

Differences in Gadolinium Retention After Repeated Injections of Macrocyclic MR Contrast Agents to Rats

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Purpose: To compare the levels of gadolinium in the blood, cerebrum, cerebellum, liver, femur, kidneys, and skin after multiple exposure of rats to the macrocyclic gadolinium-based contrast agents (GBCAs) gadoterate, gadobutrol, and gadoteridol.

Materials and Methods: Fifty male Wistar Han rats were randomized to three exposure groups ($n = 15$ per group) and one control group ($n = 5$). Animals in the exposure groups received a total of 20 GBCA administrations (four administrations per week for 5 consecutive weeks) at a dose of 0.6 mmol/kg bodyweight. After a 28-day recovery period animals were sacrificed and the blood and tissues harvested for determination of gadolinium (Gd) levels. Gd determination was performed by inductively coupled plasma mass spectrometry (ICP-MS).

Results: After 28 days' recovery no Gd was found in the blood, liver, or skin of any animal in any group. Significantly lower levels of Gd were noted with gadoteridol compared to gadoterate and gadobutrol in the cerebellum (0.150 ± 0.022 vs. 0.292 ± 0.057 and 0.287 ± 0.056 nmol/g, respectively; $P < 0.001$), cerebrum (0.116 ± 0.036 vs. 0.250 ± 0.032 and 0.263 ± 0.045 nmol/g, respectively; $P < 0.001$), and kidneys (25 ± 13 vs. 139 ± 88 [$P < 0.01$] and 204 ± 109 [$P < 0.001$], respectively). Higher levels of Gd were noted in the femur (7.48 ± 1.37 vs. 5.69 ± 1.75 and 8.60 ± 2.04 nmol/g, respectively) with significantly less Gd determined for gadoterate than for gadobutrol ($P < 0.001$) and gadoteridol ($P < 0.05$).

Conclusion: Differences exist between macrocyclic agents in terms of their propensity to accumulate in tissues. The observed differences in Gd concentration point to differences in GBCA washout rates in this setting and in this experimental model, with gadoteridol being the GBCA that is most efficiently removed from both cerebral and renal tissues.

Level of Evidence: 2

Technical Efficacy: Stage 5

J. MAGN. RESON. IMAGING 2018;47:746–752.

Gadolinium-based contrast agents (GBCAs) have been in clinical use for ~30 years and, collectively, have an excellent safety profile, with reported acute adverse reaction rates ranging from 0.01–2% with serious adverse reaction rates of only 0.01–0.04%.¹ Nevertheless, an association with the development of nephrogenic systemic fibrosis (NSF) in 2006² led to heightened awareness within the radiology community of differences among GBCAs in terms of chelate

stability and the potential for gadolinium (Gd) release in vivo. A specific consequence of the NSF crisis was that macrocyclic GBCAs were perceived as more stable in vivo and therefore “safer” than linear (open-chain) GBCAs, despite clear evidence of differences among GBCAs of the same class.^{3,4} The recent recognition of T₁-hyperintensity in the deep brain nuclei on unenhanced T₁-weighted magnetic resonance imaging (MR) images as a phenomenon associated

View this article online at wileyonlinelibrary.com. DOI: 10.1002/jmri.25822

Received May 15, 2017, Accepted for publication Jul 6, 2017.

The copyright line for this article was changed on 20 October 2017 after original online publication.

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with cumulative GBCA exposure^{5–9} has further fueled the perception that marked differences exist between macrocyclic and linear GBCAs, despite the fact that no clinical signs or symptoms associated with T₁-hyperintensity have yet been described for any GBCA,¹⁰ and despite the fact that T₁-hyperintensity has also been reported in deep brain nuclei on unenhanced T₁-weighted images after the exclusive administration of macrocyclic agents.^{11–13}

Unfortunately, given the unfeasibility of acquiring tissue samples from live human patients, most studies performed to evaluate Gd retention in the brain have utilized animal, typically rat, models and have focused primarily on quantifying T₁ signal increases and/or Gd retention after exposure to macrocyclic GBCAs versus linear GBCAs.^{14–22} To date, no studies have compared the three available macrocyclic agents: gadoterate (Dotarem; Guerbet, Roissy, France), gadobutrol (Gadovist; Bayer Pharma, Berlin, Germany), and gadoteridol (ProHance; Bracco Imaging, Milan, Italy) in terms of their susceptibility to Gd retention. Our purpose was to determine whether these three macrocyclic GBCAs differ in terms of their propensity to deposit Gd in selected rat tissues.

