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RESEARCH PAPER

Stability and Biodistribution of a Biodegradable Macromolecular MRI Contrast Agent Gd-DTPA Cystamine Copolymers (GDCC) in Rats

Xueming Wu • Yuda Zong • Zhen Ye • Zheng-Rong Lu

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ABSTRACT

Purpose The aim of this study was to evaluate stability and Gd tissue distribution of a biodegradable macromolecular MRI contrast agent, GDCC.

Methods Kinetic stability of GDCC was evaluated based on transmetallation with endogenous metal ions Zn^{2+} and Cu^{2+} in rat plasma in comparison with Omniscan, MultiHance and ProHance. *In vivo* transmetallation of GDCC was evaluated by determining metal content in the urine samples of Spague–Dawley rats. The biodistribution of the agents was determined in rats at 48 h post-injection.

Results A new method of using ultrafiltration was developed for study of kinetic stability against transmetallation of Gd(III)-based MRI contrast agents. Both *in vitro* and *in vivo* stability of the contrast agents towards transmetallation with Zn^{2+} were in the order of ProHance > MultiHance ≈ GDCC > Omniscan. No significant transmetallation with Cu^{2+} was observed for the contrast agents. GDCC had comparable retention to the control agents in most organs and tissues with slightly high retention in the liver and kidneys at 48 h post-injection.

Conclusion Ultrafiltration is efficient and accurate for characterizing the kinetic stability of Gd(III)-based MRI contrast agents. The novel biodegradable macromolecular contrast agent GDCC is promising for further development for contrast enhanced MRI.

X. Wu • Z.-R. Lu (✉)
Department of Biomedical Engineering
Case Western Reserve University
Wickenden Building, Room 427, 10900 Euclid Avenue
Cleveland, Ohio 44106-7207, USA
e-mail: zxl125@case.edu

Y. Zong • Z. Ye
Department of Pharmaceutics and Pharmaceutical Chemistry
University of Utah
Salt Lake City, Utah, USA

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INTRODUCTION

Gadolinium-based contrast agents are routinely used to enhance image contrast in magnetic resonance imaging procedures. Currently, the clinical Gd(III)-based MRI contrast agents are categorized into two distinct groups: macrocyclic Gd(III) chelates and linear chelates. In general, macrocyclic chelates, including Gd-DOTA and Gd-(HP-DO3A), are more stable than linear molecules, e.g. Gd-DTPA and Gd-(DTPA-BMA). Macrocyclic Gd(III) chelates have a higher activation energy barrier for both the complexation and dissociation than the linear chelates (1). Transmetallation is considered the main cause for *in vivo* dissociation of Gd(III)-based MRI contrast agents, especially for linear chelates, with endogenous metal ions, including Zn^{2+}, Cu^{2+} and Ca^{2+} (2–4). As a result, transmetallation can induce release of toxic Gd(III) ions from the chelates in the body and the depletion of the endogenous metal ions with subsequent elimination as a hydrophilic complex through the kidneys. Since Gd^{3+} ions are highly toxic, the stability of Gd(III) chelates is a critical parameter for design and development of new Gd(III)-based MRI contrast agents. The stability of Gd(III) chelates should include both thermodynamic and kinetic stability. If a Gd(III) chelate has high thermodynamic stability and low kinetic stability, transmetallation with endogenous metal ions, such as zinc (Zn^{2+}), copper (Cu^{2+}) and calcium (Ca^{2+}), in the plasma can result in the release of free gadolinium ions (Gd^{3+}), which can deposit in the body (5). Long-term tissue accumulation of toxic Gd^{3+} can cause toxic side effects, including nephrogenic systemic fibrosis (1,6–11).

Recently, we designed and developed a new class of biodegradable macromolecular MRI contrast agents based on polydisulfide Gd(III) chelates with improved pharmacokinetic properties (12–14). Our previous studies have shown that the biodegradable macromolecular contrast agents result in prolonged contrast enhancement in the cardiovascular system and tumor tissue and then gradually degrade *in vivo* and excrete via renal filtration, resulting in minimal long-term tissue accumulation (13–15). The agents are also effective to non-invasively characterize tumor vascularity and assess therapeutic efficacy in dynamic contrast-enhanced MRI (16–18). In this study, we developed a new and efficient method for *in vitro* evaluation of the kinetic stability against transmetallation of Gd(III)-based MRI contrast agents and evaluated the kinetic stability of a lead biodegradable macromolecular contrast agent, Gd-DTPA cystamine copolymers (GDCC), both *in vitro* and *in vivo*. Transmetallation of GDCC with endogenous ions Zn^{2+} and Cu^{2+} was evaluated in rat plasma and in rats in comparison with clinical contrast agents Omniscan, MultiHance and ProHance. The biodistribution of GDCC at 48 h after administration was also evaluated in rats in comparison with the clinical agents.

