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The Degradation and Clearance of Poly(N-hydroxypropyl-L-glutamine)-DTPA-Gd as a Blood Pool MRI Contrast Agent

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Abstract

Although polymeric magnetic resonance imaging (MRI) agents have significantly improved relaxivity and prolonged circulation time *in vivo* compared with current imaging agents, the potential for long-term toxicity prevents their translation into the clinic. The aim of this study was to develop a new biodegradable, nonionic polymeric blood pool MRI contrast agent with efficient clearance from the body. We synthesized PHPG-DTPA, which possesses two potentially degradable sites *in vivo*: protein amide bonds of the polymer backbone susceptible to enzymatic degradation and hydrolytically labile ester bonds in the side chains. After chelation with Gd³⁺, PHPG-DTPA-Gd displayed an R₁ relaxivity of 15.72 mM⁻¹ · sec⁻¹ (3.7 times higher than that of Magnevist^T). *In vitro*, DTPA was completely released from PHPG polymer within 48 h when incubated in mouse plasma. *In vivo*, PHPG-DTPA-Gd was cleared via renal route as shown by micro-single photon computed tomography of mice after intravenous injection of ¹¹¹In-labeled PHPG-DTPA-Gd. MRI of nude rats bearing C6 glioblastoma showed significant enhancement of the tumor periphery after intravenous injection of PHPG-DTPA-Gd. Furthermore, mouse brain angiography was clearly delineated up to 2 h after injection of PHPG-DTPA-Gd. PHPG-DTPA-Gd's biodegradability, efficient clearance, and significantly increased relaxivity make it a promising polymeric blood pool MRI contrast agent.

INTRODUCTION

Magnetic resonance imaging (MRI) is a powerful diagnostic technique because of its excellent soft tissue contrast and sub-millimeter resolution. To reduce ambiguity in disease diagnosis, about 25% of MRI examinations employ MRI contrast agents. All eight clinically approved gadolinium-based contrast agents, such as Magnevist (Gd-DTPA), Dotarem (Gd-

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DOTA), Prohance (Gd-HP-DO₃A), and Omniscan (Gd-DTPA-BMA), are small-molecular-weight hydrophilic contrast agents. These agents extravasate quickly and are rapidly cleared from intravascular and interstitial space, with a typical elimination half-life of about 1.5 h. This rapid clearance is generally beneficial to patient health; however, it also makes it difficult to conduct many time-dependent imaging studies. In contrast, polymeric agents with a molecular weight greater than the renal clearance threshold normally possess significantly prolonged blood circulation and preferential accumulation in solid tumors owing to an enhanced permeability and retention (EPR) effect. Moreover, polymeric contrast agents display enhanced relaxivity owing to the restricted rotation of large molecules. To date, a few polymeric contrast agents have been proposed and studied for MR angiography and cancer imaging in the preclinical setting [1–6]. However, the extended circulation time of polymeric MRI contrast agents is usually concomitant with slow excretion from the body, and prolonged retention of Gd-containing polymers in the body may increase the chances of trans-metallation between Gd³⁺ and endogenous metal ions such as Zn²⁺, Cu²⁺, and Ca²⁺, resulting in the release of free Gd³⁺, which may cause nephrogenic systemic fibrosis (NSF) [7, 8]. Thus, biodegradability that ensures the clearance of DTPA-Gd within a relatively short time after MRI scanning has become crucial for the development of polymeric MRI contrast agents.

To address this long-term toxicity issue, imparting biodegradability to the polymers that act as carriers for MRI contrast agents has proven to be a practical strategy. Ideally, polymeric contrast agents should degrade and be rapidly excreted after diagnostic scans are completed (i.e. a day). Due to the excellent biocompatibility and biodegradability of poly(L-glutamic acid) (PG), we previously conjugated DTPA-Gd to PG [9, 10]. The resulting polymer (PG-Gd) demonstrated backbone degradation in the presence of lysosomal enzymes (i.e., cathepsin B). In fact, PG-Gd could be degraded in the presence of cathepsin B even when <30% of the carboxylic acid groups in PG were substituted with DTPA-Gd. In non-human primates, PG-Gd could be completely cleared from the body within a week [6]. However, it is desirable that the biodegradability of the polymer can be adjusted and even enhanced by tailoring the polymer structures.