Materials and Methods

The study was performed at Bracco Suisse (Plan-les-Ouates, Geneva, Switzerland), according to site-specific procedures established by the relevant Quality Assurance Unit. Procedures were conducted according to national and international regulations on the use of experimental animals (L.D. 26/2014; Directive 2010/63/EU) under authorization no. GE/97/16, delivered on July 7, 2016 by the “Direction general de la santé du canton de Genève,” according to the Swiss Federal Ordinance of 23 April 2008 on the protection of animals (OPAn).

Animal Study

Fifty male Wistar Han rats (Charles River Laboratories; L'Arbresle, France), aged 6 weeks and weighing 142–202 g at the start of treatment, were utilized. Animals were housed under controlled conditions at 21°C, 50% relative humidity, and 12-hour dark/light cycles. PXS15 food pellets (Provimi Kliba, Switzerland) and filtered water from municipal services were provided ad libitum.

After 10 days of acclimation, the animals were randomized to one of four groups as follows: group A (control; $n = 5$); group B (gadoterate; $n = 15$); group C (gadobutrol; $n = 15$); group D (gadoteridol; $n = 15$). Animals in groups B, C, and D were administered the respective contrast agent Dotarem, batch no. 16GD060B02, exp. date April 2019, Gadovist, batch no. 62072H, exp. date April 2019, and ProHance, batch no. V16614, exp. date May 2019, respectively, at a dose of 0.6 mmol/kg bodyweight four times a week, for 5 consecutive weeks (ie, 20 administrations overall), for a total cumulative dose equivalent to 12 mmol/kg bodyweight. This daily dose corresponds to a clinical dose of 0.1 mmol/kg bodyweight based on the extrapolation factor for rats described in the US Food & Drug Administration (FDA) guidance for Human Equivalent Dose (HED).²³ Animals randomized to group

A were administered saline solution (0.9% w/v NaCl) at 1.2 mL/kg bodyweight four times a week for 5 consecutive weeks. Administration of GBCA or saline was performed at room temperature into the lateral vein of the tail at an injection rate of 2 mL/min using a Harvard infusion pump (Holliston, MA).

After the end of the 5-week treatment period, each animal was allowed a recovery period of 4 weeks (28 days; corresponding to ~2.5 human years²⁴) before sacrifice. At sacrifice animals were anesthetized with pentobarbital (40 mg/kg, intraperitoneal injection) and bled from the abdominal artery. After exsanguination, a complete macroscopic postmortem examination was performed; abnormal findings, if any, were recorded. Thereafter, each animal was dissected to obtain tissues (blood, cerebrum, cerebellum, liver, femur, kidneys, and skin) for inductively coupled plasma / mass spectrometry (ICP-MS) determination of gadolinium. A total of 350 tissue/blood samples were collected (50 animals; seven tissue/blood samples per animal).

Determination of Total Gadolinium

Blood samples were mixed 1:2 with nitric acid (65% w/w, Extrapure, Merck, Darmstadt, Germany). Cerebrum and cerebellum samples were weighed and freeze-dried, then suspended in 1 mL of nitric acid. Liver, kidneys, and skin samples were weighed and freeze-dried. All dehydrated organs were weighed and ground in a mortar. Approximately 0.2 g were then weighed and suspended in 1 mL of nitric acid. Femurs were weighed and dissolved in 1 mL of nitric acid. All nitric acid solutions were stored at 4°C for at least 12 hours before digestion. Sample mineralization was performed by subjecting the samples to a wet ashing process (95 min at 180°C for blood, 110 min at 180°C for the other organs) in a microwave oven system (MARS-5 CEM). The treatment of the organs as well as their mineralization were carried out at Bracco Research Centre (Colleretto Giacosa, Turin, Italy).