MATERIALS AND METHODS

Contrast Agents

Omniscan (Gd-(DTPA-BMA), 574 Da) was purchased from GE Healthcare. MutiHance (Gd-BOPTA, 1,058 Da) and ProHance (Gd-(HP-DO3A), 558.6 Da) were obtained from Bracco Diagnostics. Gd-DTPA cystamine copolymers with molecular weight of 31, 35, 50 KDa (GDCC-31, GDCC-35, GDCC-50) were prepared as previously described (12). Isotonic saline (0.9 %) solution was used as a negative control.

Animals

Male Sprague–Dawley rats weighing between 200 and 250 g were purchased from Charles River Laboratories (Wilmington, MA, USA). All animals were housed with a 12-hour day-and-night rhythm and given tap water and standard diet. The animal study was performed according to an animal protocol approved by the Institutional Animal Care and Use Committee of the University of Utah.

Complexation Stability of GDCC

GDCC-50 in phosphate-buffered saline (PBS) was incubated with 2.5 mM of $CaCl_2$, 0.11 mM of sodium citrate, 50 μM of $ZnCl_2$ and 1 μM of $CuCl_2$ (8 mM of Na_2HPO_4, 2 mM of KH_2PO_4 and 140 mM NaCl) at pH 7.4 and 37°C. This incubation was replicated three times. After 10, 20, 40, 60, 80, 120 and 180 min incubation, incubation mixtures were filtrated through 0.22 μM filters. Then the filtered incubation mixtures were run through PD-10 columns and the polymer fractions were collected and freeze-dried. Gd^{3+}, Zn^{2+}, Cu^{3+} and Ca^{2+} contents in these freeze-dried samples were analyzed using inductive coupling plasma-optical emission spectrometry (ICP-OES).

In Vitro Transmetallation

Aqueous solution (0.1 ml, 2 mM-Gd) of GDCC-31, Omniscan, MultiHance or ProHance was mixed with 0.9 ml fresh rat plasma and incubated at room temperature for 2 h. The plasma mixtures were transferred to the sample reservoir of a centrifugal filter CF-50 (molecular weight cut-off 50 KDa) and centrifuged at 4,000 rpm and 25°C for 120 min. Since all Zn^{2+} or Cu^{2+} ions bound to proteins, mainly albumin and globulins (>60 KDa), in the plasma, only the chelates smaller than 50 KDa, including Zn^{2+} or Cu^{2+} chelates, after transmetallation could be filtered through (Fig. 1). The protein-bound Zn^{2+} or Cu^{2+} ions still remained in the upper reservoir of the filter. The efficiency of the ultrafiltration was tested with the aqueous solution of the contrast agents. Ultrafiltration was also performed for rat plasma to test the binding of Zn^{2+} or Cu^{2+} ions to plasma proteins. During centrifugation, the solution in the upper reservoir was agitated using a pipette every 20 min for the plasma mixtures. The content of metal ions in both upper reservoir and the filtrates was determined by ICP-OES after appropriate dilution. The degree of transmetallation of the contrast agents with Zn^{2+} or Cu^{2+} ions in the plasma was evaluated using the percentage of Zn^{2+} or Cu^{2+} ions filtered through the membrane, which was calculated as Zn(Cu)% = (concentration of Zn^{2+} or Cu^{2+} in the filtrates)/(total Zn^{2+} or Cu^{2+} concentration before centrifugal filtration) × 100%.

In Vivo Transmetallation

The rats were randomly divided into five groups ($n=5$ each) for four contrast agents, GDCC-31, Omniscan, MutiHance, ProHance and a saline control. The rats were anesthetized with an intraperitoneal injection of a mixture of ketamine (45 mg/kg) and xylazine (6 mg/kg). The contrast agents were injected at a dose of 0.1 mmol-Gd/kg via a tail vein. The rats were immediately placed into metabolic cages after the injection. Urine samples were collected at 12 h pre-injection, 8 h post-injection (0–8 h) and then 24 h post-injection (8–24 h) from the metabolic box. The collected urine samples were centrifuged at 4,000 rpm for 15 min. The contents of Gd(III), Zn(II), Cu

