

## OPEN

# Long-Term Evaluation of Gadolinium Retention in Rat Brain After Single Injection of a Clinically Relevant Dose of Gadolinium-Based Contrast Agents

Izabela Strzeminska, MS,\*† Cécile Factor, PhD,\* Philippe Robert, PhD,\* Anne-Laure Grindel, MS,\* Pierre-Olivier Comby, MD,‡ Joanna Szpunar, PhD, DSc,† Claire Corot, PharmD, PhD,\* and Ryszard Lobinski, PhD, DSc†

**Purpose:** The aim of this study was to investigate the presence and chemical forms of residual gadolinium (Gd) in rat brain after a single dose of Gd-based contrast agent.

**Methods:** Four groups of healthy rats (2 sacrifice time-points,  $n = 10/\text{group}$ , 80 rats in total) were randomized to receive a single intravenous injection of 1 of the 3 Gd-based contrast agents (GBCAs) (gadoterate meglumine, gadobenate dimeglumine, or gadodiamide) or the same volume of 0.9% saline solution. The injected concentration was 0.6 mmol/kg, corresponding to a concentration of 0.1 mmol/kg in humans after body surface normalization between rats and humans (according to the US Food and Drug Administration recommendations). Animals were sacrificed at 2 washout times: 1 (M1) and 5 (M5) months after the injection. Total Gd concentrations were determined in cerebellum by inductively coupled plasma mass spectrometry. Gadolinium speciation was analyzed by size-exclusion chromatography coupled to inductively coupled plasma mass spectrometry after extraction from cerebellum.

**Results:** A single injection of a clinically relevant dose of GBCA resulted in the detectable presence of Gd in the cerebellum 1 and 5 months after injection. The cerebellar total Gd concentrations after administration of the least stable GBCA (gadodiamide) were significantly higher at both time-points (M1:  $0.280 \pm 0.060$  nmol/g; M5:  $0.193 \pm 0.023$  nmol/g) than those observed for macrocyclic gadoterate (M1:  $0.019 \pm 0.004$  nmol/g, M5:  $0.004 \pm 0.002$  nmol/g;  $P < 0.0001$ ). Gadolinium concentrations after injection of gadobenate were significantly lower at both time-points (M1:  $0.093 \pm 0.020$  nmol/g; M5:  $0.067 \pm 0.013$  nmol/g;  $P < 0.05$ ) than the Gd concentration measured after injection of gadodiamide. At the 5-month time-point, the Gd concentration in the gadoterate group was also significantly lower than the Gd concentration in the gadobenate group ( $P < 0.05$ ). Gadolinium speciation analysis of the water-soluble fraction showed that, after injection of the macrocyclic gadoterate, Gd was still detected only in its intact, chelated form 5 months after injection. In contrast, after a single dose of linear GBCAs (gadobenate and gadodiamide), 2 different forms were detected: intact GBCA and Gd bound to soluble macromolecules (above 80 kDa). Elimination of the intact GBCA form was also

observed between the first and fifth month, whereas the amount of Gd present in the macromolecular fraction remained constant 5 months after injection.

**Conclusions:** A single injection of a clinically relevant dose of GBCA is sufficient to investigate long-term Gd retention in the cerebellar parenchyma. Administration of linear GBCAs (gadodiamide and gadobenate) resulted in higher residual Gd concentrations than administration of the macrocyclic gadoterate. Speciation analysis of the water-soluble fraction of cerebellum confirmed washout of intact GBCA over time. The quantity of Gd bound to macromolecules, observed only with linear GBCAs, remained constant 5 months after injection and is likely to represent a permanent deposition.

**Key Words:** gadolinium-based contrast agent, cerebellum, gadolinium species, gadolinium retention, gadodiamide, gadobenate, gadoterate

(*Invest Radiol* 2020;55: 138–143)

Gadolinium (Gd)-based contrast agents (GBCAs) constitute a major research topic in the field of clinical magnetic resonance imaging (MRI). Since 2014, numerous clinical and preclinical studies have demonstrated increased signal intensity (SI) on unenhanced T1-weighted MRI images in specific structures of the healthy brain after repeated administrations of linear GBCAs.<sup>1–4</sup> The quantitatively and qualitatively variable presence of Gd in cerebral parenchyma according to the molecular class of the GBCA was confirmed using inductively coupled plasma mass spectrometry (ICP-MS) and transmission electron microscopy–energy dispersive x-ray spectrometry.<sup>5,6</sup> Gadolinium retention in the brain raises important safety concerns because of the acute and chronic toxicities of ionic Gd<sup>3+</sup> and its deleterious role in the pathophysiology of nephrogenic systemic fibrosis.<sup>8</sup> Nephrogenic systemic fibrosis is an adverse reaction primarily related to administration of linear GBCAs in patients with severe renal disease.<sup>9</sup>