The mineralized samples were quantitatively transferred in disposable Falcon tubes, diluted to 20 mL with 2% nitric acid, filtered at 0.45 µm, and then analyzed by ICP-MS for Gd content.

ICP-MS. ICP-MS analysis of Gd (¹⁵⁷Gd) content was performed on an ELAN 6100 Perkin Elmer Spectrometer (Waltham, MA) at Bracco Research Centre. Internal standardization was performed using ¹⁵³Eu. The calibration blanks, calibration standards, and control standard solutions for each analytical sequence were prepared in 2% nitric acid by dilution of a gadolinium oxide (Gd₂O₃) standard solution (1000 mg/mL in 2% HNO₃, Certipur, Merck).

The limit of quantitation (LOQ) for gadolinium was 0.1 nmol/mL for blood, 0.1 nmol/g for cerebrum/cerebellum, 0.5 nmol/g for femur, 1 nmol/g for liver and skin, and 1.7 nmol/g for kidney. The LOQ for each tissue was verified for accuracy and precision by spiking in triplicate explanted blank organs with the corresponding amounts of gadolinium and determining the respective percent recoveries.

Statistical Analysis

Gadolinium concentration was expressed as nmol/g wet tissue in the case of cerebrum, cerebellum, liver, femur, kidneys, and skin and as nmol/mL in the case of blood. The Dixon test²⁵ was used before formal data analysis to highlight possible anomalous data points. Data were analyzed based on the distribution and homogeneity of variance amongst groups. If the data were normally

TABLE 1. Gadolinium Content in Cerebellum, Cerebrum, Femur, Kidneys, Liver, Skin, and Blood (Mean Values and SD, $n = 14-15$)

	Group A Saline mean	Group B Dotarem mean \pm SD	Group C Gadovist mean \pm SD	Group D ProHance mean \pm SD
Cerebellum nmol/g (LOQ = 0.1)	< LOQ	0.292 \pm 0.057	0.287 \pm 0.056	0.150 \pm 0.022
Cerebrum nmol/g (LOQ = 0.1)	< LOQ	0.250 \pm 0.032	0.263 \pm 0.045	0.116 \pm 0.036
Femur nmol/g (LOQ = 0.5)	< LOQ	5.69 \pm 1.75	8.60 \pm 2.04	7.48 \pm 1.38
Kidneys nmol/g (LOQ = 1.7)	< LOQ	139 \pm 88	204 \pm 109	25 \pm 13
Liver nmol/g (LOQ = 1)	< LOQ	< LOQ	< LOQ	< LOQ
Skin nmol/g (LOQ = 1)	< LOQ	< LOQ	< LOQ	< LOQ
Blood nmol/mL (LOQ = 0.1)	< LOQ	< LOQ	< LOQ	< LOQ

LOQ: limit of quantitation.

distributed based on Q-Q plots and Shapiro–Wilk’s test,²⁶ Levene’s test²⁷ was utilized to test the homogeneity of variance among groups. To test the null hypothesis that treatment groups have the same distribution, the analysis of variance (ANOVA) test was applied in the case of homogeneous variances. If variances were not homogeneous the Welch test²⁸ was used. If the results of the ANOVA or Welch tests were significant, pairwise multiple comparisons were performed with the method proposed by Tukey in the case of homogeneous variances, and Dunnett’s T3 test in the case of nonhomogeneous variances.²⁹ If data were not normally distributed, the null hypothesis was tested by nonparametric Kruskal–Wallis one-way analysis of variance. If significant differences between treatment groups were detected, pairwise multiple comparisons were performed with the method proposed by Dunn.³⁰ Adjusted P -values were calculated for multiple comparisons as $p_{adj} = pK(K-1)/2$, where K is the number of treatments to be compared. All analyses were performed with $\alpha < 0.05$ considered significant, using SPSS v. 24.0 (SPSS, Chicago IL).