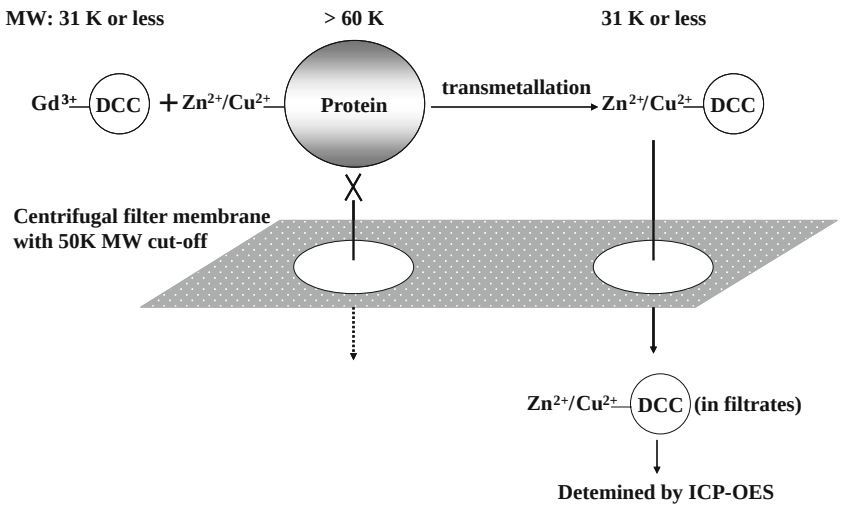

Fig. 1 Schematic illustration of the separation mechanism of Zn (II) or Cu(II) exchanged with GDCC-31 in plasma.

(II) and Ca(II) in the supernatant of the urine samples were determined by ICP-OES after appropriate dilutions.

Biodistribution of the Contrast Agents in Rats

The animals in the above study were sacrificed with an overdose of isoflurane at 48 h post-injection. The organ and tissue samples, including femur, heart, lung, liver, muscle, spleen and kidney, were collected and weighed. The tissue samples were then cut into small pieces and mixed with ultra-pure nitric acid (1.0 ml, 70%, EMD, Gibbstown, NJ). The tissue samples were liquefied within 2 weeks, and the solution was transferred to a centrifuge tube and centrifuged at 14,000 rpm for 15 min. The supernatant (0.2 ml) was diluted 10 times with de-ionized water and further centrifuged at 14,000 rpm for 15 min. The Gd(III) concentration in the final supernatant was measured by ICP-OES. The average Gd(III) content in each organ or tissue was calculated from the measured Gd(III).

Storage Stability of GDCC

The storage stability of solid and liquid forms of GDCC-35 was tested at different storage temperature for up to 14 months. Freeze-dried GDCC was stored in nitrogen at room temperature (~25°C), in the refrigerator (~5°C) and the freezer (~−20°C). GDCC saline solution at a Gd concentration of 0.33 M was stored in nitrogen at room temperature (~25°C) and in the refrigerator (~5°C). All samples were protected from light with aluminum foil. The molecular weight distributions of GDCC-35 under different storage conditions were analyzed with fast protein liquid chromatograph (FPLC, AKTA®) equipped with an analytical Superose 12 column at 1, 2, 5 and 14 months in storage. Gd(III) content in the GDCC was also analyzed by ICP-OES.

Statistical Evaluation

Statistical analysis was performed using an unpaired two-tailed Student's *t*-test (GraphPad Prism; GraphPad Software, San Diego, CA). A confidence interval of 95% ($P < 0.05$) was considered statistically significant.

RESULTS

Complexation Stability of GDCC

The complexation stability of GDCC and transmetallation effect was first evaluated in PBS buffer and under physiological concentrations with endogenous metal ions, including 2.5 mM of Ca^{2+}, 50 μM of Zn^{2+}, 1 μM of Cu^{2+} and 0.11 mM of citrate. The dynamic stability of the agent in PBS and in the presence of the metal ions is shown in Fig. 2. GDCC gradually lost ~15% of Gd(III) ion within approximately 45 min incubation and remained steady throughout the duration of study. In comparison, the Gd content in GDCC incubated with PBS did not change during the period of experiment, indicating high complexation stability of GDCC in the absence of the endogenous metal ions.

