamidoamine dendrimers modifies the pharmacokinetics of the contrast agent in an animal model (14). The blood pool retention increases dramatically with increasing size of the dendrimers and the contrast enhancement in the vasculature improves correspondingly (14). Albumin-Gd-DTPA (92 kDa) is able to differentiate benign and malignant tumors based on the hyperpermeability of tumor vasculature (15). However, clinical application of these macromolecular agents is limited by safety concerns due to their slow excretion after the MRI examination (8,16,17). The slow excretion of macromolecular Gd(III) complexes may result in the metabolism of complexes leading to the release and retention of highly toxic Gd(III) ions.

In order to alleviate the potential safety problem associated with macromolecular Gd(III) complexes, we have re-

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cently designed and prepared novel polydisulfide-based biodegradable macromolecular Gd(III) complexes, (Gd-DTPA)-cystamine copolymers (GDCC) (18). The macromolecular agent is designed to be degraded into smaller Gd(III) complexes by cleaving the disulfide bonds in the polymer chains via disulfide-thiol exchange reaction with endogenous thiols (Fig. 1). The preliminary studies have demonstrated that GDCC can provide more significant blood pool contrast enhancement than a low molecular weight control agent and are readily degraded and excreted in animal models.

In this study, the blood pharmacokinetics and long-term Gd(III) accumulation in major organs and tissues were investigated for GDCC of different molecular weights (18 kDa and 60 kDa) in Sprague-Dawley rats. A clinically available low-molecular-weight MRI contrast agent, Gd-(DTPA-BMA) (DTPA-BMA: diethylenetriaminepentaacetic acid-bismethylamide), was used as a control. MRI studies were also performed with the agents to relate the contrast enhancement in the vasculature to the pharmacokinetic properties of the agents.

## MATERIALS AND METHODS

Gd-(DTPA-BMA) (Omniscan, gadodiamide) was obtained from Nycomed Inc. (Princeton, NJ, USA). (Gd-DTPA)-cystamine copolymers (GDCC) with molecular weight of 18 kDa (GDCC-18) and 60 kDa (GDCC-60) were similarly prepared as previously described (18). Ketamine and xylazine were purchased from Ben Venue Labs (Bedford, OH, USA) and Vedco Inc. (St. Joseph, MO, USA), respectively. Male Sprague-Dawley rats (190–250 g; Charles River Laboratories, Wilmington, MA, USA) were used for the studies of pharmacokinetics, Gd(III) tissue biodistribution and MRI.

### Pharmacokinetic Study

A group of six rats were used for pharmacokinetic study of each contrast agent including GDCC-18 and GDCC-60 and a control agent Gd-(DTPA-BMA). The rats were anesthetized by an intramuscular injection of a mixture of ketamine (45 mg/kg) and xylazine (6 mg/kg). A heparinized catheter was inserted into the jugular vein for the injection of the contrast agent and for blood sampling. The contrast agent was injected at a dose of 0.1 mmol Gd/kg to the jugular vein via the catheter. Blood samples were collected from the catheter pre-injection and at various time points post-injection and transferred to centrifuge tubes. The centrifuge tubes were centrifuged at 10,000 rpm for 10 min to obtain plasma. The plasma was diluted with sterile water (Baxter) and the Gd content was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES, Perkin Elmer Optima 3100XL, Boston, MA, USA). A two-compartment pharmacokinetic model was used to simulate the contrast agent concentration in the blood after an intravenous bolus injection. WinNonLin (Pharsight Corporation) was used to fit the Gd concentration data and calculate the pharmacokinetic parameters.