Two types of GBCAs are distinguished according to the chemical structure of the ligand associated with Gd: linear and macrocyclic GBCAs. An overview of various stability parameters of 3 GBCAs is presented in Table 1. Thermodynamic stability (which reflects the affinity of Gd for its ligand) and kinetic stability (which reflects the rate at which the equilibrium between the metal and its ligand is reached) are generally both used to describe the physicochemical characteristics of GBCAs.<sup>10</sup> High kinetic stability provided by the macrocyclic structure minimize dechelation both in vitro and in vivo. Compared with macrocyclic GBCAs, linear chelates present moderate or poor kinetic stability with a higher likelihood of dechelation.<sup>11,12</sup> Numerous clinical and preclinical studies have suggested that Gd retention in tissues is inversely correlated with the thermodynamic and kinetic stability of the GBCA injected.<sup>13–18</sup> Thus, linear GBCAs result in more intense and more persistent Gd retention than macrocyclic GBCAs. Indeed, 2 studies of long-term elimination kinetics from the rat brain tissues showed rapid washout of Gd after repeated injections of macrocyclic GBCAs.<sup>14,16</sup> Conversely large amount of Gd remained in the brain 1 year after the administration of the linear GBCAs. Gadolinium species present in the cerebellar parenchyma of rats have been studied by size-exclusion chromatography (SEC) coupled to ICP-MS. These studies also

Received for publication July 10, 2019; and accepted for publication, after revision, August 24, 2019.

From the \*Guerbet Research and Innovation Department, Aulnay-sous-Bois; †Institute of Analytical and Physical Chemistry for the Environment and Materials, IPREM - UMR 5254, CNRS, Université de Pau et des Pays de l'Adour (UPPA), E2S, Pau; and ‡Department of Neuroradiology, François-Mitterrand University Hospital, Dijon, France.

Conflicts of interest and sources of funding: I.S., C.F., P.R., A.L.G., and C.C. are (or were) Guerbet employee at the time of the study. J.S. and R.L. are affiliated with the Institute of Analytical and Physical Chemistry for the Environment and Materials. IPREM received funding from Guerbet within a common research project and disclosed no relevant relationships. R.L. was the academic tutor of PhD thesis of I.S. This study was funded by Guerbet.

Correspondence to: Izabela Strzeminska, MS, Research and Innovation Department, Guerbet Group, BP57400, 95943 Roissy CDG Cedex, France. E-mail: izabela.strzeminska@guerbet.com.

Copyright © 2020 The Author(s). Published by Wolters Kluwer Health, Inc. This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

ISSN: 0020-9996/20/5503-0138

DOI: 10.1097/RLI.0000000000000623

**TABLE 1.** Overview of the Various Parameters Reflecting the Stability of GBCAs

Trade Name	Generic Name	Type	Ionic/Nonionic	Excess Ligand	Log $K_{ML}^*$	Log $K_{ML}$ pH = 7.4*	Half-Lives $T_{1/2}$ pH = 1†	Half-Lives $T_{1/2}$ pH > 5†
Dotarem	Gadoterate meglumine	Macrocyclic	Ionic	No	25.6	19.3	26.4 h (37°C)	37 y (pH = 5.3, 25°C)
MultiHance	Gadobenate dimeglumine	Linear	Ionic	No	22.6	18.4	<5 s (25°C)‡	5–7 d (pH = 7.4, 25°C)‡
Omniscan	Gadodiamide	Linear	Nonionic	Ca-DTPA-BMA (Na <sup>+</sup> salt) 5% (25 mmol/L)	16.9	14.9	<5 s (25°C) ‡	5–7 d (pH = 7.4, 25°C)‡

\*The values of thermodynamic constants were taken from Port et al.<sup>10</sup>

†The values of half-lives were taken from Frenzel et al,<sup>11</sup> and were determined in absence of a biological matrix.

‡The same value was attributed for each linear GBCA, as the linear GBCA for which this parameter was determined was not specified.

demonstrated a class effect, resulting in presence of Gd species bound to soluble macromolecules only after repeated injections of linear GBCAs.<sup>13,14</sup> In addition, the time-dependent elimination of Gd from the brain was associated with the clearance of the intact GBCA form. Although the clinical consequences of Gd retention have not yet been demonstrated, the European Medicines Agency has suspended all linear GBCAs in the European Union (with few exceptions for the liver indications).<sup>19</sup> The US Food and Drug Administration (FDA) has acknowledged the presence of a GBCA class effect, but, as no toxicological effect has yet been associated with Gd retention, it decided not to suspend the use of linear GBCAs in the United States. However, the FDA requested changes in the package inserts with the same wording for all marketed GBCAs and invited GBCA manufacturers to conduct further studies to assess the safety of these agents.<sup>20</sup>

The current knowledge of brain Gd retention is largely based on retrospective studies documented for patients who received multiple injections of GBCAs or using animal models that reproduce repeated injection conditions. However, patients who occasionally undergo an MRI examination with injection of a GBCA must not be neglected. Few researchers have addressed the issue of residual Gd after injection of a single clinically relevant dose.<sup>21–23</sup> Recently, Radbruch et al published laser ablation ICP-MS data comparing the amount and the distribution of retained Gd of linear and macrocyclic GBCAs in the deep cerebellar nuclei of a sheep after a single injection at a dose comparable to a human patient. These results revealed that after linear GBCAs administration, the mean Gd concentrations were between 58 ng Gd/g tissue and 167 ng Gd/g tissue in the deep cerebellar nuclei, whereas no significant difference of Gd concentrations between 3 marketed macrocyclic GBCAs and the control group was found. However, there is still a need for the investigation of the residual Gd and Gd speciation after a single injection of a clinically relevant dose of different GBCAs. Moreover, the evaluation of the total Gd and different Gd species should be done after a longer period than in the described publication (10 weeks). This study was therefore designed to provide the complete information about the identity of the chemical forms of the residual Gd 1 and 5 months after a single injection of a clinically relevant dose of 3 different GBCAs. In addition, the kinetics of elimination of different Gd species from the cerebellum was investigated.