In Vitro Transmetallation in Rat Plasma

The percentage of GDCC-31, Omniscan, MultiHance and ProHance in aqueous solution filtered through was 96.0%, 98.7%, 96.8% and 98.6%, respectively (Table I), indicating that the contrast agents could completely filter through CF-50 membrane in ultrafiltration. On the other hand, no Zn^{2+} and Cu^{2+} ions were detected in the filtrates of blank plasma, verifying that the Zn^{2+} and Cu^{2+} ions bound to the proteins in the plasma and could not filter through CF-50

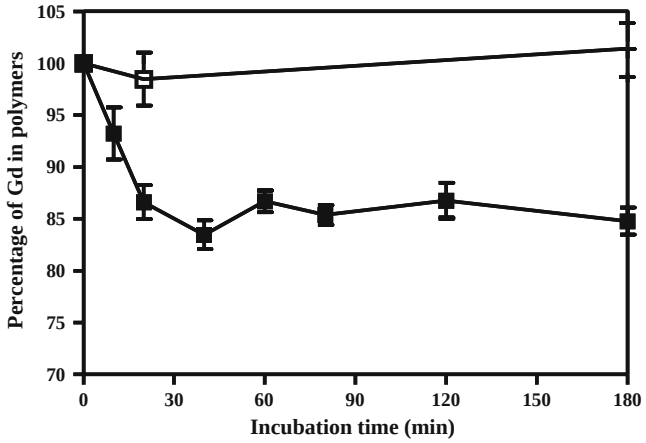

Fig. 2 Complex stability of GDCC in PBS (□) or in a PBS buffer containing 2.5 mM of $CaCl_2$, 0.11 mM of sodium citrate, 50 μM of $ZnCl_2$ and 1 μM of $CuCl_2$ at pH 7.4 and 37°C (■). Data presented as mean ± SD.

membrane. The results of the control study validated the effectiveness of the ultrafiltration for evaluating the transmetallation of the contrast agents in the plasma. After transmetallation, the chelates of Zn^{2+} and Cu^{2+} formed with ligands of the contrast agents could be readily filtered through the filtration membrane. The degree of transmetallation could be readily determined by measurement of Zn^{2+} and Cu^{2+} contents in the filtrates.

Fig. 3 shows the degree of transmetallation of the contrast agents with Zn^{2+} and Cu^{2+} in the rat plasma. The linear neutral contrast agent Omniscan resulted in the highest degree of transmetallation with Zn^{2+} (13.1%± 0.1%, Fig. 3A). The transmetallation of Omniscan was almost 10-fold higher than that of anionic linear agent MultiHance (1.4%±0.6%). No significant transmetallation with Zn^{2+} was observed for the macrocyclic agent ProHance. The biodegradable macromolecular contrast agent GDCC-31 resulted in similar degree of transmetallation (1.6%±0.1%) with Zn^{2+} as MultiHance. Transmetallation of Omniscan, MultiHance and GDCC-31 with Cu^{2+} ions in the plasma was similar to that of ProHance, much less than transmetallation with Zn^{2+} (Fig. 3B).

Table I Gd^{3+} Filtration Ratio of Different Contrast Agents from Original Aqueous Solution and Zn^{2+}/Cu^{2+} Filtration Ratio from Rat Plasma

	Gd^{3+}	Zn^{2+}	Cu^{2+}
MultiHance	96.8%±2.6%	–	–
ProHance	98.6%±0.7%	–	–
Omniscan	98.7%±2.1%	–	–
GDCC31K	96.0%±3.1%	–	–
Rat plasma	–	−1.2%±0.3%	−0.5%±0.2%

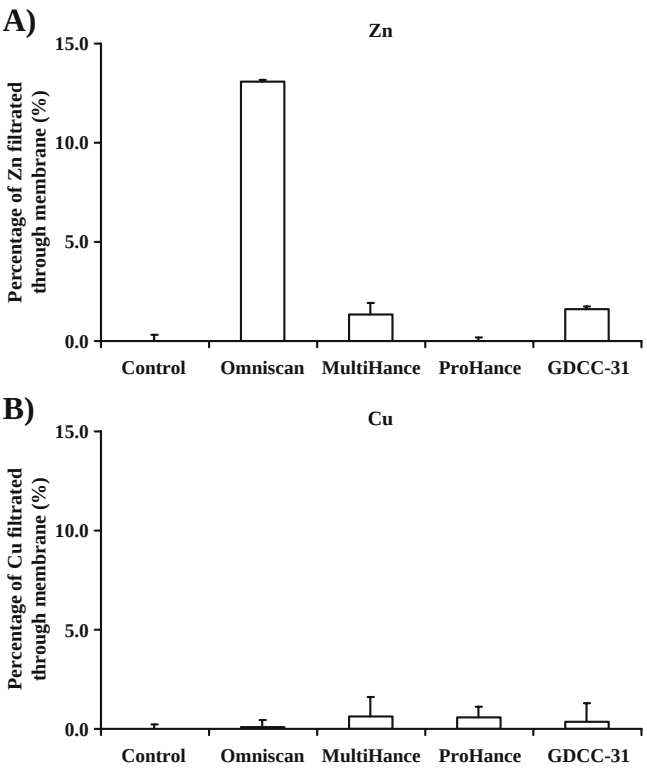

Fig. 3 Transmetallation of Gd chelating agents with Zn^{2+} (**A**) and Cu^{2+} (**B**) in rat plasma.