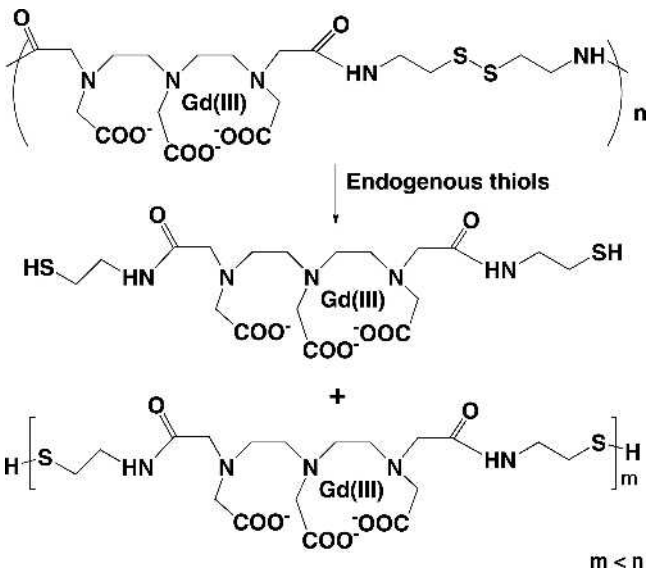


**Fig. 1.** Chain cleavage of (Gd-DTPA)-cystamine copolymers (GDCC) by reduction of disulfide bonds with endogenous thiols.

### Gd Tissue Retention

A group of six rats were used in the study of long-term Gd(III) retention in major organs and tissues for each agent. The rats were anesthetized by intramuscular injection of a mixture of ketamine (45 mg/kg) and xylazine (6 mg/kg) and contrast agent was injected at a dose of 0.1 mmol Gd/kg via a tail vain. The rats were then placed in metabolic cages and urine samples were collected at 4 and 8 h postinjection during the first day and once per day for the following 10 days. The rats were then sacrificed with an overdose of isoflurane and the organ and tissue samples (femur, heart, lung, liver, muscle, spleen and kidney) were collected and weighed. All samples except femur were mixed with 1.0 ml of sterile water and homogenized at 9500 rpm for 1 min or until there was no visible solid tissues. Femur was dissolved in 1.0 ml of nitric acid (70%, UN2031, EMD, Gibbstown, NJ, USA) overnight, and the solution was transferred to a centrifuge tube. Sterile water (2.0 ml) was added and centrifuged at 3000 rpm for 12 min. The supernatant (1.0 ml) was taken and further centrifuged at 13,000 rpm for 10 min. The supernatants were used for determination of Gd content by the ICP-AES. To convert the unit of Gd concentration (mg/L) from ICP-AES measurements to percentage of injected dose (ID) per organ/tissue, the ratios of organ or tissue over body weight used here were: lung (0.661%), liver (4.064%), spleen 0.267%), kidneys (0.88%), heart (0.447%) (19). The femurs (both left and right) are estimated to be 0.40% of the body weight. The muscle is 40% of body weight (20).

### MRI in Rats

A group of three rats were used in contrast enhanced MR blood pool imaging for each agent. The rats were anesthetized by the intramuscular administration of a mixture of ketamine (45 mg/kg) and xylazine (6 mg/kg). The contrast agents were injected intravenously at a dose of 0.1 mmol Gd/kg. MR images were acquired before and at 2, 5, 10, and 15 min after the injection of the contrast agents on a Siemens (Malvern, PA, USA) Trio 3T scanner using 3D FLASH (FL3D) pulse sequence. The system body coil was used for RF excitation and a human wrist coil was used for RF reception. The imaging parameters used were 1.64 ms echo time (TE), 4.3 ms repetition time (TR), 19° RF flip angle, and 0.5-mm coronal slice thickness. MR images were analyzed

and 3D maximum intensity projection (MIP) images were constructed with Osirix software (http://homepage.mac.com/rossetantoine/osirix/). Regions of interest were set at the left ventricle of the heart, the liver, the iliac vein, and the muscle around the iliac vein. Signal intensity (SI) was measured in the regions of interests. The ratios of the SI of the heart, liver, and iliac vein to that of the muscle at various time points were calculated for each animal and averaged among the animals in the same experimental group, respectively.