The aim of this study was to develop and characterize a new biodegradable nonionic polymeric MRI contrast agent with an imaging window of a few hours before it is cleared from the body. We chose poly(*N*-hydroxypropyl-L-glutamine) (PHPG) as the backbone on which to construct a polymeric MRI contrast agent. PHPG has been proven to be biodegradable, possesses excellent water solubility, and is neutral (nonionic), with low osmolality as well as numerous functional hydroxyl groups for chemical conjugation [11, 12].

2. Materials and methods

2.1. Materials

Poly-N⁵-(3-hydroxypropyl)-L-glutamine (PHPG, molecular weight, 5.1 x 10⁵ dalton), succinic anhydride, gadolinium acetate, 1,3-diisopropylcarbodiimide (DIC), 4-dimethylaminopyridine (DMAP), *NN*, -diisopropylethylamine (DIPEA), trifluoroacetic acid (TFA) and other reagents were obtained from Sigma-Aldrich (St. Louis, MO). *p*-(*S*-2-[4-aminobenzyl]-diethylenetriamine penta-*tert*-butyl acetate) (NH₂-Bz-DTPA[*t*-butyl ester]) was synthesized according to previously reported procedures [13]. The radioactive tracer ¹¹¹InCl₃ was obtained from Iso-Tex Diagnostics (Houston, TX).

2.2. Characterization

¹H nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 300 spectrometer (Billerica, MA), and chemical shifts were referenced to the solvent peak ($\delta =$

2.54 ppm for dimethyl sulfoxide [DMSO] and 4.79 ppm for H₂O). The Gd³⁺ content was determined by inductively coupled plasma optical emission spectroscopy (ICP-OES), performed by Galbraith Laboratories, Inc. (Knoxville, TN). Gel permeation chromatography (GPC) was carried out using a Viscotek TDA (model 301; Houston, TX) equipped with a 7.8mm x 30 cm GMH_{HR}-M TSK-gel column (Tosoh Biosep, Japan). Phosphate-buffered saline (PBS) (pH = 7.4) was used as the mobile phase (flow rate, 1.0 mL/min). The GPC system was equipped with a triple detector array, including a differential refractive index, a differential viscometer, and a right angle laser light scattering detector. A linear dextran standard was used for the calibration of the GPC apparatus. The molecular weight and distribution were calculated using Viscotek TriSEC GPC software.

2.3. Synthesis of *p*-succinamidobenzyl-diethylenetriamine-penta-(acetic acid-*t*-butyl ester) [DTPA(O-*t*-Bu)₅-Bz-NH-SA]

DTPA(O-*t*-Bu)₅-Bz-NH-SA was synthesized by following a published procedure with minor modifications [14]. Briefly, succinic anhydride (1.0 mmol) and DIPEA (0.2 mL) were added to the solution of DTPA(O-*t*-Bu)₅-Bz-NH₂ (1.0 mmol) in 10 mL of dimethylformamide (DMF)/dichloromethane (1/5, v/v). The mixture was stirred at room temperature for about 4 h. The solvent was then removed under a vacuum and the residue dissolved in ethyl acetate, washed with 2% KHSO₄ and brine, and dried over MgSO₄. After removal of the solvent, the product was purified by flash column chromatography to yield 2.02 g of DTPA(O-*t*-Bu)₅-Bz-NH-SA (83% yield) as a white solid: ¹H NMR (CHCl₃-d₃) δ (ppm): 1.41-1.47(m, 45H), 2.53(t, J=10.8 Hz, 2H), 2.68(t, J=10.8 Hz, 2H), 2.81-2.84(m, 3H), 3.04(s, 2H), 3.21-3.46(m, 12H), 7.04(d, J=8.4Hz, 2H), 7.64(d, J=8.4Hz, 2H). Electrospray ionization mass spectrometry: Calcd. for (M+H)⁺ C₄₅H₇₄N₄O₁₃: 879.53; Found: 879.56.