## MATERIALS AND METHODS

All animal experiments were conducted in full compliance with European Union Directive 2010/63/EU on the protection of animals used for scientific purposes.

Analytical reagent grade chemicals were used for this study. Ultrapure deionized water (18 MΩ cm) was obtained with the Milli-Q water purification system (Merck, Darmstadt, Germany).

## Animal Model and GBCA Administration

Four groups of 9-week-old, healthy female Sprague-Dawley rats, each weighing between 214 and 290 g at the beginning of the study (SPF/OFA rats; Charles River, L'Arbresle, France, total of 80 rats, n = 10/group, 2 sacrifice time-points) randomly received a single intravenous injection of either a Gd-based contrast agent (0.6 mmol/kg, corresponding to 0.1 mmol/kg in humans after adjusting for body surface area according to FDA guidances<sup>24</sup>) or 0.9% saline solution (saline chaser was not used). To cover the various GBCA classes available on the global market, animals received an injection of a macrocyclic ionic GBCA (gadoterate meglumine, Dotarem; Guerbet, Villepinte, France), a linear ionic GBCA (gadobenate dimeglumine, MultiHance; Bracco, Milan, Italy), and a linear nonionic GBCA (gadodiamide, Omniscan; GE Healthcare, Chalfont St Giles, United Kingdom). These 3 GBCAs are characterized by different thermodynamic and kinetic stabilities.<sup>10</sup> They were used in the form of the commercially available solution for injection (500 mM solutions). Intravenous injections (injection volume: 1.2 mL/kg) were performed in a tail vein under isoflurane anesthesia (IsoFlo; Axience, Pantin, France). Animals were sacrificed at 2 washout time-points: 1 month and 5 months after injection, referred to as M1 and M5 throughout this article. For the long-term follow-up, the time-point of 5 months after the last GBCA administration was chosen according to Robert et al.<sup>25</sup> At completion of each washout period, the rats were euthanized by exsanguination under isoflurane anesthesia. The brain was removed and dissected to sample a half cerebellum. Half cerebella were stored at -80°C before sample preparation. Special care was taken to avoid experimental Gd contamination between groups at each step of the study. Animals were raised by groups to protect against potential urine and feces contamination between groups (coprophagous species), as recommended elsewhere.<sup>25</sup> Rats were always handled by using new gloves, including for cage changes. The entire procedure (injection, sacrificed, and tissue sampling) was performed per group, with disposable surgical drapes, gloves, gowns, masks, and tools.

## Sample Preparation

One half of the cerebellum was homogenized using an Ultra-Turrax disperser (IKA-Werke GmbH & Co KG, Staufen, Germany) in water (dilution factor of 6) in an ice bath. The tissue homogenate was then centrifuged using the Centrifuge 5804 R (Eppendorf AG, Hamburg, Germany) for 60 minutes at 20,800g at 4°C, and the supernatant was

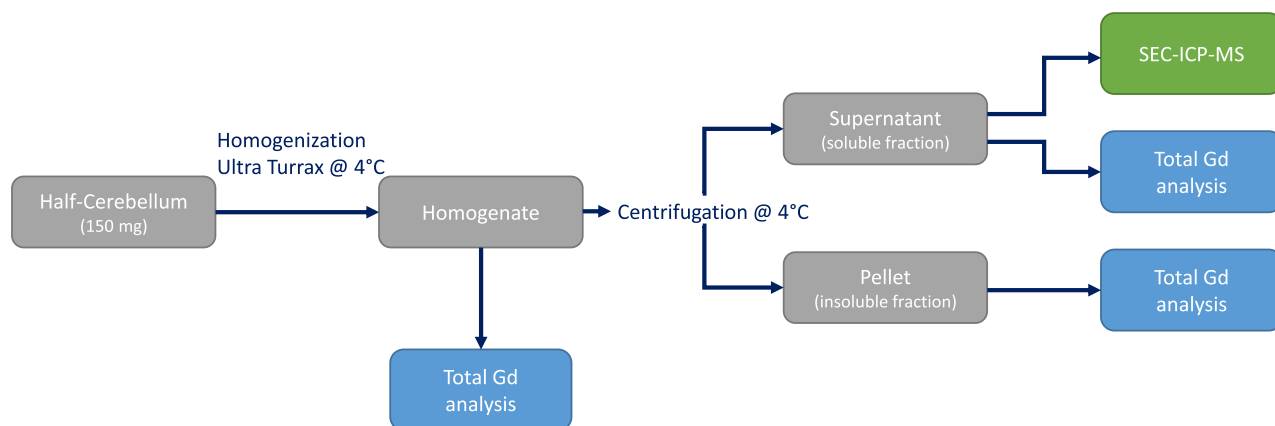


FIGURE 1. Sample preparation process and resulting fractions. Analytical methods corresponding to each fraction are shown.

separated from the pellet. The supernatant was collected and stored at  $-80^{\circ}\text{C}$  before speciation analysis by SEC coupled to ICP-MS. Aliquots of the homogenate, the supernatant, and the pellet were stored in  $-20^{\circ}\text{C}$  before determination of the total Gd concentration in these fractions. The sample preparation procedure is outlined in Figure 1.