### Statistical Analysis

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

## RESULTS

### Pharmacokinetics

The pharmacokinetics of the extracellular biodegradable macromolecular contrast agent, GDCC, in blood circulation was investigated in Sprague-Dawley rats. The structure of GDCC is shown in Fig. 1. GDCC with a low molecular weight ($M_w$ = 18 kDa, GDCC-18) and a high molecular weight ($M_w$ = 60 kDa, GDCC-60) were selected to study the impact of molecular weight on pharmacokinetics. Gd-(DTPA-BMA) is a non-ionic, clinically used low molecular weight (574 Da) contrast agent, in which two carboxylic groups of DTPA are modified by methyl amide. The agent has a short blood pool retention time and is rapidly excreted through renal glomerular filtration. It was selected as a low molecular weight control because it has a similar structure as the repeat units in GDCC. The complexes are neutral compounds with Gd(III) chelated with three carboxylates and two amides. Figure 2 shows the time-dependent Gd(III) concentration profile in the blood plasma over a period of 6 h after intravenous bolus injection of the contrast agents at a gadolinium equivalent dose of 0.1 mmol/kg. Gd-(DTPA-BMA) rapidly extravasated from the vasculature, while the macromolecular GDCC of both molecular weights maintained relatively high plasma concentrations at the initial period postinjection. At 2 min postinjection, GDCC of both molecular weights had much higher Gd plasma concentration than Gd-(DTPA-BMA) ($p < 0.05$). GDCC-60 had a higher plasma concentration than GDCC-18 ($p < 0.05$). The plasma concentration of GDCC of both molecular weights then decreased rapidly over time. At 20 min postinjection, the plasma Gd concentration for GDCC-60 (17.1 mg/L) was about the same level as that of GDCC-18 (13.3 mg/L) ($p = 0.09$). The *in vivo* degradation of GDCC resulted in relatively rapid blood clearance of GDCC.

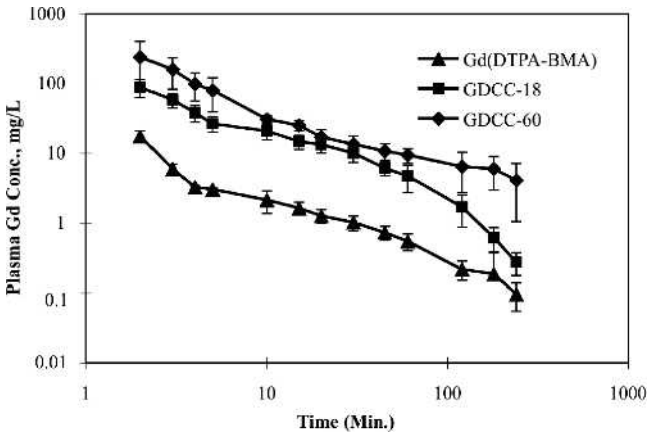


**Fig. 2.** Blood clearance of Gd(III) complexes in rats after intravenous injection of Gd-(DTPA-BMA), GDCC-18, and GDCC-60 at a dose of 0.1 mmol Gd/kg. Data presented as mean ± SD.

The time-dependent Gd(III) plasma concentrations of the agents over a period of 6 h postinjection were fitted to a two-compartment pharmacokinetic model. The pharmacokinetic parameters including half-life ($t_{1/2}$) of blood α and β phases, and the volume of distribution of the central compartment ($V_c$) for Gd-(DTPA-BMA), GDCC-60 and GDCC-18 are listed in Table I. The half-lives of the agent increased with increasing molecular weight. The α phase $t_{1/2}$ of GDCC-18 was significantly longer than that of Gd-(DTPA-BMA) ($p < 0.05$) and that of GDCC-60 was significantly longer than GDCC-18 ($p < 0.05$). The β phase $t_{1/2}$ of GDCC-60 was significantly longer than those of Gd-(DTPA-BMA) and GDCC-18 ($p < 0.05$). The difference between β phase $t_{1/2}$ of Gd-(DTPA-BMA) and GDCC-18 was not statistically significant ($p = 0.13$).