2.4. Synthesis of PHPG-DTPA

To the solution of PHPG (0.5 g, 2.69 mmol OH-) and DTPA(O-*t*-Bu)₅-Bz-NH-SA (0.7 g, 0.79 mmol) in 10 mL of anhydrous DMF were added DIC (112 mg, 0.886 mmol) and DMAP (20 mg, 0.16 mmol). The reaction mixture was stirred under argon (g) for 36 h at room temperature. After removal of most of the solvent under a vacuum, the product was precipitated into cold methanol. The resulting white solid was dried under a vacuum. To remove protective groups, 0.8 g of the as-prepared PHPG-DTPA(O-*t*-Bu)₅ polymer was dissolved in 2 mL of TFA and stirred for 4 h. TFA was removed under a vacuum after completion of the deprotection reaction, and the reaction process was monitored by ¹H NMR. The deprotected polymer was purified using centrifugal filtration (10,000 MWCO, Millipore, Billerica, MA). The product was lyophilized to afford 0.5 g PHPG-DTPA (yield, 85%). ¹H NMR (D₂O), δppm: 7.36-7.21 (AA'BB'-system, aromatic), 4.25(s, -CH-of PHPG), 3.08, 3.21, 1.68(-N-CH₂CH₂CH₂O-of PHPG), 1.85, 2.09, 2.32(-CH₂CH₂CO-of PHPG), 2.71-4.10(m, DTPA).

2.5. Chelation of PHPG-DTPA with Gd³⁺ Ions

To chelate Gd³⁺ ions, 0.04 M Gd(Ac)₃ in 10 mM sodium acetate buffer (pH = 5.8) was added in small fractions to 10 mg PHPG-DTPA solution in 10 mM sodium acetate buffer. The chelation progress was monitored by using a [4-(2-pyridylazo)-resorcinol monosodium salt] (PAR) color reaction. After extensive dialysis against 1 mM ethylenediamine tetraacetic acid (EDTA) solution and water (MWCO = 3000), the resulting Gd-chelated polymer solution was lyophilized to yield 8.5 mg of white powder. The absence of free Gd³⁺ ions was confirmed using PAR color reaction.

2.6. MR Relaxivity

Solutions of PHPG-DTPA-Gd were prepared in deionized water at equivalent Gd concentrations of 0.005, 0.01, 0.02, 0.04, 0.08, 0.16, and 0.32 mM. Spin-lattice (T_1) was measured at 4.7 T on a Biospec small animal MRI system (Bruker Biospin Corp., Billerica, MA) using an inversion-recovery pulse sequence. Relaxivity (R_1 in $\text{mM}^{-1} \text{s}^{-1}$) values were obtained using linear least-squares regression analysis of the slopes of a $1/T_1$ versus [Gd] plot.

2.7. ^{111}In Labeling of PHPG-DTPA-Gd

Aliquots of 0.5 mg PHPG-DTPA-Gd (90% of DTPA chelated with Gd^{3+}) in 200 μL of 0.1 M sodium acetate solution (pH 5.5) were mixed with an aqueous solution of $^{111}\text{InCl}_3$ (800 μCi) and incubated at room temperature for 30 min. The ^{111}In -labeled PHPG-DTPA-Gd (PHPG-DTPA-Gd(^{111}In)) was analyzed using instant thin-layer chromatography (ITLC). The paper strips were developed with PBS (pH 7.4) containing 4 mM EDTA and quantified using a Bioscan IAR-2000 thin-layer chromatography imaging scanner (Washington, DC). Free $^{111}\text{In}^{3+}$ moved to the solvent front (relative front = 0.9–1.0), and the polymer remained at the original spot (relative front = 0.0). The labeling efficiency was > 98%.

2.8. Biodegradation of PHPG-DTPA-Gd(^{111}In)

PHPG-DTPA-Gd(^{111}In) incubated in mouse plasma at 37°C was analyzed using ITLC at designated time-points. The paper strips were developed with sodium acetate buffer solution (pH 5.8) containing 4 mM EDTA and analyzed using a Bioscan IAR-2000 thin-layer chromatography imaging scanner. Enzymatic degradation of PHPG-DTPA-Gd(^{111}In) was performed by incubating PHPG-DTPA-Gd(^{111}In) in cathepsin B solution (100 units) at 37°C for 12 h.

2.9. Biodistribution

All animal studies were carried out in the Small Animal Imaging Facility at The University of Texas MD Anderson Cancer Center in accordance with institutional guidelines. Healthy Swiss mice were randomly allocated into 4 groups, with each group consisting of 6 mice. Mice in each group were injected intravenously with PHPG-DTPA-Gd(^{111}In) at a dose of 20 $\mu\text{Ci}/\text{mouse}$ and killed at 1, 24, 48, and 72 h after injection. Blood, heart, liver, spleen, kidney, lung, stomach, intestine, brain, muscle, bone, and bladder tissues were removed and weighed. Radioactivity was measured with a gamma counter. Uptake of contrast agent in the various tissues was calculated as the percentage of the injected dose per gram of tissue (%ID/g).