### Total Gd Determination

Samples (either 100  $\mu\text{L}$  or the whole pellet) were digested on a hot plate with a mixture (3:1, vol/vol) of nitric acid and hydrogen peroxide (either 400  $\mu\text{L}$  or a volume calculated to respect the dilution factor of 5) at  $80^{\circ}\text{C}$  for 8 hours followed by dilution with water. In this last step, indium was added as internal standard to obtain 1 ng/mL final concentration. Total Gd concentrations were measured in a homogenate, a supernatant, and a pellet with ICP-MS using an Agilent Model 7500 or 7700x ICP MS (Agilent Technologies, Santa Clara, CA). The ICP-MS instrument used for total Gd determination was optimized daily using a multielemental solution. A standard curve of inorganic Gd in 4.2%  $\text{HNO}_3$  was used by monitoring the response of the  $^{158}\text{Gd}$  isotope. The quantification range of the method was 0.010 to 1.0 ng/mL in solution for the first time-point (M1) and 0.001 to 0.5 ng/mL for the second time-point (M5). The Gd concentration in each fraction of cerebellum (homogenate, supernatant, and pellet) was expressed as nanomole per gram and was calculated by multiplying the Gd concentration measured in the final solution by the dilution factor applied during sample preparation, including the homogenization step for calculation for the cerebellum. The corresponding lower limits of quantification (LLOQs) of Gd in the cerebellum were 0.018 nmol/g in wet tissue (M1) and 0.002 nmol/g in wet tissue (M5), complying with the criterion that the analyte response at the LLOQ should be at least 5 times the analyte response of the zero calibrator.<sup>26</sup>

### Gd Speciation Analysis in the Cerebellum

To investigate the presence of Gd species in the cerebellum after a single dose of GBCA, samples taken at the M1 and M5 time-points were analyzed by SEC-ICP-MS using the 1200 or 1260 HPLC separation system (Agilent) with detection by an ICP-MS Agilent Model 7700x. Gadolinium species were isocratically eluted from a Superdex-75 column (300  $\times$  10 mm; GE Healthcare, Chalfont St Giles, United Kingdom) with 100 mM ammonium acetate (pH 7.4) over 45 minutes at a flow rate of 0.7 mL/min. The injection volume was 100  $\mu\text{L}$ . The size-exclusion column was calibrated using a series of different molecular weight markers to correlate with the elution volume (retention time). The molecular weight markers were Blue Dextran, transferrin, bovine serum albumin, ovalbumin, carbonic anhydrase, cytochrome C, and Gly-Tyr. The LLOQ of SEC-ICP-MS was estimated using standard GBCA solutions and set at 0.318 pmol/mL for all 3 GBCAs for both time-points (M1 and M5),

complying with the criterion that the signal-to-noise ratio must be at least 5. To control for the background level of Gd, the supernatants obtained from the samples of the control group were run between the samples of the GBCA group as blanks. The average blank chromatogram per run was calculated (6 runs in total) and subtracted from the chromatograms of the corresponding runs of the GBCA groups. To evaluate the amount of water-soluble Gd extracted from the cerebellum and analyzed by SEC-ICP-MS, the extraction efficiency was calculated using the following equation:  $\text{extraction efficiency} = \frac{n_{\text{Gd, supernatant}}}{n_{\text{Gd, supernatant}} + n_{\text{Gd, pellet}}}$ , where  $n_{\text{Gd}}$  is the number of moles of Gd. In addition, the mass balance was verified using the following equation:  $\text{mass balance} = \frac{n_{\text{Gd, supernatant}} + n_{\text{Gd, pellet}}}{n_{\text{Gd, homogenate}}}$ .

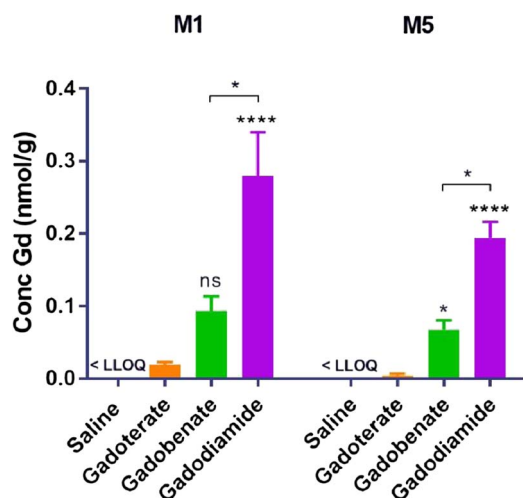
### Statistical Analysis

Data are expressed as mean  $\pm$  standard deviation (SD). Due to the nonhomogeneous variance of total Gd concentrations, and the nonnormal distribution of some groups (D'Agostino-Pearson test),<sup>27</sup> the differences between these data were analyzed using a Kruskal-Wallis test followed by a Dunn test. For calculation of means and SDs, and for statistical analysis of total Gd concentration in the cerebellum, values less than LLOQ were arbitrarily replaced by the LLOQ value. Outliers for all data were detected and excluded from the analysis according to the ROUT method<sup>28</sup> (Q = 1%). Statistical analyses were carried out using GraphPad Prism 7 software (GraphPad Software Inc, San Diego, CA). Differences were considered significant at  $P \leq 0.05$ .