### Renal Clearance and Gd Tissue Retention

The excretion of Gd(III) complexes in urine for Gd-(DTPA-BMA), GDCC-18, and GDCC-60 is plotted as the percentage of injected dose during a period of 10 days postinjection in Fig. 3. Most of the Gd(III) complexes for the low molecular weight control and the two GDCC agents were excreted in urine within the first 4 h postinjection. Over a period of 10 days, approximately 67% of the injected dose of Gd-(DTPA-BMA) was measured in urine, and 61% and 54% were measured for GDCC-18 and GDCC-60, respectively.

Figure 4 shows the biodistribution of Gd(III) in the major organs and tissues, including the femur, heart, kidneys, liver, lung, muscle, and spleen of rats 10 days after the injection of either Gd-(DTPA-BMA), GDCC-18, or GDCC-60 at a dose of 0.1 mmol Gd/kg. The accumulation of Gd(III) in the tissues measured was at the same minimal level for the control agent and both macromolecular agents, except that GDCC had a slightly higher Gd accumulation in the kidneys. The differences in Gd(III) kidney accumulation between Gd-(DTPA-BMA) and GDCC ($p < 0.05$) were significant, but the accumulations were in a comparable minimal level. The Gd(III) kidney accumulation in GDCC-18 and GDCC-60 was similar ($p = 0.58$).

### Contrast-Enhanced MRI in Rats

Figure 5 shows the 3D maximum intensity projection (MIP) images of rats contrast enhanced by Gd-(DTPA-BMA), GDCC-18, and GDCC-60 before and at 2, 5, 10, and 15 min after injection of the agents via a tail vein at a dose of

**Table I.** Pharmacokinetic Parameters of Gd-(DTPA-BMA), GDCC-18, and GDCC-60 After Intravenous Injection at a Dose of 0.1 mmol Gd/kg in Rats

| | Gd-(DTPA-BMA) | GDCC-18 | GDCC-60 |
|---|---|---|---|
| $t_{1/2}$, α (min) | 0.48 ± 0.16 | 1.08 ± 0.24 | 1.74 ± 0.57 |
| $t_{1/2}$, β (min) | 21.2 ± 5.5 | 26.5 ± 5.9 | 53.7 ± 15.9 |
| $V_c$ (L/kg) | 0.072 ± 0.066 | 0.069 ± 0.040 | 0.046 ± 0.031 |

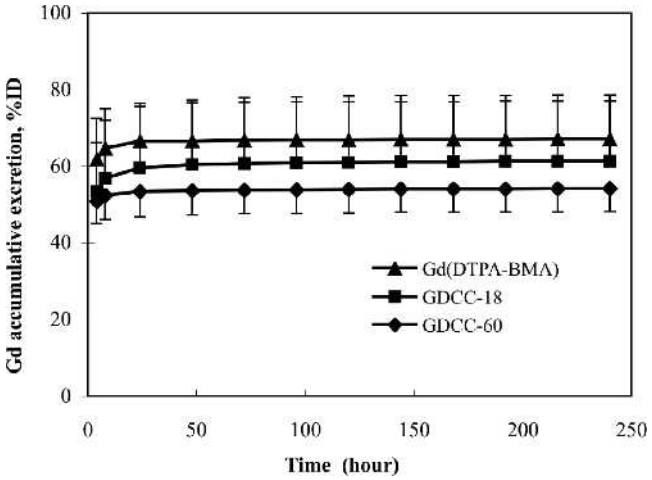


**Fig. 3.** Accumulative urinary clearance of Gd(III) complexes in rat after intravenous injection of Gd-(DTPA-BMA), GDCC-18, and GDCC-60 at a dose of 0.1 mmol Gd/kg. Data presented as mean ± SD.