2.10. Pharmacokinetics

Eight healthy female BALB/c mice (22–25 g; Charles River Laboratories, Wilmington, MA) were injected intravenously with 20 μCi of PHPG-DTPA-Gd(^{111}In). At predetermined intervals, blood samples (10 μL) were taken from the tail vein, and the radioactivity of each sample was measured with a Cobra Autogamma counter (Packard, Downers Grove, IL). The blood pharmacokinetic parameters of the radiotracer were analyzed with a two-compartment model using WinNonlin 5.0.1 software (Pharsight Corporation, Palo Alto, CA). The animals were euthanized by CO_2 exposure at the end of the study. Values are expressed as means $\pm$ standard deviations calculated for 8 mice.

2.11. $\mu\text{SPECT}/\text{CT}$ Imaging

PHPG-DTPA-Gd(^{111}In) (0.2 mg/mouse, 250 $\mu\text{Ci}/\text{mouse}$) was injected intravenously via the tail vein in 3 mice. At designated time points (1 h and 48 h) after administration of PHPG-DTPA-Gd(^{111}In), single-photon computed tomography (SPECT) and computed tomography

(CT) images were acquired using a μ SPECT-CT scanner (Gamma Medica, Northridge, CA). During each imaging session, mice were anesthetized with 2% isoflurane gas (Iso-Thesia, Rockville, NY) in oxygen. SPECT (radius of rotation 3 cm, 32 projections, 20 s per projection) and CT (512 projections, 75 kV, 500 mA) scans were acquired and co-registered for image fusion and presentation of 3D anatomical localization of the tracer signal. Acquired SPECT and CT data sets were processed with AMIRA 5.1 (San Diego, CA).

2.12. In Vivo MRI

For the tumor imaging and clearance study, rats bearing C6 glioma were separated into 2 groups ($n=3$ /group). The rats were then anesthetized using 2% isoflurane in oxygen and placed inside the magnet. MRI was performed on a 7.0-Tesla Biospec scanner using a transmit/receive RF volume coil with 72 mm inner diameter. High-resolution T1-weighted MR images were obtained using a 3Dfast spoiled gradient echo sequence (TE = 1.8 ms, TR = 5.3 ms, 20° excitation angle) with a matrix size of 256×256×48 and spatial resolution of 0.23×0.23×0.94 cm³. After pre-contrast MRI, the rats were injected intravenously with PHPG-DTPA-Gd at a dose of 0.04 mmol Gd/kg. A T1-weighted FSPGR sequence with higher temporal resolution (TE = 1.8 ms, TR = 5.3 ms, 20° excitation angle, 128×96×12 matrix, requiring 6.1s per repetition) was repeatedly acquired for 30 minutes beginning approximately one minute prior to contrast injection. Twenty-four hours later, the rats were scanned again using the high-resolution sequence described above.

To demonstrate the advantages of a long-circulating contrast agent in magnetic resonance angiography, 3 female nude mice 4–6 weeks old were injected with PHPG-DTPA-Gd at a dose of 0.04 mmol Gd/kg and anesthetized with 2% isoflurane during the imaging study. High-resolution mouse brain angiography was performed using a Bruker 9.4T Biospec MRI system (Billerica, MA) with a linear transmit volume coil and a four-element phased array receiver coil. The imaging parameters for the 3D FLASH sequence acquisition are as follows: repetition time/echo time = 15 ms/2.2 ms, flip angle = 20°, and matrix size = 256×96×72 at a spatial resolution of 0.2×0.1×0.2 mm³. The total scan time was 1.7 min. The scan was repeated at 10, 25, 40, 60, 75, 90, 105, 120, and 135 minutes after contrast injection. The presence of contrast in the blood pool was evaluated with region-of-interest analyses at the sagittal sinus and carotid vein, both of which are large veins that are less affected by inflow than arteries. To display the brain vasculature, the non-brain structures were masked from the images, and the maximum intensity projection method was used to project the blood vessels to a two-dimensional plane using ImageJ software (National Institutes of Health, Bethesda, MD).

2.13. Statistical Analysis

Statistical analysis of pharmacokinetic parameters was conducted using analysis of variance with Tukey's post hoc test (SYSTAT for Windows 11, SYSTAT Software, Inc., San Jose, CA). Differences in biodistribution data were analyzed using a two-tailed unpaired Student's *t*-test. *P* values < 0.05 were considered statistically significant.