Results

All animals successfully underwent all aspects of the study and were sacrificed as foreseen in the study protocol. No unexpected changes in bodyweight were noted and no clinical signs or symptoms were observed for any animal in any group. No gross pathological tissue changes were noted at sacrifice.

A total of 350 tissue samples were analyzed, each sample corresponding to an individual tissue/organ excised at necropsy. Among these 350 samples, data points from seven samples were considered outliers based on the Dixon test. No more than a single outlier was identified in each 15-sample group of animals and organs. After close

examination, these data points were excluded from further elaboration. Exclusion of these seven outliers did not affect the significance of the differences between groups (Supplementary Table 1).

After the 28-day recovery period no Gd was found in the blood, liver, or skin of any animal in any group (all but five values below the LOQ for these samples, Table 1). Significantly ($P < 0.001$; all evaluations) lower levels of Gd were noted with gadoteridol compared to gadoterate and gadobutrol in both the cerebellum (0.150 \pm 0.022 nmol/g vs. 0.292 \pm 0.057 nmol/g and 0.287 \pm 0.056 nmol/g, respectively) and cerebrum (0.116 \pm 0.036 nmol/g vs. 0.250 \pm 0.032 nmol/g and 0.263 \pm 0.045 nmol/g, respectively) (Table 1, Fig. 1).

Significantly ($P < 0.001$) higher Gd concentration in the femur was noted with gadobutrol (8.60 \pm 2.04 nmol/g) compared to gadoterate (5.69 \pm 1.75 nmol/g). The mean value for gadoteridol (7.48 \pm 1.37 nmol/g) fell between the values for gadobutrol and gadoterate and was only marginally significantly higher than the mean value for gadoterate ($P < 0.05$). The mean Gd concentration found for gadoteridol in the cerebellum was almost 50 times lower than that found in femurs. The Gd concentrations found for gadoterate and gadobutrol were \sim 20 and 30 times lower in the cerebellum than in femurs, respectively.

The Gd concentrations in the kidneys were significantly lower with gadoteridol (25 \pm 13 nmol/g) than with gadoterate (139 \pm 88 nmol/g; $P < 0.01$) and gadobutrol (204 \pm 109 nmol/kg; $P < 0.001$).