0.1 mmol Gd/kg. The group of animals injected with the same agent exhibited similar contrast enhancement in the MR images. The contrast enhancement in the heart, liver or iliac vein with the agents at various time points is shown as the SI ratio of these organs or tissue to the muscle (Fig. 6). The biodegradable macromolecular agents, GDCC-18 and GDCC-60, resulted in more significant contrast enhancement in the heart and vasculature than Gd-(DTPA-BMA) in first 5 min postinjection. There was no significant difference between GDCC-18 and GDCC-60 ($p > 0.05$). The contrast enhancement was strong at 2 min postinjection for both GDCC-18 and GDCC-60, and the signal intensity was then gradually decreased over time. Significant contrast enhancement was still visible in the artery at 15 min postinjection. It appears that GDCC-60 does not have obvious advantage over GDCC-18 on elongating blood pool contrast enhancement in rats even though it has a larger initial size. The results are consistent with the pharmacokinetic data. All agents produced significant contrast enhancement in the kidneys. The contrast enhancement in the urinary bladder gradually increased over time for Gd-(DTPA-BMA) and the macromolecular agents, indicating that the agents were excreted in urine.

## DISCUSSION

The biodegradable macromolecular MRI contrast agent, (Gd-DTPA)-cystamine copolymers, has been designed to facilitate the clearance of the contrast agent by gradual breakdown of disulfide bonds in the macromolecules. It is expected that the contrast agent can circulate in the vasculature for an acceptable time window for effective contrast enhanced MR imaging and then clear from the body with minimal long-term tissue retention of toxic Gd(III) ions. The study has confirmed that the biodegradable macromolecular agent has a relatively long blood circulation for effective blood pool MR contrast enhancement and then clears rapidly with minimal Gd(III) tissue retention.

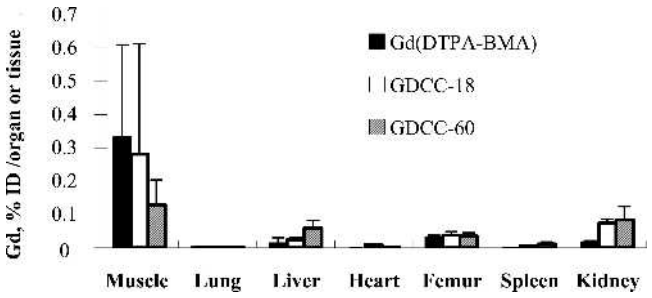


**Fig. 4.** Biodistribution of gadolinium(III) in rats 10 days after intravenous injection of Gd-(DTPA-BMA), GDCC-18, and GDCC-60 at a dose of 0.1 mmol Gd/kg. Data presented as mean ± SD.

The plasma pharmacokinetics demonstrated that the low molecular weight control agent, Gd-(DTPA-BMA), rapidly extravasated from the vasculature, while the degradable macromolecular agent had a higher plasma concentration in the first few minutes (approximately 5 to 15 min) postinjection. The macromolecular agent was then gradually cleared from the vasculature. The larger size (or molecular weight) of GDCC resulted in higher plasma concentration of the contrast agent in the initial period after the injection but did not significantly affect the blood clearance of the agent. The higher plasma concentration of GDCC-60 at 5 min postinjection did not result in more significant blood pool contrast enhancement than GDCC-18 (Fig. 6) because of the limitation of the imaging protocol we used. The signal intensity is not linearly correlated to the concentration of the contrast agents (21). At 20 min postinjection, the plasma concentration of GDCC-60 reached a level similar to that of GDCC-18 ($p = 0.09$). The results also validated the MR contrast enhancement in rat vasculature by GDCC-60 and GDCC-18. With GDCC, the higher blood concentration of contrast agent resulted in clearer definition of the blood vessels at two minutes post-injection. The contrast enhancement in the blood pool decreased over time with decreasing plasma gadolinium concentration. High-molecular-weight GDCC-60 did not provide prolonged blood pool contrast enhancement when compared with GDCC-18. At 15 min postinjection, similar vascular contrast enhancement was observed for GDCC-60 and GDCC-18.