3. Results and Discussion

3.1. Synthesis and characterization of PHPG-DTPA-Gd

Scheme 1 depicts the synthesis of PHPG-DTPA and PHPG-DTPA-Gd. PHPG-DTPA was prepared via esterification between PHPG and DTPA(OtBu)₅-Bz-NH-SA and subsequent de-protection in TFA. The protection groups were quantitatively removed with pure TFA without breakage of the polymer backbone. The chelation of Gd³⁺ ions was carried out by adding Gd(Ac)₃ in sodium acetate buffer (pH = 5.8) in small fractions to an aqueous solution of PHPG-DTPA. The process was monitored by using PAR color reaction. PHPG-

DTPA-Gd was obtained after extensive dialysis against 1 mM EDTA solution and water to ensure complete removal of free Gd. Figure 1A shows the ^1H -NMR spectrum of PHPG-DTPA. On the basis of the ratio of peak intensities between the methine protons in PHPG ($-\text{NHCH}_2\text{CH}_2\text{CH}_2\text{O}-$, $\delta = 1.68$ ppm) and the benzyl proton in DTPA-Bz-NH- ($-\text{CH}_2-\text{C}_6\text{H}_4-$, $\delta = 7.20$ ppm), it was estimated that about 20% of the hydroxyl groups of PHPG were conjugated to DTPA-Bz-NH-SA. Figure 1B shows GPC profiles of PHPG-DTPA and its corresponding Gd-chelated polymer PHPG-DTPA-Gd. The number-average molecular weight (M_n) and the molecular weight distribution of PHPG-DTPA were 1.02×10^5 and 1.20, respectively. Chelation with Gd increased the molecular weight of the resulting polymer PHPG-DTPA-Gd to 1.11×10^5 but did not change the molecular weight distribution. The molecular weight data from GPC analysis is consistent with the degree of polymerization for PHPG (250) and the degree of Gd substitution (20% of side chains). Gd^{3+} content in PHPG-DTPA-Gd was found to be 7.50 % (w/w) by the ICP-OES method. These data are also consistent with the ^1H -NMR finding that about 20% of PHPG's side chain hydroxyl groups were conjugated with SA-Bz-DTPA-Gd.

The relaxivity of PHPG-DTPA-Gd was calculated using linear least-squares regression analysis of the slopes of a $1/T_1$ versus $[\text{Gd}]$ plot, in which the spin-lattice relaxation time (T_1) was measured at 4.7 T MRI. It was found that PHPG-DTPA-Gd had an R_1 value of $15.72 \text{ mM}^{-1}\text{s}^{-1}$, which was 3.7 times higher than the relaxivity of Magnevist (Gd-DTPA) measured under the same conditions. The increased relaxivity of polymeric contrast agents can be ascribed to an increased rotational correlation time due to restricted rotational movement when DTPA-Gd moieties are lodged in polymer chains. Thus, PHPG-DTPA-Gd is capable of producing the same contrast enhancement at one third the dose of Magnevist.

3.2. ^{111}In labeling of PHPG-DTPA-Gd and degradation behavior of PHPG-DTPA-Gd in plasma

^{111}In decays by electron capture and emits photons useful for detection and imaging, with a physical half-life of 2.8 days. Here, ^{111}In was used to study the degradation, biodistribution, pharmacokinetics, and SPECT/CT imaging properties of PHPG-DTPA-Gd. ^{111}In labeling was achieved by chelating approximately 90% of the DTPA groups in PHPG-DTPA with Gd and using the remaining 10% of the DTPA groups for ^{111}In labeling (Scheme 1). The radiolabeling efficiency was greater than 98% without purification, and the specific activity of the resulting PHPG-DTPA-Gd(^{111}In) was $\sim 5 \text{ mCi/mg}$.

Figure 2 shows the degradation of PHPG-DTPA-Gd(^{111}In) in mouse plasma using the ITLC method. The intact PHPG-DTPA-Gd(^{111}In) was located at the 75 mm position on the 150 mm scale (Fig. 2A), and the free ^{111}In -DTPA was found at about 120 mm (data not shown). The release of ^{111}In -DTPA from PHPG-DTPA-Gd(^{111}In) was evident as early as 1 h after incubation of the polymer in mouse plasma at 37°C . By 48 h after incubation, almost all radioactivity was dissociated from PHPG-DTPA-Gd(^{111}In) (Fig. 2E). On the other hand, when PHPG-DTPA-Gd(^{111}In) was incubated in PBS at 37°C , no free ^{111}In -DTPA was detected within 24 h (data not shown). These results suggest that Gd-DTPA in PHPG-DTPA-Gd was cleaved from PHPG-DTPA-Gd, likely through both enzymatic degradation of the PHPG backbone and hydrolysis of ester bonds in the side chains [15]. When PHPG-DTPA-Gd was incubated with cathepsin B, polymeric and oligomeric species were observed in the ITLC chromatogram (Fig. 2F). This result indicates that PHPG-DTPA-Gd(^{111}In) is degradable in the presence of lysosomal enzymes. Further work is needed to pinpoint which enzyme(s) played a key role in the release of DTPA-Gd(^{111}In) from the PHPG polymer and were involved in the backbone degradation of PHPG.