## RESULTS

### Total Gd Determination

Total Gd concentrations in the cerebellum are shown in Figure 2. At the 2 time-points, total Gd concentrations for gadodiamide, the least stable GBCA, were significantly higher than the concentration measured for gadoterate ( $P < 0.0001$ ). Similarly, the highest Gd concentration was found for gadodiamide (M1:  $0.280 \pm 0.060$  nmol/g; M5:  $0.193 \pm 0.023$  nmol/g) followed by gadobenate dimeglumine (M1:  $0.093 \pm 0.020$  nmol/g; M5:  $0.067 \pm 0.013$  nmol/g). The lowest total Gd concentration was therefore detected for gadoterate meglumine (M1:  $0.019 \pm 0.004$  nmol/g, M5:  $0.004 \pm 0.002$  nmol/g). At both time-points, Gd concentrations after injection of gadobenate, a linear GBCA, were significantly lower ( $P < 0.05$ ) than those measured after injection of gadodiamide. At M5, the Gd concentration after injection of gadoterate was significantly lower than that measured after injection of gadobenate ( $P < 0.05$ ). A similar but nonsignificant trend was observed at M1, which could be due to the presence of 5 results situated below the LLOQ. Five months after a single injection of gadoterate meglumine, the Gd concentration decreased (0.004 nmol/g) and was



Kruskal-Wallis test for M1 and M5  $p < 0.0001$

Comparison with Dunn's test results in graph:

\*\*\*\* ( $p \leq 0.0001$ ) gadodiamide M1/M5 vs. gadoterate M1/M5

\* ( $p \leq 0.05$ ) gadobenate M5 vs. gadoterate M5

ns (not significant) gadobenate M1 vs. gadoterate M1

For M1: LLOQ<sub>M1</sub> = 0.018 nmol/g; for M5: LLOQ<sub>M5</sub> = 0.002 nmol/g.

**FIGURE 2.** Total gadolinium concentration in cerebellum after a single injection of various gadolinium-based contrast agents 1 month (M1) and 5 months (M5) after injection. Group sizes are 10 rats per product per time-point, except for groups in which outliers were excluded (total of 6 outliers for 80 values were excluded). Therefore, data points for each group are, for M1: gadoterate  $n = 8$ , gadobenate  $n = 10$ , gadodiamide  $n = 10$ , saline  $n = 9$ ; for M5: gadoterate  $n = 9$ , gadobenate  $n = 10$ , gadodiamide  $n = 9$ , saline  $n = 9$ .

close to the LLOQ of the method (LLOQ = 0.002 nmol/g). At this time-point, the residual Gd concentrations in the cerebellum were 48-fold and 17-fold higher for the linear GBCAs gadodiamide and gadobenate, respectively, than for the macrocyclic GBCA gadoterate.

## Gd Speciation Analysis in Cerebellum

Table 2 shows the extraction efficiencies of water-soluble fraction of cerebellum for each GBCA obtained after the sample preparation procedure for each of the 2 time-points. The extraction efficiency varied significantly with the molecular category of GBCA for both time-points. The highest extraction efficiency was found for gadoterate (greater than 50%). The extraction efficiency for gadobenate and gadodiamide was much lower (not greater than 25%). The Gd mass balance was also verified between the various fractions obtained. By taking into account the initial concentrations in the homogenates,  $89\% \pm 8\%$  (expressed as the mean  $\pm$  SD of 49 samples from the GBCA group) of the Gd was found in the various fractions (supernatant + pellet), indicating that virtually no Gd was lost during the sample preparation process (data not shown). For the remaining 11 samples, the values of Gd concentration for some fractions were below LLOQ and therefore were not included in the mass balance calculation.

Representative examples of SEC-ICP-MS chromatograms of the water-soluble fraction extracted from cerebellum are shown in Figure 3. After injection of gadoterate, at both M1 and M5, only one Gd species was eluted at 26.4 minutes. According to column calibration, all species eluting before 13.1 minutes should be larger than 80 kDa, whereas species eluting after 20.1 minutes should be associated with molecules

smaller than 12 kDa. The signal observed for gadoterate therefore corresponds to low molecular weight molecules, such as the form of GBCA initially injected. This retention time is in good agreement with the retention time detected for the standard GBCA solution. For the linear GBCAs, SEC-ICP-MS analysis of the soluble fraction showed 2 significant Gd peaks eluted at 11.7 minutes and 26.4 minutes. Similar to gadoterate, the peaks eluting at 26.4 minutes could be attributed to the form of Gd chelate initially injected. The signal observed at 11.7 minutes was assigned to a fraction of Gd bound to macromolecules heavier than 80 kDa. Five months after injection, the SI of intact GBCA (retention time, 26.4 minutes) decreased for all 3 GBCAs. However, the Gd signal at 11.7 minutes, representing Gd bound to macromolecules and detected only for the linear GBCAs, seemed to have remained constant 5 months after injection. Moreover, for both time-points, the proportion of the peak at 11.7 minutes was higher for gadodiamide than for gadobenate.