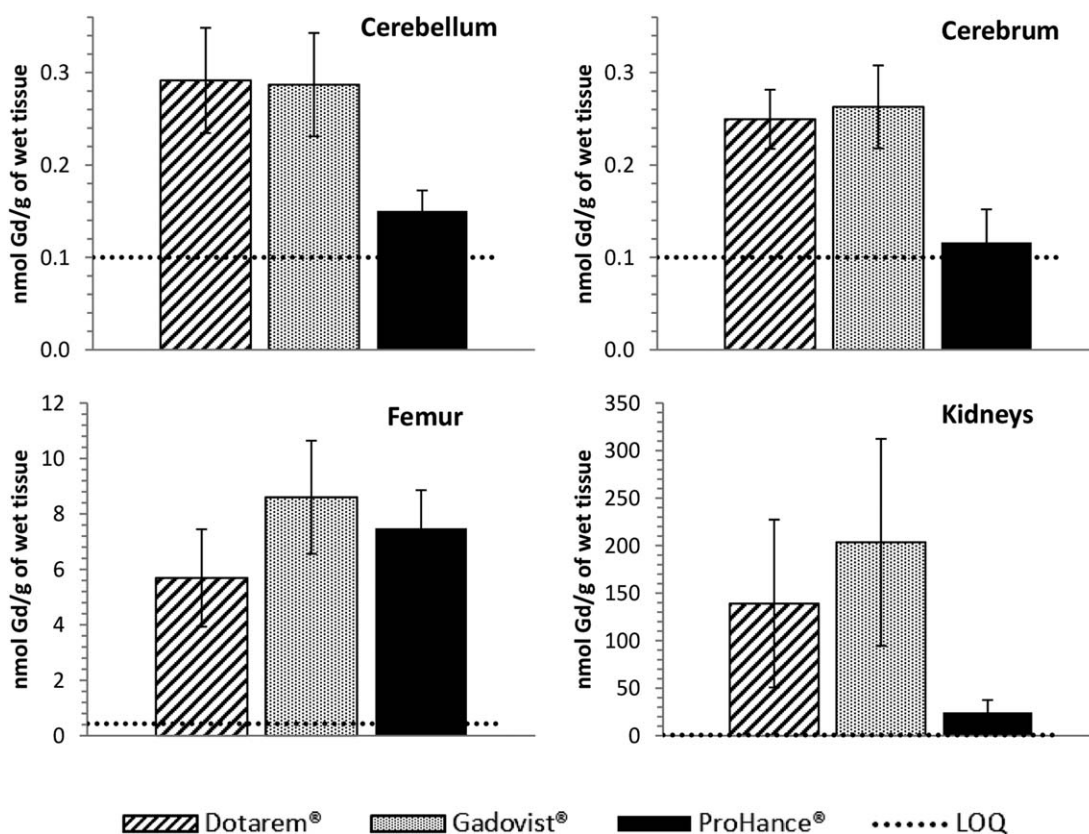


FIGURE 1: Gadolinium content (mean \pm SD) in cerebellum, cerebrum, femur, and kidneys.

Discussion

The mechanisms underlying the penetration and the subsequent retention of GBCAs in the deep brain nuclei for extended periods are still largely unknown. Animals, particularly rats and other lower mammal species, differ from humans in terms of anatomy, physiology, and biochemistry, and findings from studies on animals should not be considered entirely representative of the situation in humans. On the other hand, animal models may help to identify the causative aspects of very relevant human pathologies, as was the case with early investigations of NSF.^{31,32} In the case of Gd retention in human brain, animal models may help to shed light on the mechanisms, and indicate possible approaches to circumventing or reducing this phenomenon.

The results of our study reveal statistically significant differences between the three macrocyclic GBCAs in terms of Gd accumulation in different organs and tissues at 4 weeks after the last GBCA injection. Most notably, significantly less Gd was determined in the cerebellum and cerebrum of animals exposed to gadoteridol than in animals exposed to either gadoterate or gadobutrol. Less pronounced differences were noted in the femur, possibly reflecting the much greater overall accumulation in this tissue than in other tissues and the limited and less rapid GBCA washout. Nevertheless, significant differences were noted, with less Gd determined in femurs of animals exposed to gadoterate than in animals

exposed to gadobutrol ($P < 0.001$) or gadoteridol ($P < 0.05$). A surprising finding was the levels of Gd determined in the kidneys: significantly less Gd was determined in animals exposed to gadoteridol than in animals exposed to either gadoterate or gadobutrol. The kidneys are the only excretory organ for these GBCAs³³ and thus high levels of Gd are to be expected until the GBCA has entirely cleared from the blood. Nevertheless, it was surprising that relatively high levels of Gd (up to 204 nmol/g) were noted after gadoterate and gadobutrol; about 6 and 8 times higher, respectively, than after gadoteridol at 28 days after the last GBCA exposure, when the levels in blood had fallen to below the LOQ (0.1 nmol/mL) for all GBCAs.

It is unclear why elevated Gd levels were determined in the kidneys after gadoterate and gadobutrol injections, but it may reflect vacuolization in the cytoplasm of the proximal tubular epithelial cells and the subsequent entrapment of GBCA molecules. This phenomenon has been observed previously after repeated administrations of gadobutrol to rats at intermediate to high doses, and, importantly, was not shown to have any cytotoxic degenerative effects on cell organelles.³⁴ Reasons for the differences in measured Gd levels may reflect differences in the physicochemical characteristics, in particular viscosity, of the GBCAs. Thus, if GBCA molecules become entrapped in vacuoles, more highly viscous molecules may take longer to clear.³⁵ Among the three GBCAs utilized in this study,

