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Original contribution

The use of a binary chelate formulation: Could gadolinium based linear contrast agents be rescued by the addition of zinc selective chelates?

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ABSTRACT

Tissue and bone retention of gadolinium based contrast agents (GBCAs) has become a clinical concern because of the potential short and long term toxic effects of free gadolinium. This is a critical problem for most open-chain agents that more readily transmetallate in vivo, in comparison to macrocyclic compounds. Gadolinium diethylene tri-aminepentaacetic acid bis-glucosamide (Gd-DTPA-BIGA) is an experimental, open-chain contrast agent which has a significantly increased relaxivity coefficient in comparison to other GBCAs. This results in greater signal intensity and improved contrast enhancement. These superior imaging qualities initiated a search for a solution to the transmetallation of this agent. Plasma zinc is a well-known GBCA transmettalation agent. Since the base chelate of Gadodiamide (Gd-DPTA-Bis-Methylamide or Omniscan), DTPA-Bis-Methylamide (DTPA-BMA), readily transmettalates with and binds serum zinc, we hypothesized that a plasma "zinc sink," may significantly reduce transmetallation of linear agents. 5% DTPA-BMA was added to a formulation of Gd-DTPA-BIGA, which was tested against the original formulation of Gd-DTPA-BIGA with 0.2% of the base chelate DTPA-BIGA. These formulations, including gadodiamide, were labeled with $^{153}GdCl_3$ followed by infusion into cohorts of Sprague Dawley rats which were sacrificed at 1, 30 and 60 days. Internal organs were harvested, along with blood, skin and femur, and analyzed for residual gadolinium. A subset of tissues were also interrogated with ICP-MS. Labeled Gadodiamide and saline where used as controls.

Conclusion: The addition of 5% DTPA-BMA, as a zinc binding agent, reduced the transmetallation of the linear agent Gd-DTPA-BIGA, in comparison to its original formulation supplemented with 0.2% BIGA. This result indicates that supplementing linear GBCAs with ancillary chelates may hold promise for reducing, or eliminating the biological archiving of gadolinium in tissues. In addition, this paper provides valuable animal data on the long term retention of gadolinium from linear based contrast agents.

1. Introduction

GBCAs are invaluable adjuvants for magnetic resonance imaging (MRI). Since 1985, in excess of 460 million doses of GBCAs have been used in conjunction with MRI [1]. Adverse reactions were, and still are, extremely low [2]. We initially reported that GBCAs left gadolinium behind in bone tissue of humans undergoing hip replacement in 2005 [3,4]. In 2006 it was discovered that, in patients with poor renal function (i.e. GFR $< 30\,mL/min/1.73\,m^2$), the inability to efficiently excrete the gadolinium based contrast agent could lead to a buildup of free gadolinium, triggering nephrogenic systemic fibrosis (NSF) [5]. These clinical cases are rare and occur in a small number of renally compromised patients. More recently, gadolinium deposition has been discovered in the dentate nucleus (DN) and the globus pallidus (GP) after serial contrast enhanced MRI procedures. However, the clinical significance of this accumulation, if any, is unknown [6]. Quantitatively, macrocyclic GBCA deposition is far less than linear GBC deposition [6–8]. These revelations have prompted the FDA to require manufacturers of all GBCAs, both linear and macrocyclic, to provide additional cautionary information in their product inserts [9,10]. In addition, the European Medicines Agency has either restricted, or suspended the use of linear GBCAs in the European Union [11]. More

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recently it has been suggested that the newly hypothesized "glympahtic" system is responsible for both deposition and clearance of gadolinium in and from brain tissue [12]. In general, these conditions are recognized as a "family of disorders" which are associated with the clinical use of GBCAs [13]. These disorders are grouped into NSF; severe acute adverse events, thought to be caused by an allergic or a physiological reaction to a GBCA; gadolinium storage condition, or gadolinium accumulation in tissues and gadolinium deposition disease. Gadolinium deposition includes symptoms characteristic of these disorders, but which occur in patients with normal, to near normal renal function [13].