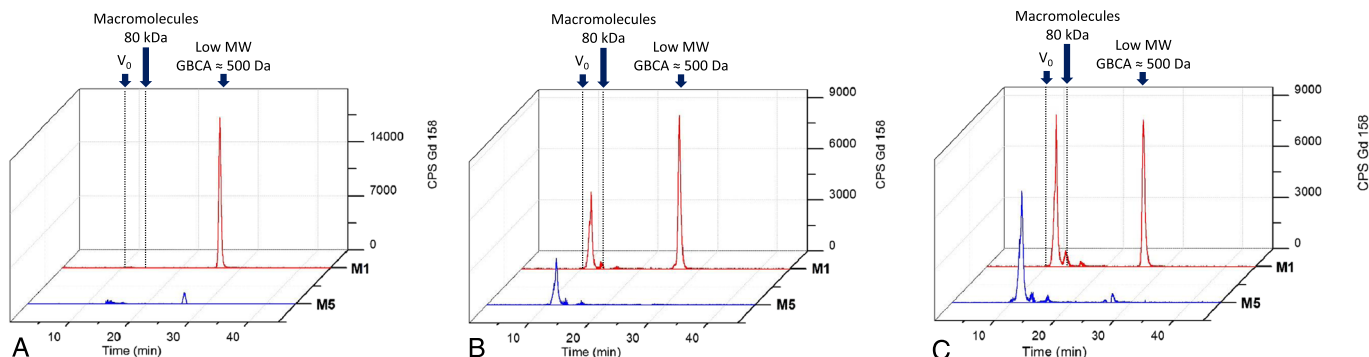
## DISCUSSION

This study was based on the methodological approach reported by Frenzel et al<sup>13</sup> to investigate whether residual Gd in the rat brain was present in the form of intact GBCA or in other chemical form(s) 3 and 24 days after 10 repeated injections of different GBCAs (cumulative dose, 25 mmol/kg). A similar methodology was subsequently used in a 1-year study of the long-term brain elimination kinetics and Gd species in rats after 5 injections of GBCAs (cumulative dose, 12 mmol/kg).<sup>14</sup> These analytical approaches are based on tissue fractionation and analysis of the water-soluble fraction extracted from different parts of the brain by HPLC-ICP-MS. In these studies, SEC was used for the detection of Gd species. Size-exclusion chromatography allows separation of analytes mainly according to their hydrodynamic volume, although other nonspecific mechanisms may also be concurrently involved. Smaller molecules flow more slowly through the column because they penetrate into the pores of the stationary phase, whereas large molecules are rapidly eluted through the column because they do not enter the pores. Hydrophilic interaction liquid chromatography, which allows unambiguous detection of small and hydrophilic molecules such as intact GBCA, was also used in 2 studies.<sup>14,15</sup> Hydrophilic interaction liquid chromatography ICP-MS therefore provides complementary information to SEC-ICP-MS, allowing identification of intact GBCA in the soluble fraction but was not used in current study. These techniques allow a better understanding of Gd speciation and Gd elimination from the brain parenchyma after repeated injections of GBCAs. This study demonstrates, for the first time, the presence of different Gd species in rat cerebellum after a single injection of a clinically relevant dose of macrocyclic versus linear GBCAs. Because of very low brain Gd concentration expected after a single dose of 0.6 mmol/kg of GBCA, special precautions were taken to control for

**TABLE 2.** Gadolinium Extraction Efficiency of Soluble Fraction of Cerebellum

Contrast Agent	Type	Extraction Efficiency (%)	
		Month 1	Month 5
Gadoterate meglumine	Macrocyclic	70 $\pm$ 2 (n = 5)	51 $\pm$ 10 (n = 5)
Gadobenate dimeglumine	Linear	25 $\pm$ 10 (n = 3)	9 $\pm$ 2 (n = 10)
Gadodiamide	Linear	8 $\pm$ 1 (n = 10)	8 $\pm$ 1 (n = 9)

Differences in number of replicates are due to the fact that, for some samples, the Gd concentration in the supernatant was <LLOQ. Note that the LLOQ for the M5 point (0.002 nmol/g) were much lower than for M1 (0.018 nmol/g) because of a different ICP-MS system used.



**FIGURE 3.** Typical SEC-ICP-MS chromatograms of the water-soluble fraction of cerebellum extracts after single injection of gadoterate meglumine (A), gadobenate dimeglumine (B), gadodiamide (C) 1 month (M1) and 5 months (M5) after injection. The chromatograms are shown after subtraction of average blanks.  $V_0$  indicates void volume, determined using Blue Dextran (2000 kDa).

the background level of Gd and to avoid cross-contamination at each step of the experimental work. The procedure used in this study proved to be robust for the analysis of a large number of complex biological samples containing trace levels of Gd. Regarding the outliers, one sample showed an extremely high Gd concentration, strongly suggesting a contamination during the experimental work for this sample. Some of the 5 other outliers may be due to the fact that, for the purpose of the statistical treatment, the LLOQ value was arbitrarily assigned to the concentrations below LLOQ, which may have induced “artificial” outliers by the ROUT test.