TABLE 2. Osmolality, Viscosity, and Lipophilicity of Macrocyclic GBCAs

GBCA	Molecular weight	Osmolality ^a (osmol/kg)	Viscosity ^a (mPa·s)	Log P butanol:water ^b
Gadoterate (Dotarem)	580.6 ^c	1.35	2.0	−2.87
Gadobutrol (Gadovist)	604.7	1.60	4.96	−2.0
Gadoteridol (ProHance)	558.7	0.63	1.3	−1.98

^aFrom Bellin MF, Van Der Molen AJ. Extracellular gadolinium-based contrast media: An overview. *Eur J Radiol* 2008;66:160-167.
^bFrom “The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging” 2nd Ed. Merbach AS, Helm L, Toth E, eds. Hoboken, NJ: Wiley 2013.
^cWithout meglumine.

gadoteridol has the lowest viscosity, coupled with the lowest osmolality and lowest molecular weight (Table 2), all properties that might contribute to increasing the rate of elimination from membrane-enclosed vacuoles.

Whereas several studies in animals have looked at gadoterate and gadobutrol in terms of Gd accumulation in the brain and other organs,^{14–18} comparatively few studies have evaluated gadoteridol after a prolonged off-dose period. In one recent study aimed at investigating the differential effects on histology between macrocyclic and linear GBCAs, Lohrke et al²⁰ found only minimally different levels of Gd in the brains of animals administered gadobutrol compared with animals administered gadoteridol (0.7 ± 0.4 vs. 0.5 ± 0.2 nmol Gd/g tissue, respectively; evaluation of significance not presented). Conversely, a more recent independent study by McDonald et al²¹ found higher median concentrations of Gd in brain tissue of gadobutrol-injected rats than in brain tissue of gadoteridol-injected rats (1.6 vs. 0 mg/g tissue, respectively). Notably, McDonald et al further corroborated our findings in demonstrating markedly higher median concentrations of Gd also in the kidney, liver, and spleen of rats administered gadobutrol than in rats administered gadoteridol. Of note, however, is that whereas Lohrke et al²⁰ allowed 8 weeks' recovery after the last GBCA administration before animal sacrifice, McDonald et al²¹ allowed only 7 days. However, this again points to a more rapid and efficient elimination mechanism for gadoteridol, possibly reflecting advantageous physicochemical properties.

In another study that compared all three macrocyclic GBCAs,²² the aim was to clarify the entry pathway to the central nervous system (CNS). Therefore, the animals were sacrificed shortly after GBCA administration, ie, after 4.5 or 24 hours. In this setting, no significant differences were apparent among the different GBCAs, even between linear and macrocyclic GBCAs, except for a slightly (albeit not statistically tested) higher Gd concentration in the cerebellum of rats exposed to gadodiamide at 24 hours after injection. In that study,²² the blood–CSF barrier was indicated as a potential entry pathway of GBCAs into the CNS, with subsequent distribution within

the brain parenchyma determined by the glymphatic system³⁶ in a manner similar to that postulated for gadopentetate after intrathecal administration in man.³⁷ Although hypothetical at present, corroboration of this potential distribution mechanism comes from observations of enhanced perivascular space (PVS) at 4 hours after the intravenous administration of GBCAs in human volunteers.³⁸ The PVS is part of the brain glymphatic system, through which the interstitial solutes, coming also from the CSF, are cleared from the brain. In their study, Naganawa et al³⁸ attributed entry into the CSF to permeation from the peripheral part of the cranial nerve or nerve sheath. Since no differences have been found in CSF Gd levels up to 24 hours after injection of either linear or macrocyclic GBCAs,²² the differences we observe both in the cerebellum and cerebrum after macrocyclic GBCA exposure must be due to a more efficient clearance of gadoteridol. As noted above, such clearance may reflect differences in molecular features, such as lower molecular weight and lower viscosity. An additional physicochemical feature to consider is the lipophilicity of the molecule. Although the lipophilicity of gadoteridol is only slightly greater than that of gadobutrol, it has been reported that liquid in the paravascular space (ie, the glymphatic system) efficiently transports lipophilic molecules, reducing their diffusion into the brain parenchyma.³⁹