In Vivo Transmetallation

In vivo transmetallation of the contrast agents with Zn^{2+} or Cu^{2+} ions in the plasma was evaluated based on the metal ion contents in the urine samples after intravenous administration of the contrast agents. Fig. 4 shows the Gd (III) concentration in the urine samples collected before and during 0–8 h and 8–24 h after injection of the contrast agents. It appears that the contrast agents mostly excreted in the first 8 h post-injection. The macrocyclic agent ProHance had the highest concentration in the urine samples.

Fig. 5 shows the Zn(II) concentration in the urine samples collected before and during 0–8 h and 8–24 h

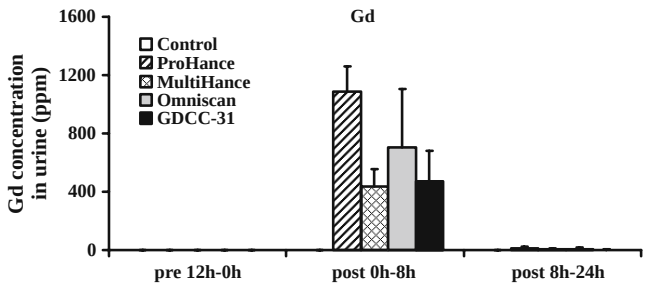

Fig. 4 Concentrations of gadolinium(III) excreted in urine before and after administration of the contrast agents. Data presented as mean ± SD.

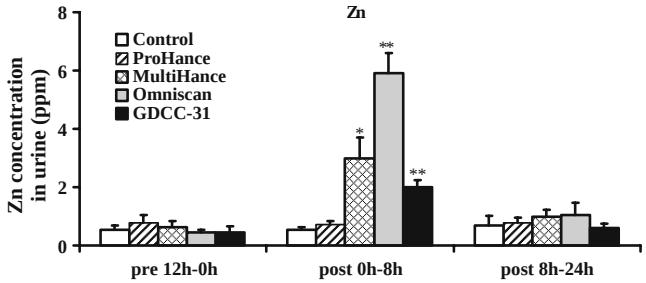

Fig. 5 Concentrations of zinc(II) excreted in urine before and after administration of the contrast agents (**$P<0.001$, *$P<0.005$). Data presented as mean ± SD.

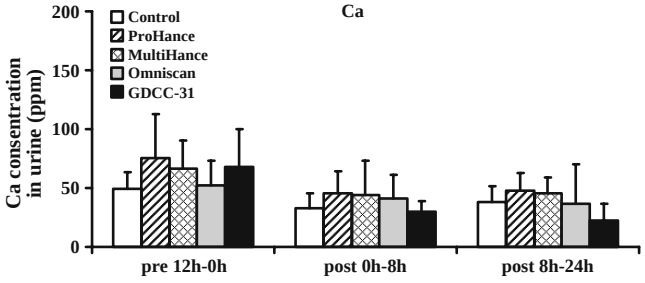

Fig. 7 Concentrations of calcium(II) excreted in urine before and after administration of the contrast agents. Data presented as mean ± SD.

after injection of the contrast agents. Significant increase of Zn(II) concentration was detected in the urine samples collected in the first 8 h post-injection for the linear agents, Omniscan ($p<0.001$) and MultiHance ($p<0.005$), and GDCC-31 ($p<0.001$), as compared to the control group injected with saline and the samples collected before injection. Omniscan resulted in the highest Zn(II) concentration in the urine samples. GDCC-31 resulted in a slightly lower Zn(II) concentration than MultiHance in the urine samples. No significant difference was observed between the ProHance group and the control group ($p=0.92$). The Zn(II) concentration in the urine samples collected after 8 h returned to the baseline level for all agents. No significant change of the Cu(II) or Ca(II) concentration was observed in the urine samples collected after injection of the contrast agents, Figs. 6 and 7.