3.3. Pharmacokinetics

Figure 3A shows the radioactivity-time curve after intravenous injection of PHPG-DTPA-Gd(^{111}In). The data were well fitted into a 2-compartment model, as indicated by the solid line over a period up to 48 h (Fig. 3A). The following observations were made. First, PHPG-DTPA-Gd(^{111}In) had an initial distribution phase with a mean half-life of 2.16 h after dosing, followed by a terminal elimination half-life of 8.40 h. The tracer activity in blood at 2 h (11.75 ± 2.75 ID/g) was still as high as 40% of its activity at 5 min after injection (29.23 ± 6.20 ID/g). In comparison, Magnevist has a terminal elimination half-life of about 20 min [16]. Thus, PHPG-DTPA-Gd(^{111}In) has a prolonged half-life that allows for a longer imaging window. Second, PHPG-DTPA-Gd(^{111}In) was mainly distributed to the central compartment, with a volume of distribution of 4.19 mL, approaching the volume of distribution at steady state (5.87 mL). Thus, the polymer was largely confined to the vascular system. Small-molecular-weight contrast agents, on the other hand, are known to be rapidly distributed to the extravascular fluid space.

3.4. Biodistribution and $\mu\text{SPECT/CT}$ Imaging

Figure 3B summarizes the biodistribution of PHPG-DTPA-Gd(^{111}In). The highest tracer concentration 1 h after intravenous injection was found in the blood. Other organs with high blood perfusion, such as the heart, liver, kidney, and lung, also showed a high level of polymer accumulation at 1 h. PHPG-DTPA-Gd(^{111}In) was rapidly excreted from the body. The polymer concentration in the kidney decreased from 6.74 ± 1.62 %ID/g at 1 h postinjection to 1.92 ± 0.75 %ID/g at 24 h (3.5-fold reduction) to 0.52 ± 0.26 %ID/g at 72 h (13.0-fold reduction). Similarly, the tracer concentration in the spleen decreased from 2.49 ± 0.38 %ID/g at 1 h to 0.34 ± 0.04 %ID/g at 72 h postinjection (7.3-fold reduction), and the tracer concentration in the liver decreased from 6.58 ± 0.65 %ID/g at 1 h to 1.30 ± 0.24 %ID/g at 72 h postinjection (5.1-fold reduction). Clearly, the ester linkage contributed to the effective excretion of DTPA-Gd(^{111}In) moiety from the body. At 1 h and 24 h postinjection, significant radioactivity was found in the bladder and urine, indicating renal clearance of the degradation products.

Figure 4 shows $\mu\text{SPECT-CT}$ images of a mouse acquired at 1 h and 24 h after intravenous injection of PHPG-DTPA-Gd(^{111}In). At 1 h, radioactivity was distributed throughout the body as a result of the blood pooling activity of the polymer. Moreover, radioactive degradation products were collected in the bladder. By 24 h after injection, most of the radioactivity was cleared from the body; the only organs showing retention of radioactivity were the liver, spleen, and kidneys. Taken together, our findings support the notion that DTPA-Gd(^{111}In) moiety was efficiently cleaved from the polymeric carrier, and was largely excreted via the renal system.

3.5. In Vivo MRI

Figure 5 shows representative MR images of a nude rat bearing subcutaneous C6 glioblastoma obtained at different time points after injection of PHPG-DTPA-Gd at a dose of 0.04 mmol Gd/kg. Uptake of PHPG-DTPA-Gd in the kidneys was visualized as early as 3 min postcontrast, and by 30 min, the kidneys showed a high level of contrast enhancement. Importantly, activity in the kidney gradually decreased. By 24 h postcontrast, most of the contrast agent had been excreted from the kidney. Enhancement of the C6 tumor rim was also observed during the 30 min - 24 h window. However, it appears that the tumor rim was better delineated at 30 min postcontrast than at 24 h postcontrast. This may be explained by the degradation of the polymer and removal of small-molecular-weight Gd-DTPA-containing moieties from the tumor site. Therefore, there are two phases of tumor delineation after contrast injection: the initial accumulation phase due to blood pool activity and the clearance phase when polymer degradation becomes a dominant factor.