In past experiments we have looked at the transmetallation of gadolinium chelates with zinc citrate. It was noted with some interest that Gd-DPTA-Bis-Methylamide (Gadodiamide, or Omniscan) readily transmetallates. It is hypothesized that gadolinium is released by Gadodiamide and that zinc is bound by the free chelate in the presence of zinc citrate. Gadolinium was readily transmetallated to the citrate molecule at a molar ratio of 3 to 1, as determined by HPLC [14]. Gadodiamide has been widely used in millions of human doses and is the linear agent most commonly associated with NSF [4]. We theorize that the base chelate of Gadodiamide, DTPA-BMA, might be better as an agent for scavenging zinc from the body than to actually carry gadolinium. Furthermore, in previous experiments using Gadodiamide with 5% DTPA-BMA, about 70% of the serum zinc was extracted from a single clinical dose in human volunteers [14]; so it is clearly capable of binding zinc. Gadodiamide is commercially formulated with 5% excess DTPA-BMA. Two formulations of Gd-DTPA-BIGA were studied in comparison to commercial Gadodiamide. The original GdDTPA-BIGA formulation includes 0.2% DTPA-BIGA, but in this study the free DTPA-BIGA was replaced with 5% DTPA-BMA. The idea is to create a binary compound using 5% DTPA-BMA as an in vivo "zinc sink" to enhance the safety of linear agents which have excellent contrast properties and very low acute toxicities. In previous attempts to measure objectively and differentially the retention of Gadolinium in animal models, we have used inductively coupled plasma atomic emission spectroscopy (ICP-AES). Unfortunately, the amount of gadolinium left behind is small and it is difficult to measure significant differences in the retention of gadolinium between different agents as it is close to the limit of detection with ICP-AES without giving multiple high dose contrast injections. Hence, another aim of this study was to look at gadolinium retention in a much more sensitive fashion from a single gadolinium dose. Here we use $^{153}GdCl_3$ labeled GBCAs as well as inductively couple plasma mass spectroscopy (ICP-MS) both of which are much more sensitive than ICP-AES, in the hope of being able to more effectively discriminate between agents using a single dose.

Prior research has also utilized ^{153}Gd to follow the retention of both open chain and macrocyclic compounds. Tweedle et al. analyzed gadolinium deposition, over a relatively short period of 14 days, in both rats and mice with a ^{153}Gd dose of 1 μCi per rat [15]. The Tweedle et al. study also included Gadodiamide.

In this study we decided to significantly increase the amount of $^{153}GdCl_3$ to approximately 60 μCi per rat to improve sensitivity in order to accurately access long term gadolinium deposition. Utilizing $^{153}GdCl_3$, which has a relatively long half-life of 241.6 days, allows one to accurately measure retained Gd over a period of months. It should be noted that the accumulation of gadolinium with linear chains in recent animal studies, is in general, clinically unrealistic, as few patients will undergo the number of contrast enhanced MRIs reported in this literature (i.e. 20 injections over 4 weeks) [12,16].

Given the potential long term residual effects of gadolinium deposition in the brain and other organs by the use of linear agents, we explore whether gadolinium transmetallation can be precluded, or reduced by the addition of a supplemental chelate/compounds to Gd-DTPA BIGA, which is being explored as a potential pre-clinical candidate with improved relaxivity. This strategy would allow the continued clinical use of open-chain agents with superior imaging qualities and specific clinical applications.

In the current study, we investigate the potential of "rescuing" a linear agent, Gd-DTPA-BIGA with the supplemental chelate DTPA-BMA. We chose Gd-DTPA-BIGA as it has twice the relaxivity and has also shown better kinetic stability than Gd-DTPA-BMA in vitro [17].

2. Materials and methods

2.1. Contrast agents

Gadodiamide, (GE Healthcare, Chicago, Ill; J100, expiration: 13 Jan 2020) and two formulations of Gd-DTPA-BIGA (Magnetic Research Inc., Provo, Utah, USA) were used in this study. One Gd-DTPA-BIGA formulation included an additional 0.2% DTPA-BIGA of the base chelate, while the second was supplemented with 5% DTPA-BMA. Original animal studies and formulation of Gd-DTPA-BIGA were performed with added 0.2% DTPA-BIGA [14]. Earlier experiments demonstrate that the addition of the two glucose molecules to an open-chain compound to create Gd-DTPA-BIGA, resulted in a 2-fold enhancement increase in both liver and renal tissue compared to the linear agent gadopentetate dimeglumine (Gd-DTPA, or Magnevist; Bayer HealthCare, Whippany, NJ) [18]. In addition, Gd-DTPA-BIGA shows better T1 relaxation than either Magnevist, or the macrocyclic Gd-HPDO3A (ProHance) [18].