The rapid blood clearance of GDCC was attributed to the breakdown of the macromolecules into smaller Gd(III) complexes by the reduction of the disulfide bonds in the polymer backbone with endogenous thiols, which was confirmed in our previous work (18). The degradation and reduction of the molecular weight of GDCC is a gradual process as shown in the incubation of GDCC with 15 μM cysteine (18). The high-molecular-weight GDCC-60 may produce some relatively large molecules at the early stages of degradation, which might remain in the blood circulation for a longer time. The large molecules would eventually degrade into smaller complexes that could be extravasated from the vasculature and excreted through renal glomerular filtration. The detection of Gd in the muscle (Fig. 4) was a result of the extravasation of smaller Gd complexes after degradation of GDCC. This correlates well with the pharmacokinetic behavior and blood pool contrast enhancement of GDCC-60.

The Gd(III) complexes formed from degradation of GDCC were mainly cleared in urine via renal glomerular filtration, similar to Gd-(DTPA-BMA). More than 50% of injected GDCC of both low and high molecular weights was found in the urine samples collected in the first four hours post-injection. In comparison, the clearance of non-degradable macromolecular Gd(III) can be much slower. For example, less than 25% of injected PAMAM dendrimer-based

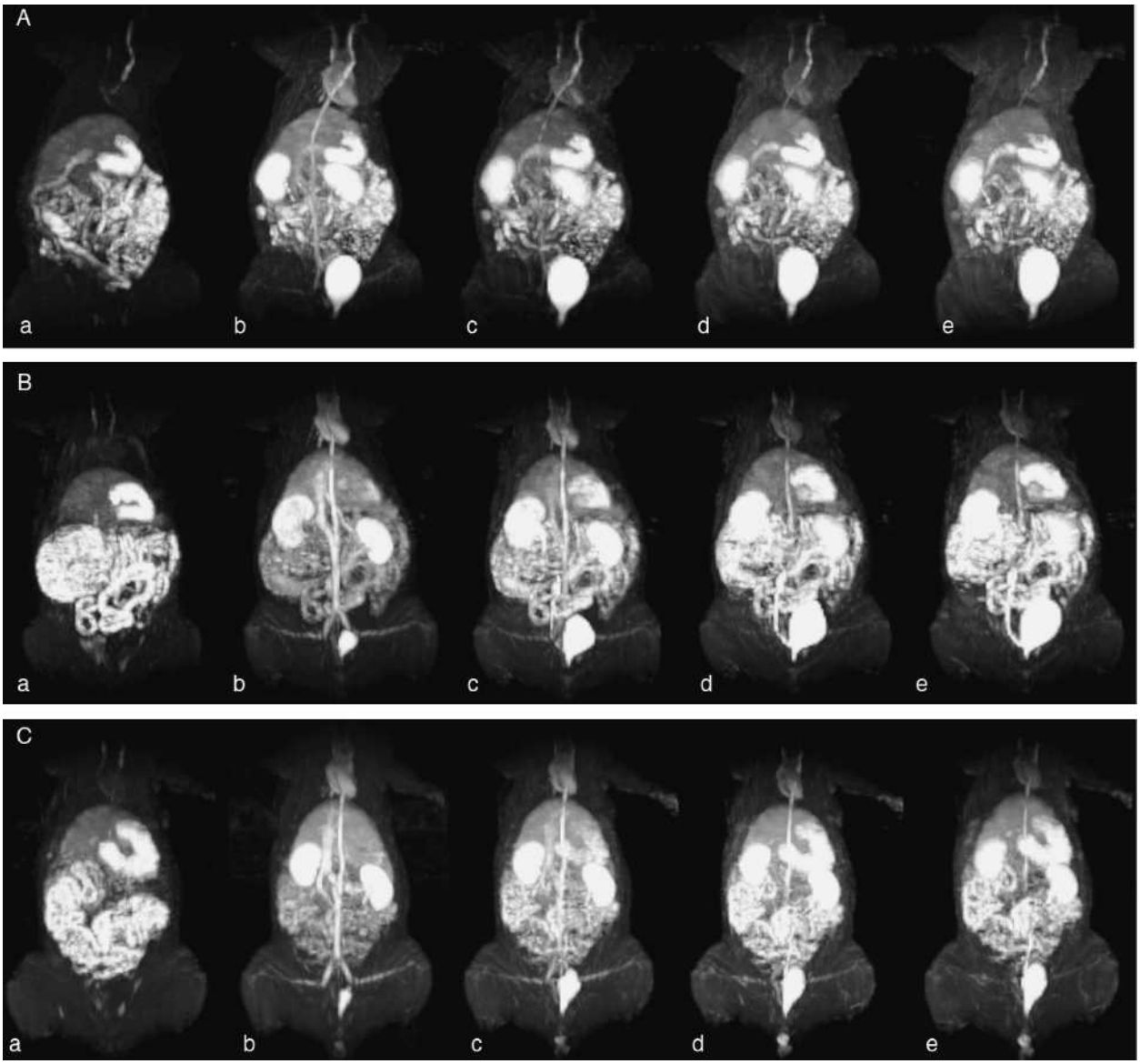


**Fig. 5.** Three-dimensional maximum intensity projection (MIP) MR images of rats before (a) and at 2 (b), 5 (c), 10 (d), and 15 (e) min after intravenous injection of Gd-(DTPA-BMA) (A), GDCC-18 (B), and GDCC-60 (C) at a dose of 0.1 mmol Gd/kg.