Biodistribution in Rats

Fig. 8 shows the biodistribution of Gd(III) in the major organs and tissues, including the femur, heart, kidneys, liver, lung, muscle, and spleen of rats 2 days after a single injection of MultiHance, ProHance, Omniscan and GDCC-31 at a dose of 0.1 mmol-Gd/kg. Approximately 1.5% and 0.8% of injected GDCC-31 was measured in the liver and kidneys at 48 h post-injection, higher than the small molecular contrast agents ($p<0.05$). The retention of GDCC-31 in other organs and tissues was at a comparable minimal level as the low molecular weight contrast agents. Omniscan also had a significantly higher retention in the liver and kidneys than other small molecular contrast agents ($p<0.05$).

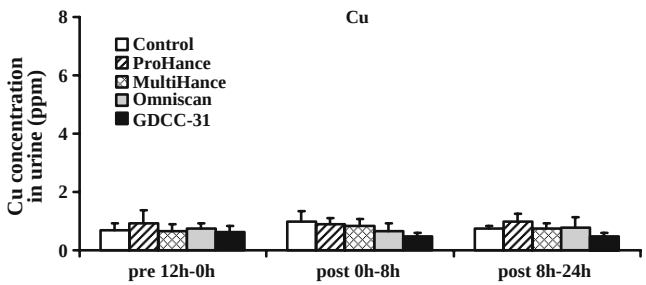

Fig. 6 Concentrations of copper(II) excreted in urine before and after administration of the contrast agents. Data presented as mean ± SD.

Storage Stability of GDCC

Fig. 9 shows the molecular weight distribution of the biodegradable macromolecular contrast agent GDCC after storage for 14 months under different conditions in both solid form and solution form. Since disulfide bonds may be sensitive to oxidation and photo-oxidation, the samples were stored in dark and in the presence of nitrogen. No significant change of the molecular weight distribution was observed for the sample after the long-term storage, as shown in size exclusion chromatograms, indicating no breakdown of the polymer chains. The Gd content in GDCC did not change after storage for 14 months. The polydisulfides exhibited good stability for long-term storage in dark and in nitrogen.

DISCUSSION

Polydisulfide Gd(III) complexes have been designed as biodegradable macromolecular MRI contrast agents to alleviate the safety concerns related to the slow excretion of Gd(III)-based macromolecular MRI contrast agents. Macromolecular MRI contrast agents have shown superior enhancement for blood pool imaging and cancer imaging over low molecular weight contrast agents. However, the safety concern related to their slow excretion has limited further development of macromolecular contrast agents. The polydisulfide Gd(III) complexes provide strong blood pool enhancement and can readily breakdown into small Gd(III) chelates via reduction of the disulfide bonds in the polymer chains by endogenous free thiols in the plasma. The resulted small Gd(III) chelates can be readily excreted via renal filtration with minimal long-term tissue accumulation after the contrast-enhanced MRI.

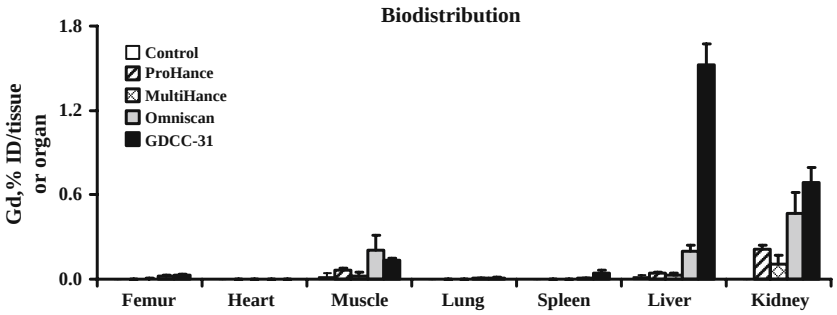

Fig. 8 Biodistribution of gadolinium(III) in rats 2 days after intravenous injection of MultiHance, ProHance, Omniscan and GDCC-31 at a dose of 0.1 mmol Gd/kg. Data presented as mean ± SD.

Our previous studies have shown that Gd-DTPA cystamine copolymer (GDCC) is a promising lead biodegradable macromolecular MRI contrast agent. GDCC initially acts as a macromolecular contrast agent, resulting in superior contrast enhancement over low molecular weight contrast agent, e.g. Gd(DTPA-BMA), for blood pool imaging and tumor imaging. It is effective to determine vascular permeability of angiogenic tumor microvessels, while low molecular weight contrast agents often result in overestimated vascular permeability (15–18). GDCC can also accurately assess therapeutic efficacy of anti-angiogenic therapy and other cancer treatment. *In vivo* complexation stability of GDCC is a critical parameter for further development of biodegradable macromolecular contrast agent. The Gd(III) chelates approved for clinical applications have high thermodynamic stability. However, transmetallation of these agents with endogenous Zn^{2+}, Cu^{2+} and Ca^{2+} may result in dissociation of the chelates and release of toxic Gd(III) ions (3,4).