2.2. Formulation of $^{153}GdCl_3$ labeled GBCAs

10 mCi of $^{153}GdCl_3$ in 2 × 0.5 ml volumes were purchased from Eckert & Ziegler (Valencia, Ca; product code: 6153). The labeled gadolinium concentration was 1.01 mmol per liter. The volumes were combined to 1 ml and diluted to 10 ml with physiological saline (Baxter, Deerfield. Ill; 2F7124). 2 ml of this dilution was added to each GBCA/chelate formulation for a total of 133 μM, in a total volume of 15 ml for each formulation.

Both BIGA and Gd-DTPA-BIGA were synthesized using previously reported methodology (see Appendix A for synthesis of BIGA and Gd-DTPA-BIGA). Purity was analyzed by HPLC, thin layer chromatography (TLC), IR, mass spectroscopy, and elemental analysis. Gd-DTPA-BIGA contained 2 waters for a formula weight of 751.71 g per mole. A 0.5 M solution of Gd-DTPA-BIGA was prepared and to this was added 0.2%, by molar weight, BIGA. An additional 1.4 μmol of DTPA-BIGA was added to the 15 ml stock solution containing Gd-DTPA-BIGA and the $^{153}GdCl_3$, to compensate for the added label. Each stock solution for the chelates was prepared identically.

Gd-DTPA-BIGA, mixed with 5.0 M percent excess of DTPA-BMA. DTPA-BMA was synthesized according to published literature references (see Appendix A for synthesis of DTPA-BMA). It was a hydroscopic solution and was created by adding 0.03 mmol of a 10% DTPA-BMA stock solution to a final diluted volume of 15 ml of solution containing $^{153}GdCl_3$ as well as unlabeled GdDTPA-BIGA.

Gadodiamide, prepared by G.E. Healthcare was formulated by adding the 0.002 μmol of $^{153}GdCl_3$. All agents were incubated at room temperature for > 24 h. Since the amount of labeled gadolinium was negligible compared to the 5% BMA, no additional BMA was added. The agents were chromatographed using TLC (Whatman 28419593) with a solvent of 0.1 M NaAc and methanol (1:1.5, pH 6.1). Two separate methods were used to detect the presence of free gadolinium in the chelate formulations. In one method TLC strips were developed with xylenol orange. Here the movement of free gadolinium was noted qualitatively. Samples of gadolinium chloride were used as unchelated controls. In the second method, the previous TLC plate was scored into a matrix. The matrix squares were excised/cut into individual squares, taking care to ensure that observed migration points were located in the center of their individual squares. Each square was individually counted in a Wallac Wizard 1470 Gamma Counter. No radioactivity was found, above background, in the corresponding squares of the formulated products in comparison to the control. Thus, any Gadolinium

present in the formulations would be below the LOD which is the background count of radioactivity. This was repeated three times.

The final chelate preparations were brought to a pH of between 6.5 and 7 by adding a tiny amount of 1.25 N sodium hydroxide.

2.3. Animals

Seventy-two Sprague Dawley Rats (Crl:SD, strain 400) age 6 weeks (125–150 g), to ensure skeletal maturity, were purchased from Charles River (Wilmington, Massachusetts, USA) [19]. Animals were kept at 22 °C at 24% humidity with a 12 h night and day cycle. Water and standard rodent food were supplied ad libitum. Animals were maintained in accordance with the Animal Welfare Act and Animal Welfare Regulations (USDA 2013).

2.4. Study setup

Animals were divided into 12 groups of 6 rats (3 females and 3 males/group). These numbers were selected based on FDA recommendations [20]. Each group of 6 animals was assigned to a specific GBCA or a control: Omniscan (commercial formulation contains 5% DTPA-BMA), Gd-DTPA-BIGA 0.2% DTPA-BIGA, Gd-DTPA-BIGA 5% DTPA-BMA and saline. Animals were placed in groups to be sacrificed at intervals of 1, 30 and 60 days (Table 1).

Rats were tail infused (Terumo SURFLO® Winged Infusion Set, 25Gx3/4″) by a pediatric anaesthesiologist to assure accurate tail vein access. A small amount of saline was injected to test the catheter. A total dose of 0.1 mmol/kg of animal weight of a ^{153}Gd labeled GBCA in saline (0.9% Sodium Chloride, Irrigation, Baxter 2F7124), chased by 1 ml of saline was injected.

Bedding was frequently changed during days 1–4, or until counts could no longer be detected in the feces or shavings by a hand held monitor.