(from generation 3 to 6, MW = 6909 to 43,451 Da) macromolecular Gd(III) complexes were excreted in both feces and urine two days after injection into mice (22). The urine excretion of GDCC was clearly shown by contrast enhanced MR images of the urinary bladder. The time-dependent increase of signal intensity in the bladder revealed the excretion of the contrast agents in urine. The *in vivo* degradation of GDCC clearly facilitated the excretion of Gd(III) complexes. As a result, only a minimal amount of Gd(III) was retained in the major organs and tissues for the biodegradable macromolecular contrast agent 10 days postinjection. The gadolinium residual in the organs and tissues measured for GDCC-18 and GDCC-60 was at the same level as the low molecular weight control, Gd-(DTPA-BMA). The minimal *in vivo* gadolinium retention suggested that a considerable amount of the contrast agents were also excreted into feces.

The biodegradability of (Gd-DTPA)-cystamine copolymers was confirmed in our previous study (18). Monomeric and dimeric Gd(III) complexes, after the reduction of disulfide bonds and further metabolism, were detected in the urine samples. The pharmacokinetic results have further demonstrated that the biodegradable macromolecular MRI contrast agent acts as macromolecules in the blood pool at the early stage after injection and then degrades into smaller Gd(III) complexes that excrete rapidly from the body. Because of the biodegradability of the macromolecular agent, its molecular weight or size has little impact on elongating blood pool retention time. GDCC provides a time window of at least 5 min for effective contrast enhanced MR imaging of the vasculature in rats. The time window might be longer in patients because the blood circulation is slower in humans, providing enough time for many MRI procedures of the cardiovascular system. However, the degradation and blood clearance of GDCC might be too fast for some imaging procedures. Currently, we are attempting to modify the structure of the polydisulfide based biodegradable macromolecular Gd(III) com-