In this study, we further investigated the transmetallation stability of a lead biodegradable macromolecular MRI contrast agent, Gd-DTPA cystamine copolymers (GDCC), both *in vitro* and *in vivo* in comparison with the clinical contrast agents, MultiHance, Omniscan and ProHance. The results have shown that the structure of the contrast agents has a significant effect on transmetallation with endogenous metal ions, consistent with previous reports (1–3). The biodegradable macromolecular contrast agent GDCC had similar *in vitro* and *in vivo* kinetic stability as the linear agent MultiHance. GDCC also showed good stability for long-term storage in dark and the absence of oxygen.

The *in vitro* transmetallation of the agents was investigated by ultrafiltration in rat plasma, a newly developed method in this study. The control study showed that the endogenous metal ions Zn^{2+} and Cu^{2+} did not pass through the filtration membrane of the selected size because they bound to the proteins in the plasma. These ions were only detected in the filtrate when they were removed from the proteins after transmetallation. The method was validated by measuring the concentration of the contrast agents in the filtrate after filtration. Centrifugal filtration was a convenient and accurate alternative approach for the study of transmetallation in the plasma along with other reported methods, including instant thin-layer chromatography-silica gel (ITLC-SG) (19), high-performance liquid chromatography (HPLC) (20,21), and relaxometric method (22).

It has been reported that Zn(II) ions are the main cause of transmetallation of gadolinium-based MRI contrast agents, especially the linear agents, both *in vitro* and *in vivo* (3,4,19–22). We showed here that GDCC was stable in the absence of the endogenous metal ions. Zn(II) in rat plasma resulted in significant release of Gd(III) ions from Omniscan, MultiHance and GDCC via transmetallation. Omniscan resulted in the most transmetallation with Zn(II), and GDCC had similar transmetallation as the MultiHance. The macrocyclic agent ProHance was stable against transmetallation. It was noticed that the degree of transmetallation induced by Zn(II) in the plasma was much less than that by free Zn(II) ions in buffer (19–22). The binding of Zn (II) in the proteins might inhibit further transmetallation with the contrast agents. The order of transmetallation of all the tested agents with plasmic Zn(II) was Omniscan > MultiHance ≈ GDCC > ProHance (Fig. 3A).

It was speculated that Cu^{2+} in the plasma might cause significant transmetallation. The transmetallation of GDCC, MultiHance and Omniscan with plasmic Cu(II) was much less significant than that with Zn(II) (Fig. 3B), although the concentrations of Zn^{2+} and Cu^{2+} in rat plasma was similar as determined by ICP-OES (data not

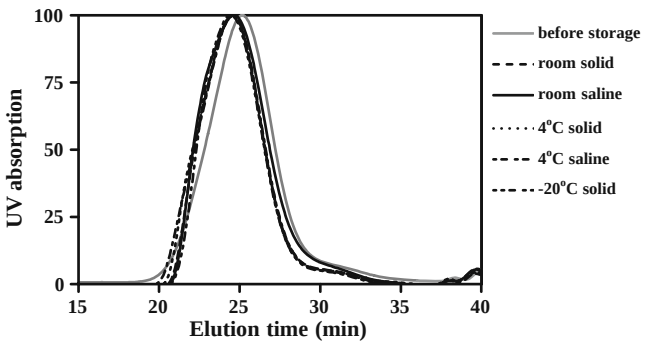

Fig. 9 Size-exclusion chromatograms of GDCC before and after 14 months storage under various conditions.

shown). Slight transmetallation with Cu(II) was observed for the macrocyclic agent ProHance in the plasma. The results indicated that the Gd(III)-based contrast agents were generally stable against transmetallation with Cu(II) in plasma. The transmetallation of the contrast agents with Ca^{2+} in the plasma could not be determined with centrifugal filtration because Ca^{2+} ions in plasma were mostly in free form and could readily filter through. Nevertheless, the thermodynamic stability constants of the chelates of Ca(II) and the ligands of the MRI contrast agent are approximately 10 orders of magnitude lower than the corresponding Gd(III) chelates (2). It is considered that Ca^{2+} is inert for transmetallation with Gd(III)-based contrast agents.