Animals from the GBCA and saline control groups were sacrificed at 1, 30 and 60 days after being euthanized via 2–3 min in a container saturated with CO_2 via evaporation of dry ice.

Animals were immediately dissected and the following organs were removed and weighed: blood, skin, brain, lungs, liver, kidneys, intestine, fat, muscle, spleen and femur. A Wallac Wizard 1470 Gamma Counter was use to count samples. The instrument was calibrated with ^{125}I as per the manufacturer's recommendation. Accurate calibration was confirmed by an ELS Life Sciences Technologies Inc. (Missouri City, Texas) qualified Field Technician. A control solution of the same lot of $^{153}GdCl_3$ used in GBCA formulations, with known CPM, was used in all counting events to monitor ^{153}Gd decay. Back ground was monitored with 3 blank tubes per each counting event. All samples were counted 3 times for 1 min each. An average score for these 3 counts was taken and normalized to represent average counts per gram of tissue. CPM were converted to ng-Gd/g-tissue by calculating the mass of gadolinium in the initial dose and the remaining amount inferred by the drop in counts at 1, 30 and 60 days, represented as the percent of the remaining initial dose. A blank tube was utilized as a negative control as well as the organs for animals treated with saline. Data at 30 and 60 days were adjusted for decay of $^{153}GdCl_3$. Finally, results were converted to µg-Gd/kg tissue as a comparable to the original dosing of 0.1 mmol/kg of animal weight.

Table 1
Experimental design; distribution of animals and formulations.

	Gadodiamide	Gd-DTPA-BIGA 5% BMA	Gd-DTPA-BIGA 0.2% BIGA	Saline
1 day	3F/3M	3F/3M	3F/3M	3F/3M
30 days	3F/3M	3F/3M	3F/3M	3F/3M
60 days	3F/3M	3F/3M	3F/3M	3F/3M

2.5. ICP-MS

A subset of frozen samples of target organs: femur, kidney, brain and liver were received in 5 ml tubes closed by parafilm. All samples had been previously counted for ^{153}Gd. Samples were thawed in a refrigerator overnight prior to preparation. 1.0 ± 0.3 g of each sample was transferred into a weighed, acid-leached, 50 ml PTFE block-digester vial using a metal spatula, tweezers, and/or scalpel. Samples were weighed to the nearest 0.1 mg before adding 10.0 ml of concentrated trace metal grade nitric acid and refluxed at 120 °C for 2 h in a graphite block digester (SCP Science, Quebec, Canada). Digested samples were then transferred into acidleached polypropylene centrifuge tubes and stored until all organ samples completed the digestion process.

All samples from the same organ (plus six chemical blanks) were diluted 1:20 by volume (or 1:40 for the femur samples in order to reduce the matrix from Ca) in polystyrene test tubes using 2.4% trace metal grade nitric acid and calibrated pipettes (Eppendorf, Hauppauge, New York, USA). An internal standard of 10 ppb Indium was added to each sample dilution. Actual dilution factor by weight was calculated assuming a density for the digest of 1.41 g/ml. A total dilution factor from the wet tissue of about 250 (or 500 for femur samples) was then run in an inductively coupled plasma mass spectrometer (Agilent 7500ce, Santa Clara, California, USA), together with a calibration curve containing 0, 0.1, 0.5, 1.0, 5.0 and 10.0 ng/mL of Gd, Eu and Ba. Samples and calibration curves were introduced in the mass spectrometer using a syringe driven loading system at 50 µl/min (syringeFAST) through a PFA nebulizer, dual-pass quartz spray chamber, sapphire injector (Elemental Scientific, Omaha, USA), and platinum cones (Spectron, Ventura, California, USA). Serial dilutions (1:10, 1:20, 1:40 and 1:100) were studied for one femur and one kidney sample, with results agreeing within 5% of the average value (see Appendix B for detailed ICP-MS methods).

2.6. Statistical techniques

All CPM data was corrected for decay, including the formulations, based on the manufacturer's activity date of $^{153}GdCl_3$ and the date on which the samples were counted. Analysis of variance was used to compare mean count values for the different solutions. Statistical significance was assessed using the *t*-test, with corresponding *p* values reported in the results. Post hoc comparisons of the means were performed using the Student-Newman-Keuls (SNK) test at the 0.05 level of significance. Statistics were computed in SAS 9.4 (SAS Institute, Cary, NC, USA, 2012). The means and standard deviations were calculated for the ICP-MS values for the formulation groups in Table 1. A ratio of ICP-MS to CPM values, converted to µg/kg of tissue, was calculated for each organ at each time point.