Our data are qualitatively consistent with the results of previous studies of repeated injections in rats<sup>13–18</sup> and with the study of single dose injection of GBCAs in a large animal model (ie, sheep).<sup>23</sup> Linear GBCAs leave more residual total Gd in the cerebellum than macrocyclic GBCAs. Gadolinium elimination between M1 and M5 is much faster for the macrocyclic GBCA gadoterate (79% decrease in total, elementary Gd concentration) than for the linear GBCAs gadobenate and gadodiamide, with a 28% and 31% decrease, respectively, which confirms previous findings.<sup>14,16</sup> Analysis of Gd speciation in the soluble fraction 1 month after a single injection of linear GBCAs showed that Gd was present in at least 2 distinct chemical forms: (1) small molecules including intact GBCA, and (2) soluble macromolecules. As reported by Robert et al,<sup>14</sup> we confirmed that the Gd detected in soluble macromolecules should be associated with the class of macromolecules larger than 80 kDa. However, we observed only one significant Gd species, whereas Robert et al reported 2 peaks. On the other hand, Frenzel et al suggested that only a single class of macromolecules contained Gd, but with significantly higher molecular weights (200–300 kDa). The different number of Gd signals bound to macromolecules, detected on SEC-ICP-MS chromatograms, could be explained by the use of different chromatographic columns. In this study, we used the same type of stationary phase as that used by Frenzel et al, which is a composite of cross-linked agarose and dextran, whereas Robert et al used a stationary phase composed of silica. The difference in molecular weight of the macromolecular Gd fraction detected (80 vs 300 kDa) is probably due to the use of different molecular weight markers for column calibration. It should also be noted that if the nature of the molecular weight marker used for column calibration differs from that of the analyte, molecular weights cannot be accurately calculated.

Compared with the Gd speciation observed for linear GBCAs, Gd was only detected in the form of intact GBCA after a single injection of the macrocyclic GBCA gadoterate. As previously described,<sup>14</sup> washout of the intact GBCA can be observed for all GBCAs between M1 and M5. In the present study, we report an 89% to 100% decrease of the levels of the intact GBCA. In contrast, the amount of Gd bound to soluble macromolecules observed for gadobenate and gadodiamide

remained constant 5 months after injection of a single dose of GBCA. It is possible that this fraction of Gd generates the persistent SI enhancement observed on MRI images. Consequently, the binding of Gd to soluble macromolecules could maximize its relaxivity and produce visible SI enhancement despite the very low Gd concentration. Another key point is that the extraction efficiency varies for each GBCA, as reported elsewhere.<sup>13,15,29</sup> After injection of macrocyclic GBCAs, Gd was mostly found in the soluble brain fraction in the form of low molecular-weight molecules (Frenzel et al), corresponding to the intact GBCA form (Gianolio et al). Conversely, after injection of linear GBCAs, Gd was largely found in the pellet. The least thermodynamically stable GBCA could therefore induce other Gd forms, which are less effectively extracted by the sample preparation protocol, as reflected by our data. Interestingly, the extraction efficiency at M1 decreases in the order of stability of the GBCA: gadoterate > gadobenate > gadodiamide (Port et al<sup>10</sup>).

A limitation of this study is the fact that SEC-ICP-MS does not allow identification of the chemical nature of the macromolecules and cannot demonstrate whether the intact linear GBCA or transmetalated  $Gd^{3+}$  ion is bound to macromolecules. This information would represent a substantial progress toward a better understanding of the processes involved in Gd accumulation in brain and the putative biological and toxicological consequences of this phenomenon. Frenzel et al<sup>15</sup> suggested that intact GBCAs (except for gadobenate) do not bind to macromolecules because they do not bind to plasma proteins. Furthermore, a study comprising 15-day incubation of GBCAs in human serum showed release of 20% of free  $Gd^{3+}$  from nonionic linear GBCAs and 2% from ionic linear GBCAs, whereas no Gd release was reported for macrocyclic GBCAs.<sup>11</sup> Although these findings strongly support the theory of potential dechelation of linear GBCAs, no evidence is available concerning the chemical form of Gd bound to macromolecules.

The second limitation of this study is that the speciation method only provides information about the water-soluble fraction. The insoluble fraction (pellet) that contains a large amount of Gd (especially for linear GBCAs) cannot be analyzed in terms of speciation. The pellet contains cell debris, inorganic precipitates such as colloidal forms of hydroxide, phosphate, and carbonate or denatured macromolecules, which could bind Gd or capture GBCAs. Research into solving this problem is already underway.