To demonstrate the advantage of the long plasma half-life of the proposed contrast agent in MRI angiography, high-resolution mouse brain angiography was performed. Figure 6 shows the normalized signal intensity in two major veins, the sagittal sinus on the brain surface and the carotid vein in the neck. Both curves showed maximum contrast enhancement in the blood at or before 10 minutes after contrast injection. The contrast enhancement reduced over time with an exponential time constant of 6–7 h. The effect of the contrast agent was slightly different in the arteries, as shown in Figure 7. The enhancement in the arteries was comparable to the pre-contrast level at 2 h after contrast injection.

4. Conclusions

In summary, we have developed a new biodegradable and nonionic polymer MRI contrast agent, PHPG-DTPA-Gd, that has enhanced blood pool activity as well as desirable degradability and eventual clearance from the kidneys and the body within 24 h. Rapid clearance of the Gd-DTPA moiety from the body was attributable to two independent degradation mechanisms: backbone degradation of PHPG-DTPA-Gd by lysosomal enzyme and hydrolysis of side chains.

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References

1. Bogdanov AA Jr, Weissleder R, Frank HW, Bogdanova AV, Nossif N, Schaffer BK, et al. A new macromolecule as a contrast agent for MR angiography: preparation, properties, and animal studies. *Radiology*. 1993; 187:701–706. [PubMed: 8497616]
2. Kobayashi H, Kawamoto S, Jo SK, Bryant HL Jr, Brechbiel MW, Star RA. Macromolecular MRI contrast agents with small dendrimers: pharmacokinetic differences between sizes and cores. *Bioconjug Chem*. 2003; 14:388–394. [PubMed: 12643749]
3. Ladd DL, Hollister R, Peng X, Wei D, Wu G, Delecki D, et al. Polymeric gadolinium chelate magnetic resonance imaging contrast agents: design, synthesis, and properties. *Bioconjug Chem*. 1999; 10:361–370. [PubMed: 10346865]
4. Lu ZR, Wang X, Parker DL, Goodrich KC, Buswell HR. Poly(l-glutamic acid) Gd(III)-DOTA conjugate with a degradable spacer for magnetic resonance imaging. *Bioconjug Chem*. 2003; 14:715–719. [PubMed: 12862423]
5. Villaraza AJL, Bumb A, Brechbiel MW. Macromolecules, dendrimers, and nanomaterials in magnetic resonance imaging: the interplay between size, function, and pharmacokinetics. *Chem Rev*. 2010; 110:2921–2959. [PubMed: 20067234]
6. Tian M, Wen X, Jackson EF, Ng C, Uthamanthil R, Liang D, et al. Pharmacokinetics and magnetic resonance imaging of biodegradable macromolecular blood-pool contrast agent PG-Gd in non-human primates: a pilot study. *Contrast Media Mol Imaging*. 2011; 6:289–297. [PubMed: 21861289]
7. Sieber MA, Lengsfeld P, Frenzel T, Golfier S, Schmitt-Willich H, Siegmund F, et al. Preclinical investigation to compare different gadolinium-based contrast agents regarding their propensity to release gadolinium in vivo and to trigger nephrogenic systemic fibrosis-like lesions. *Eur Radiol*. 2008; 18:2164–2173. [PubMed: 18545998]
8. Puttagunta NR, Gibby WA, Smith GT. Human in vivo comparative study of zinc and copper transmetallation after administration of magnetic resonance imaging contrast agents. *Invest Radiol*. 1996; 31:739–742. [PubMed: 8970874]