3. Results

Mean %ID/g-tissue were calculated according to organ, agent, and days. The association between mean measures, compound and days was not dependent on sex. Hence, the results are collapsed over male and female rats. The interaction terms tested whether the association between the mean values and days varied by compound. For example, with liver the mean score on day 1 is significantly greater for Gd-DTPA-BIGA 0.2% DTPA-BIGA and similar for Gadodiamide and Gd-DTPA-BIGA 5% DTPA-BMA. There is no difference in mean scores among the compounds for days 30 and 60. The tendency is for Gadodiamide and Gd-DTPA-BIGA 5% DTPA-BMA to have lower mean scores than Gd-DTPA-BIGA 0.2% DTPA-BIGA, but the results are variable by organ; for example, for blood Gd-DTPA-BIGA 5% DTPA-BMA has lower retention at 1 day, while both Gd-DTPA-BIGA 0.2% DTPA-BIGA and 5% DTPA-BMA are less at 30 days, yet there is no difference among all 3 agents at 60 days. In brain tissue Gadodiamide and Gd-DTPA-BIGA 5% DTPA-BA

Table 2
Comparative mean values of gadolinium in μg-Gd/kg of tissue (6 animals/group) for gadodiamide and Gd-DTPA-BIGA supplemented with either 0.2% DTPA-BIGA or 5%DTPA-BMA from ^{153}Gd data. The lowest values are italicized while bolded values are significantly different from the other values at that time ($p < 0.05$).

	Day 1			Day 30			Day 60		
	Gadodiamide 5% DTPA-BMA	Gd-DTPA-BIGA 0.2% DTPA-BIGA	Gd-DTPA-BIGA 5% DTPA-BMA	Gadodiamide 5% DTPA-BMA	Gd-DTPA-BIGA 0.2% DTPA-BIGA	Gd-DTPA-BIGA 5% DTPA-BMA	Gadodiamide 5% DTPA-BMA	Gd-DTPA-BIGA 0.2% DTPA-BIGA	Gd-DTPA-BIGA 5% DTPA-BMA
Blood	**8.5**	6.0	*4.7*	**1.4**	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Brain	16.1	***10.1***	14.0	9.4	*7.5*	8.4	8.0	9.1	*6.2*
Fat	48.0	**85.3**	*34.7*	*4.8*	**21.1**	6.9	*2.7*	**9.7**	3.9
Femur	619.3	652.1	*486.9*	746.8	764.5	*736.0*	670.8[a]	741.7	***549.2***
Heart	*71.1*	**119.8**	74.9	30.7	40.6	*23.1*	*10.4*	**26.5**	13.8
Intestine	*113.3*	161.3	163.7	*9.8*	16.4	11.3	***3.8***	12.8	8.5
Kidneys	4456.1	4427.3	***3629.6***	482.4	603.8	*467.8*	*160.5*	292.4	243.3
Liver	*336.2*	**703.5**	390.2	60.3	90.8	*57.8*	*15.7*	28.2	17.5
Lungs	237.2	*233.1*	**554.5**	*40.9*	55.0	50.8	*26.7*	38.9	27.2
Muscle	51.9	74.0	*38.5*	*9.4*	26.0	12.0	*2.6*	**8.4**	4.0
Skin	*208.2*	**483.4**	260.8	*42.4*	120.1	59.5	*32.6*	**83.1**	50.5
Spleen	375.8	495.8	*371.1*	138.6	*100.4*	168.6	112.3	86.1	*63.0*

[a] This value is not significantly different from the other 2 values in this group.