Unfortunately, in this, as in the other published studies,^{14–17} the analytical technique used (ICP-MS) does not permit speciation analysis, to distinguish between different Gd-containing molecular species. Although this should be the subject of further study, we do not expect significant dechelation and Gd release from any of these GBCAs. In support of this expectation, an as-yet unpublished study in which LC-ICP-MS analysis was performed of gadoteridol-treated rat brain revealed an amount of gadoteridol that matched the total amount of gadolinium present as determined by ICP-MS (personal communication, Silvio Aime, Turin University; submitted). This finding suggests that, for gadoteridol at least, the Gd-chelate molecule remains intact after administration. Although not supported by identification and quantitation of the compound, a similar hypothesis has been made for the

macrocyclic agent gadobutrol.¹⁸ With this in mind, our results suggest that a mechanism different from dechelation may be responsible for the observed differences in Gd retention in CNS and renal tissues. Such differences were not observed in bones (femur), suggesting that different mechanisms may be responsible for GBCA absorption and release from this tissue. Unfortunately, in the skin the considerably higher LOQ prevented any meaningful comparison between GBCAs.

It is important to note that tissue sampling in our study was carried out under extremely controlled cleaning procedures to avoid cross-contamination. This permitted very accurate determination of Gd levels. The same precision, in conjunction with a relatively large group size ($n = 15$), helped minimize variation (standard error), thereby permitting accurate assessment of intergroup differences for GBCAs that are typically regarded as having superimposable behavior.

The limitations of this study are those that are common to many animal studies in that the findings can only be considered as indicative rather than representative of the complex clinical situation in humans. Furthermore, the dosing frequency (four times a week for 5 weeks) is considerably higher than that which would occur in routine clinical practice. As such, the subtle differences assessed for GBCA clearance from animal tissues may not appear in human subjects who receive multiple doses of GBCA over much longer timeframes. Nonetheless, also in a human study published by Murata et al,⁴⁰ Gd retention in the dentate nucleus was much lower in the five patients treated from 1–11 times with gadoteridol, than in the two patients treated 1–2 times with gadobutrol. A specific limitation of our study is that measurements were made on tissue samples obtained at a single timepoint (28 days) after the last GBCA administration. Hence, it is not possible to say whether the differences observed at 28 days would be maintained at later timepoints. At the time the study was performed (September 2016), most published data were based on tissue sampling at one timepoint, typically at 4–5 weeks after the end of dosing,^{14–16} and thus our experimental design was established to acquire comparable data. Future work should look at additional timepoints over a longer time period to better investigate the potential impact of different GBCA elimination rates. A second limitation of our study is that comparison was not performed with one or more linear GBCAs. However, McDonald et al²¹ recently compared the macrocyclic GBCAs gadoteridol and gadobutrol with the linear GBCAs gadobenate and gadodiamide and found differences in Gd concentration not only between the two classes but also between GBCAs within each class. These findings suggest that simplistic differentiation of GBCAs as either linear or macrocyclic is no longer valid and that it is more appropriate to evaluate each agent individually, based on actual clinical and analytical observations, as and when they become available.

In conclusion, Gd retention was determined in various tissues at 4 weeks after multiple administrations of the three

commercially available macrocyclic GBCAs. Significantly lower Gd concentrations were determined with gadoteridol compared to gadoterate and gadobutrol in the cerebellum, cerebrum, and kidneys, while less pronounced differences in favor of gadoterate were noted in bone. Since dechelation is not considered to occur with the tested agents, the observed differences point to differences in washout rates, with gadoteridol being the GBCA that is most efficiently removed from both CNS and renal tissues. Gd retention observed after repeated GBCA administrations should not be interpreted exclusively as a sign of dechelation. Accurate speciation studies are needed to understand the behavior of different GBCAs.

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