9. Jackson EF, Esparza-Coss E, Wen X, Ng CS, Daniel SL, Price RE, et al. Magnetic resonance imaging of therapy-induced necrosis using gadolinium-chelated polyglutamic acids. *Int J Radiat Oncol Biol Phys.* 2007; 68:830–838. [PubMed: 17379450]
10. Wen X, Jackson EF, Price RE, Kim EE, Wu Q, Wallace S, et al. Synthesis and characterization of poly(L-glutamic acid) gadolinium chelate: a new biodegradable MRI contrast agent. *Bioconjug Chem.* 2004; 15:1408–1415. [PubMed: 15546209]
11. Aragon CL, Read MR, Gaynor JS, Barnhart MD, Wilson D, Papich MG. Pharmacokinetics of an immediate and extended release oral morphine formulation utilizing the spheroidal oral drug absorption system in dogs. *J Vet Pharmacol Ther.* 2009; 32:129–136. [PubMed: 19290942]
12. Li XL, Adams NW, Bennett DB, Feijen J, Kim SW. Synthesis of poly(hydroxypropylglutamine-prazosin carbamate) and release studies. *Pharm Res.* 1991; 8:527–530. [PubMed: 1871052]
13. Zhang G, Zhang R, Wen X, Li L, Li C. Micelles based on biodegradable poly(L-glutamic acid)-b-poly lactide with paramagnetic Gd ions chelated to the shell layer as a potential nanoscale MRI-visible delivery system. *Biomacromolecules.* 2008; 9:36–42. [PubMed: 18047289]
14. Wang W, McMurray JS, Wu Q, Campbell ML, Li C. Convenient solid-phase synthesis of diethylenetriaminepenta-acetic acid (DTPA)- conjugated cyclic RGD peptide analogues. *Cancer Biother Radiopharm.* 2005; 20:547–556. [PubMed: 16248770]
15. Pan H, Kopeckova P, Liu J, Wang D, Miller SC, Kopecek J. Stability in plasmas of various species of HPMA Copolymer-PGE(1) conjugates. *Pharm Res.* 2007; 24:2270–2280. [PubMed: 17899324]
16. 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]

Fig. 1. Characterization of PHPG-DTPA-Gd. (A) ^1H NMR spectrum of PHPG-DTPA in $\text{DMSO-}d_6$. (B) Gel permeation chromatography profiles of PHPG-DTPA and PHPG-DTPA-Gd. The polymers were eluted with PBS 7.8mm x 30 cm $\text{GMH}_{\text{HR}}\text{-M}$ TSK-gel TSK column at a rate of 1 mL/min.

Fig. 2.

ITLC chromatograms of PHPG-DTPA-Gd(¹¹¹In) demonstrating degradation of the polymer. PHPG-DTPA-Gd(¹¹¹In) was incubated in mouse plasma for (A) 0 h, (B) 1 h, (C) 12 h, (D) 24 h, and (E) 48 h. (F) ITLC chromatogram of PHPG-DTPA-Gd(¹¹¹In) after incubation with cathepsin B at 37°C for 12 h.

Fig. 3. Pharmacokinetics (A) and biodistribution (B) of PHPG-DTPA-Gd(^{111}In) after intravenous injection in mice. Radioactivity in each organ was measured with a gamma counter ($n = 6$), and was calculated as the percentage of the injected dose per gram of tissue (%ID/g). The solid line in (A) was curve-fitted to a two-compartment model.

Fig. 4.
 μ SPECT-CT images of a mouse at 1 h and 24 h after intravenous injection of PHPG-DTPA-Gd(^{111}In) at a dose of 100 μCi /mouse.

Fig. 5. Coronal MR images of rats bearing C6 tumors acquired before contrast injection and at 2 min, 30 min, and 24 h after intravenous injection of PHPG-DTPA-Gd at a dose of 0.04 mmol Gd/kg. Arrows in the left panel: kidney. Arrows in the right panel: C6 tumor.

Fig. 6.

Normalized signal intensity curves in the carotid vein and sagittal sinus. Both curves were plotted with the mean signal intensity and one standard deviation error bar. The normalized signal intensity curves follow a monoexponential trend after 10 min, with exponential delay time constants of 6.2 h ($R^2=0.99$) and 6.9 h ($R^2=0.94$) in the carotid vein and sagittal sinus, respectively.

Fig. 7. Maximum intensity projection of brain vasculature. (A) Time-of-flight precontrast images showing the major arteries of the Circle of Willis. Subsequent images (B-E) were acquired at 10, 25, 60, 90, and 120 min. The level of detail of the Circle of Willis at 120 min after contrast injection is similar to the precontrast level.

Scheme 1.

Synthesis of PHPG-DTPA-Gd and ^{111}In -labeled PHPG -DTPA-Gd. DIC: 1,3-diisopropylcarbodiimide; DMAP: 4-dimethylaminopyridine; TFA: trifluoroacetic acid.