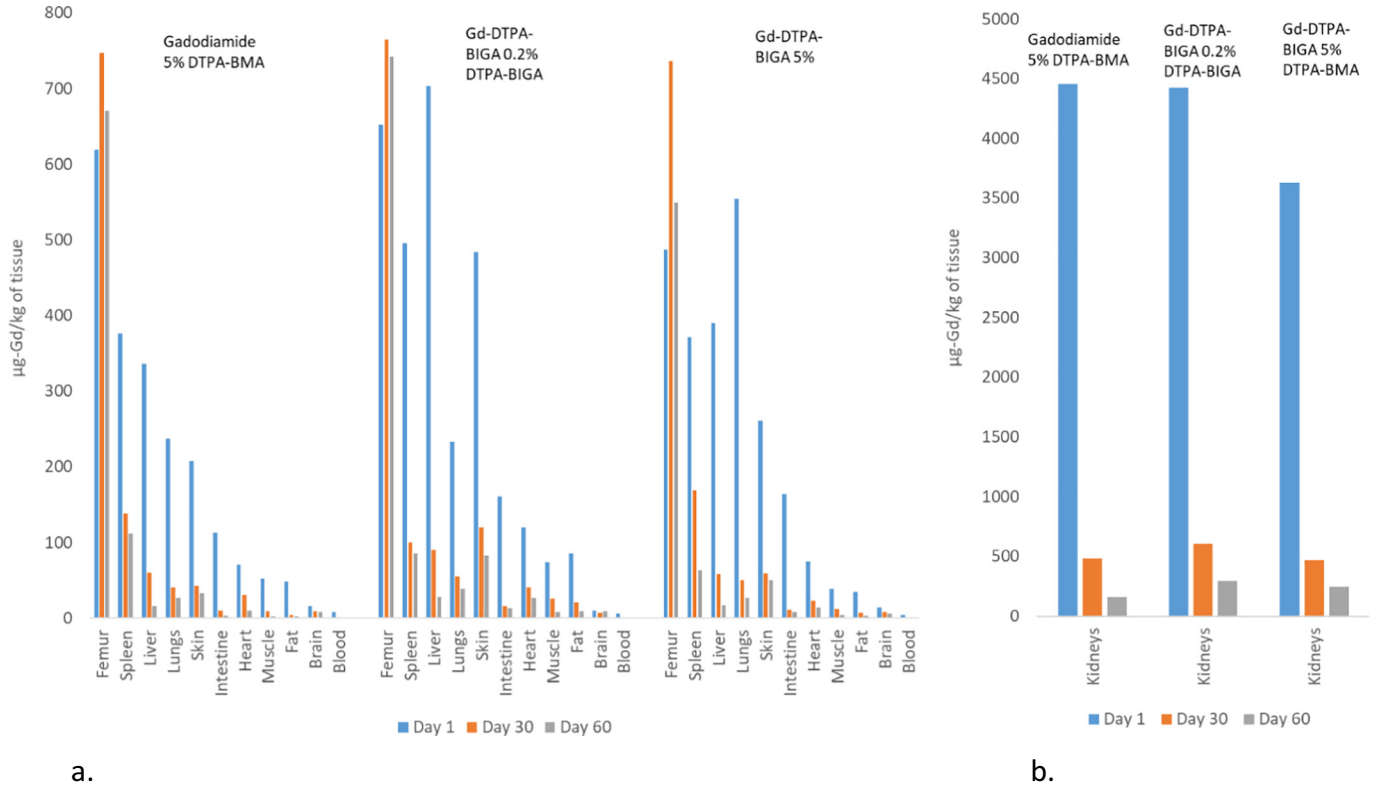

Fig. 1. a. Gadolinium concentration in μg-Gd/kg tissue graphed at days 1, 30 and 60 for each GBCA based on Table 2. b. The relatively high concentration of gadolinium in the kidneys at day 1.
Blue = Gadodiamide 5% DTPA-BMA; Orange = Gd-DTPA-BIGA 0.2% DTPA-BIGA; Gray = Gd-DTPA-BIGA 5% DTPA-BMA. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

are the lowest at 60 days. In fat, femur and heart, Gadodiamide and Gd-DTPA-BIGA 5% DTPA-BMA are lower at day 1 with the lowest retention for all 3 agents at 60 days. For kidneys, Gadodiamide and Gd-DTPA-BIGA 5% DTPA-BMA show the lowest values. In muscle and skin Gadodiamide and Gd-DTPA-BIGA 5% DTPA-BMA outperform Gd-DTPA-BIGA 0.2% DTPA-BIGA at each time point. For liver and spleen Gadodiamide and Gd-DTPA-BIGA 5% DTPA-BMA are lower at day 1, however, all agents perform the same at 30 and 60 days (Table 2, Fig. 1a and b). Saline control samples were below background. Saline controls for 30 and 60 days are free of counts.