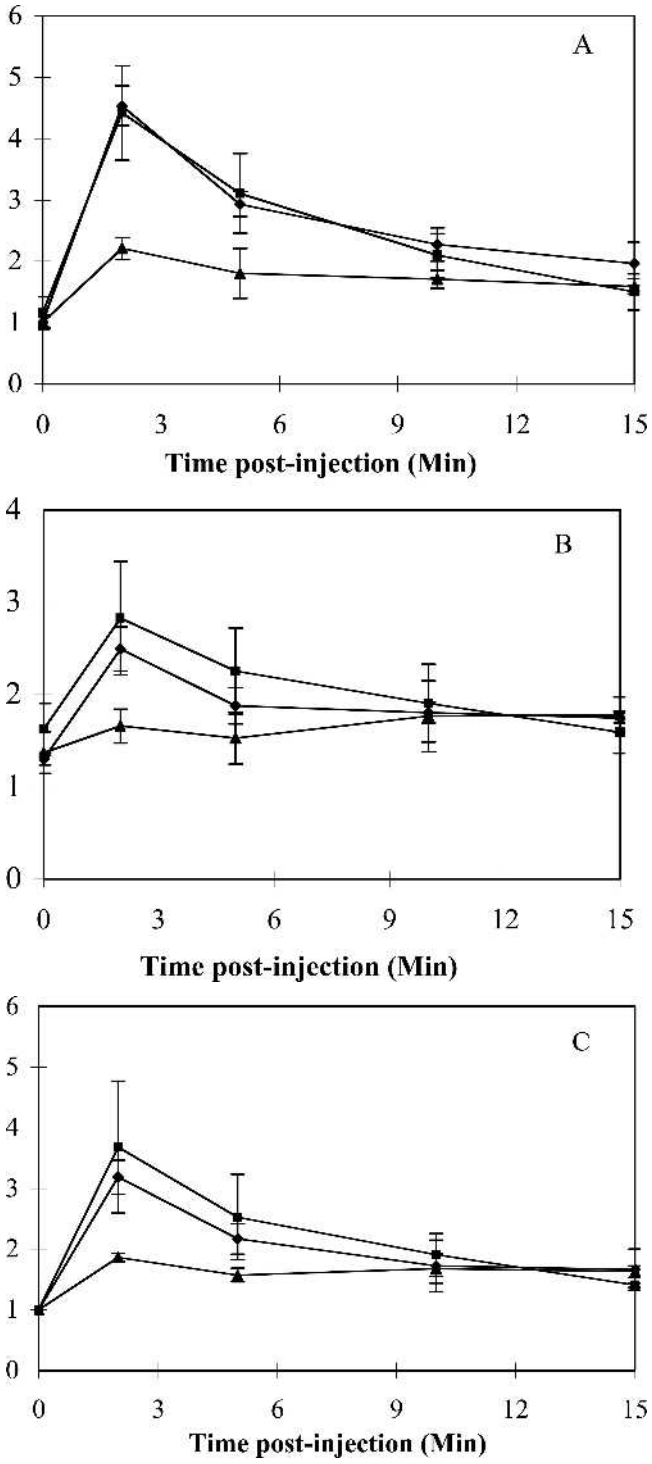


**Fig. 6.** The signal intensity ratio of the heart (A), liver (B), and iliac vein (C) to the muscle of rats before and at various time points after injection of of Gd-(DTPA-BMA) (▲), GDCC-18 (■), and GDCC-60 (♦) at a dose of 0.1 mmol Gd/kg. Data presented as mean ± SD.

plexes to control the degradation rate and blood pool retention and to satisfy various imaging purposes.

## CONCLUSIONS

The biodegradable macromolecular MRI contrast agent, GDCC, exhibited higher blood concentration and longer blood pool retention than the low molecular weight control agent, Gd-(DTPA-BMA). The biodegradable macromolecular agent can be readily degraded *in vivo* and rapidly excreted after contrast enhanced MRI. The macromolecular agent had comparable retention of gadolinium in major organs and tissues as Gd-(DTPA-BMA). The increase of the molecular weight of GDCC increased the blood concentration of the contrast agent in the early stages after injection, but did not extend its blood retention. The size of GDCC had little impact on long-term gadolinium tissue retention. GDCC demonstrated more significant and prolonged blood pool contrast enhancement than Gd-(DTPA-BMA). Because of its superior blood pool contrast enhancement and minimal gadolinium tissue retention, GDCC holds promise for further development as a safe and effective blood pool MRI contrast agent.

## ACKNOWLEDGMENTS

The research work is supported in part by the NIH grants R21/R33 CA095873 and R01 EB00489. The authors thank Prof. Dennis L. Parker for valuable discussions.

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