The results of *in vivo* transmetallation of GDCC and other tested agents were consistent with the *in vitro* data. Omniscan resulted in the highest Zn(II) concentration in the urine samples collected in the first 8 h post-injection. GDCC resulted in slightly lower Zn(II) concentration than MultiHance. No increase of Zn(II) concentration was observed in the urine samples collected from the rats injected with ProHance. No increase of Ca(II) and Cu(II) concentration was observed in all urine samples, suggesting that the endogenous Zn(II) was the main cause for transmetallation of open-chain Gd(III) chelates. The order of *in vivo* stability of the contrast agents against transmetallation was the same as what was observed in the *in vitro* study (Figs. 3A and 5). The stability of the contrast agents was clearly correlated to their structural characteristics (23–25). Macrocyclic molecules, where Gd^{3+} is caged in the pre-organized cavity of the ligand, are much more stable than linear molecules because a much higher activation energy barrier has to be overcome for both the complexation and de-complexation of macrocyclic agents. The linear open-chain Gd(III) chelates had relatively high conformational mobility, while the macrocyclic chelates had tight packing and high conformational rigidity.

The biodegradable macromolecular contrast agent showed similar stability as MultiHance against transmetallation with Zn(II) both *in vitro* and *in vivo*. Although GDCC had similar non-ionic chelate structure as Omniscan, it resulted in significantly less transmetallation with Zn(II) than Omniscan. One possible reason was that Omniscan had an excess of calcium-chelated ligand (25 mM) in its formulation. Since the thermodynamic stability constant of Zn(DTPA-BMA) is much higher than that of Ca(DTPA-BMA) (2), the excess Ca(DTPA-BMA) could readily exchange with Zn(II) ions and remove Zn(II) ions from the plasma proteins. Another possible explanation was that GDCC was a polymeric complex with multiple chelating sites in its backbone, which might increase the stability of the agent via polydentate effect.

The short-term tissue retention of GDCC was investigated in rats at 48 h after the injection. The long-term tissue accumulation of GDCC was reported previously in rats at 10 days post-injection (13). Due to its biodegradability, GDCC resulted in minimal long-term tissue retention similar to low molecular contrast agents independent of its molecular weight (13,14). Its biodistribution at 48 h post-injection would be helpful to understand how rapidly the agent could be eliminated from the body. The study showed that GDCC-31 had comparable low retention as the tested low molecular weight contrast agents in most organs and tissues, including the femur, heart, lung, muscle and spleen. Its retention in the liver and kidneys was only slightly higher than the small molecular agents at 48 h post-injection (Fig. 8), suggesting that *in vivo* degradation of GDCC macromolecules might be a gradual process. As demonstrated in our previous study, GDCC with molecular weights of 18 and 60 KDa resulted in minimal tissue accumulation comparable to Omniscan at 10 days after intravenous injection (13). In comparison, a non-degradable macromolecular agent, Gd-DTPA 1,6-hexanediamine copolymers, resulted in much higher tissue accumulation in rats at 10 days after injection (14). The results in study showed that over 95% of the injected GDCC was excreted in the first 2 days post-injection, which was much higher than the excretion of non-degradable macromolecular contrast agents after 10 days post-injection (14). The retention of GDCC was much lower than that of other macromolecular Gd(III) complexes. For example, a Gd-DTPA polypropyleneimine dendrimer (generation 2) conjugate (7 kDa) resulted in the retention of 45% of injected dose in rats 14 days after injection (26). Carboxymethyl hydroxyethyl starch-(Gd-DO3A) had approximately 47% of injected dose in the body of experimental animals seven days after injection (27). Although the biodegradable macromolecular contrast agent excreted slightly more slowly than the small molecular contrast agents, it showed much more rapid excretion than non-degradable macromolecular contrast agents and could be completely excreted in an extended period post-injection.

CONCLUSIONS

We have established a new method of using ultrafiltration to evaluate transmetallation of the Gd(III)-based MRI contrast agents with endogenous metal ions in the blood plasma. The results showed that Omniscan was most sensitive to transmetallation with Zn(II) ions in the plasma, and ProHance was most stable against transmetallation. The biodegradable macromolecular contrast agent GDCC demonstrated similar kinetic stability against transmetallation with Zn(II) as MultiHance. The same *in vivo* stability order was observed for the agents based on elevated excretion of Zn(II) in the urine samples collected within

8 h after the administration. GDCC resulted in similar short-term tissue retention as the small molecular contrast agents in most organs and tissues with slightly higher retention in the kidneys and liver. The biodegradable macromolecular contrast agent GDCC showed good kinetic stability toward transmetallation and rapid excretion via renal filtration. GDCC was stable for storage up to 14 months in dark and the presence of nitrogen. GDCC is promising for further preclinical and clinical development as a biodegradable macromolecular MRI contrast agent.

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