For all tissues, with the exception of femur, and each agent, the presence of gadolinium is highest at day 1 and decreases through 30 days and reaches the lowest level at 60 days. Across all agents, retention is highest in bone, kidneys and spleen. Bone has extensive binding capacity with both hydroxyapatite and other phosphate ligands as well as a large amount of the reticuloendothelial system (bone marrow). Spleen has a large reticuloendothelial system, likely responsible for gadolinium retention. The kidney, of all organs, gets the most exposure as the agent is concentrated and excreted through this organ. There is a surprising amount of gadolinium in the skin at 60 days, a factor that is relevant to the development of NSF.

ICP-MS identifies elemental gadolinium, but not the isotope ^{153}Gd.

Table 3
Mean comparative ICP-MS values of gadolinium in μg-Gd/kg of tissue (6 animals/group) for gadodiamide and Gd-DTPA-BIGA supplemented with either 0.2% DTPA-BIGA or 5% DTPA-BMA, based on ^{153}Gd data.

GBCA	Day 1			Day 30			Day 60		
	Gadodiamide 5% DTPA-BMA	Gd-DTPA-BIGA 0.2% DTPA-BIGA	Gd-DTPA-BIGA 5% DTPA-BMA	Gadodiamide 5% DTPA-BMA	Gd-DTPA-BIGA 0.2% DTPA-BIGA	Gd-DTPA-BIGA 5% DTPA-BMA	Gadodiamide 5% DTPA-BMA	Gd-DTPA-BIGA 0.2% DTPA-BIGA	Gd-DTPA-BIGA 5% DTPA-BMA
Brain	16.7	17.9	19.9	14.6	14.8	15.7	13.1	16.3	11.4
Femur	818.7	1108.2	835.5	1239.0	1473.8	1199.7	1033.8	1453.3	967.8
Kidney	7496.7	8455.7	7305.8	723	1012.0	707.8	223.8	565.5	415.3
Liver	729.5	1366.2	822.7	88.5	160	105.7	22.8	47.0	30.3

Table 4
Mean values of gadolinium in μg/kg of tissue calculated from CPM data and comparative values derived from ICP-MS averaged across all 3 GBCAs and associated samples.

	Day 1		Day 30		Day 60	
	Mean GBCAs	Mean ICP-MS	Mean GBCAs	Mean ICP-MS	Mean GBCAs	Mean ICP-MS
Brain	13.4	18.2	8.4	15.0	7.8	13.6
Femur	586.1	920.8	749.1	1282.9	653.9	1151.7
Kidney	4171.0	7752.7	518.0	744.8	232.1	378.4
Liver	476.6	972.8	69.6	112.8	20.5	33.4

A subset of organs was sent for comparative ICP-MS analysis. ICP-MS detected 1.7 fold more Gd in tissue than the ^{153}Gd method, in μg-Gd/g-tissue (Tables 3 and 4).

ICP-MS saline controls demonstrate the presence of small amounts of gadolinium. Presumably this represents background environmental gadolinium exposure. These values are generally equal to or < 3 μg/kg of tissue (Table 5).

Alternatively, some of this discrepancy between ICP-MS and ^{153}Gd values could be related to experimental error. However, both techniques (labeled gadolinium and ICP-MS) are remarkably sensitive and give values that are very close, given the trace amounts of gadolinium present.

4. Discussion

The results of this experiment indicate that in general, 5% DTPA-BMA reduces transmetallation of Gd-DTPA-BIGA in comparison to the base formulation of the same compound, Gd-DTPA-BIGA 0.2% DTPA-BIGA. Based on previous studies, it is likely that the diminished transmetallation in this study is because of the higher affinity which DTPA-BMA has for zinc, relative to gadolinium [11]. Plasma zinc is bound by the excess DTPA-BMA in both the Gadodiamide and Gd-DTPA-BIGA 5% DTPA-BMA.

The general trend over 60 days is higher retention of gadolinium at all time points for the original formulation of Gd-DTPABIGA 0.2% DTPA-BIGA when compared with Gadodiamide and Gd-DTPA-BIGA 5% DTPA-BMA. These results likely describe the in vivo performance of DTPA-BMA as it relates to zinc binding and the inhibition of transmetallation by the binding of plasma and other repositories of zinc, when compared against DTPA-BIGA. As a formulation supplement, the metabolic behavior of DTPA-BMA appears consistent, irrespective of the agent. Yet, the results indicate that the binary formulation decreases, but does not eliminate tramsmetallation. It could simply be that not enough DTPA-BMA was added to eliminate transmetallation by zinc (> 5% DTPA-BMA). Perhaps a future investigation could test increasing concentrations of DTPA-BMA, although Optimark (available in the U.S.A.) loses slightly more Gd than Omniscan, even though it is formulated with 10% excess calcium-ligand [21]. Alternatively, the results imply that the transmetallation process likely has other pathways than just zinc substitution. For example, the elevated retention of gadolinium in bone suggests that free gadolinium binds to the inorganic phosphate component of bone for which gadolinium has a high affinity [22,23]. Moreover, there are multiple biological conduits that result in the uptake of gadolinium in brain tissue [24]. Importantly, gadolinium can readily replace Ca^{2+} in biological systems. Gadolinium alters, or prohibits the function of enzymes that use Ca^{2+} as a co-factor and can interfere with, or block calcium channels [25]. In addition, many serum proteins bind free gadolinium or the chelate form. Free Gd^{3+} at physiological pH quickly forms Gd_2O_3; an insoluble particulate which is taken up by the reticuloendothelial system (liver, spleen, bone, lungs).

Critically, gadolinium interferes with the cellular mitochondria network by potentially promoting apoptosis [20]. There are likely a myriad of ways in which gadolinium and perhaps its chelated forms can interfere with or block cellular functions and be retained in specific organs or tissues. These toxicity issues have been reviewed elsewhere [26]. It is clear that an in depth understanding of the mechanisms of dechelation of GBCAs, is an important step in precluding gadolinium retention for both linear agents and macrocyclics; however, the addition of supplemental chelates and/or other compounds holds promise for “rescuing” linear agents, or even minimizing gadolinium left behind by macrocyclic agents. It is also abundantly clear from this very sensitive residual gadolinium data, looking at gadolinium retained over a period of 60 days, that all three of these open-chain formulations leave significant deposits of gadolinium behind. Simply put, the presence of excess DTPA-BMA does not sufficiently rescue Gd-DTPA-BIGA from transmetallation. A caution is raised by this data because gadolinium is retained in most tissues and can be detected down to parts per trillion and quadrillion. Although many comparative values at all time points lack statistical significance, μg differences may well have a biological impact, especially over many doses. However, it remains to be seen at what concentration gadolinium is toxic and how long it takes for toxicity to appear clinically, if ever it does. Furthermore, as suggested by the saline control data, small amounts of gadolinium are present in rat tissue that has not been exposed to contrast media.

The results of this study demonstrate that sensitive values can be acquired without adjusting/increasing the dose for rodents, in comparison to humans, and without multiple infusion events [12,16]. Both ^{153}Gd and ICP-MS demonstrate that in general, microgram quantities of gadolinium per kg of tissue are retained, with these agents, in most

Table 5
Background gadolinium detected in saline control animals at 30 and 60 days. Values are in μg of gadolinium/kg of tissue.

Animals	Day 30		Day 60	
	F1	M1	F1	M1
Brain	< 0.2	0.3	< 0.2	0.7
Femur	1.0	2.0	3.0	1.0
Kidney	< 2.0	< 2.0	< 2.0	< 2.0
Liver	1.1	1.7	1.0	1.3

tissues at 60 days. Perhaps higher concentrations of free chelate, or a different chelate with greater zinc selectivity, would further inhibit transmetallation. The FDA is currently questioning the potential effects of this residual gadolinium [27].

This data, which looks at retention out to 60 days, reveals minimal reduction in gadolinium levels in some tissues over this time period. Importantly, bone deposition actually increases from day 1 and remains significant out to at least 60 days. This bone reservoir of gadolinium may have clinical repercussions as bone tissue is mobilized in osteoporosis, renal failure, etc.

Linear agents have been very well tolerated by the vast majority of patients and are inexpensive to synthesize. Thus, the search for better agents continues; one which combines exceptional relaxation properties with minimal toxicity.

5. Conclusions

In this study we tested a binary chelate formulation as an approach to reducing transmetallation of an agent which has enhanced and improved imaging qualities over macrocyclic compounds. The addition of 5% DTPA-BMA, as a zinc binding agent, reduced the transmetallation of the linear agent Gd-DTPA-BIGA, in comparison to its original formulation supplemented with 0.2% BIGA. Hence, the addition of compounds to inhibit/preclude the transmetallation of valuable linear chain agents merits further study and consideration.

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Ethics

Sprague Dawley Rats (Crl:SD, strain 400) were maintained in accordance with the Animal Welfare Act and Animal Welfare Regulations (USDA 2013).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mri.2019.01.001.

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