## CONCLUSIONS

Even a single injection of a clinically relevant dose of Gd-based contrast agent resulted in long-term Gd detection in the cerebellar parenchyma of healthy rats. Administration of linear GBCAs (gadodiamide and gadobenate) resulted in higher residual Gd concentrations than

administration of the macrocyclic agent gadoterate (15-fold higher at M1 to 48-fold higher at M5 for gadodiamide vs gadoterate, 5-fold higher at M1 to 17-fold higher at M5 for gadobenate vs gadoterate). Speciation analysis of the water-soluble fraction of cerebellum confirmed washout of the intact GBCA form with time. The amount of Gd bound to macromolecules, only observed with the linear GBCA, remained constant 5 months after injection and likely represents a permanent deposition.

## REFERENCES

- Kanda T, Ishii K, Kawaguchi H, et al. High signal intensity in the dentate nucleus and globus pallidus on unenhanced T1-weighted MR images: relationship with increasing cumulative dose of a gadolinium-based contrast material. *Radiology*. 2014;270:834–841.
- Radbruch A, Weberling LD, Kieslich PJ, et al. Gadolinium retention in the dentate nucleus and globus pallidus is dependent on the class of contrast agent. *Radiology*. 2015;275:783–791.
- Runge VM. Safety of the gadolinium-based contrast agents for magnetic resonance imaging, focusing in part on their accumulation in the brain and especially the dentate nucleus. *Invest Radiol*. 2016;51:273–279.
- Kanda T, Nakai Y, Oba H, et al. Gadolinium deposition in the brain. *Magn Reson Imaging*. 2016;34:1346–1350.
- McDonald RJ, McDonald JS, Kallmes DF, et al. Intracranial gadolinium deposition after contrast-enhanced MR imaging. *Radiology*. 2015;275:772–782.
- Robert P, Lehericy S, Grand S, et al. T1-weighted hypersignal in the deep cerebellar nuclei after repeated administrations of gadolinium-based contrast agents in healthy rats: difference between linear and macrocyclic agents. *Invest Radiol*. 2015;50:473–480.
- Palasz A, Czekaj P. Toxicological and cytophysiological aspects of lanthanides action. *Acta Biochim Pol*. 2000;47:1107–1114.
- Grobner T. Gadolinium—a specific trigger for the development of nephrogenic fibrosing dermopathy and nephrogenic systemic fibrosis? *Nephrol Dial Transplant*. 2006;21:1104–1108.
- Idée JM, Fretellier N, Robic C, et al. The role of gadolinium chelates in the mechanism of nephrogenic systemic fibrosis: a critical update. *Crit Rev Toxicol*. 2014;44:895–913.
- Port M, Idée JM, Medina C, et al. Efficiency, thermodynamic and kinetic stability of marketed gadolinium chelates and their possible clinical consequences: a critical review. *Biometals*. 2008;21:469–490.
- Frenzel T, Lengsfeld P, Schirmer H, et al. Stability of gadolinium-based magnetic resonance imaging contrast agents in human serum at 37°C. *Invest Radiol*. 2008;43:817–828.
- Fretellier N, Idée JM, Dencausse A, et al. Comparative in vivo dissociation of gadolinium chelates in renally impaired rats: a relaxometry study. *Invest Radiol*. 2011;46:292–300.
- Frenzel T, Apte C, Jost G, et al. Quantification and assessment of the chemical form of residual gadolinium in the brain after repeated administration of gadolinium-based contrast agents: comparative study in rats. *Invest Radiol*. 2017;52:396–404.
- Robert P, Fingerhut S, Factor C, et al. One-year retention of gadolinium in the brain: comparison of gadodiamide and gadoterate meglumine in a rodent model. *Radiology*. 2018;288:424–433.
- Gianolio E, Bardini P, Arena F, et al. Gadolinium retention in the rat brain: assessment of the amounts of insoluble gadolinium-containing species and intact gadolinium complexes after repeated administration of gadolinium-based contrast agents. *Radiology*. 2017;285:839–849.
- Jost G, Frenzel T, Boyken J, et al. Long-term excretion of gadolinium-based contrast agents: linear versus macrocyclic agents in an experimental rat model. *Radiology*. 2019;290:340–348.
- Sieber MA, Lengsfeld P, Frenzel T, 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.
- Rasschaert M, Emerit A, Fretellier N, et al. Gadolinium retention, brain T1 hyperintensity, and endogenous metals: a comparative study of macrocyclic versus linear gadolinium chelates in renally sensitized rats. *Invest Radiol*. 2018;53:328–337.
- European Medicines Agency. EMA's final opinion confirms restrictions on use of linear gadolinium agents in body scans. Available at: [http://www.ema.europa.eu/docs/en\\_gb/document\\_library/referrals\\_document/gadolinium\\_contrast\\_agents\\_31/european\\_commission\\_final\\_decision/wc500240575.pdf](http://www.ema.europa.eu/docs/en_gb/document_library/referrals_document/gadolinium_contrast_agents_31/european_commission_final_decision/wc500240575.pdf). Accessed March 5, 2019.
- Food and Drug Administration. FDA drug safety communication: FDA warns that gadolinium-based contrast agents (GBCAs) are retained in the body; requires new class warnings. Available at: <https://www.fda.gov/drugs/drugsafety/ucm589213.htm>. Accessed March 5, 2019.
- Tweedle MF, Wedeking P, Kumar K. Biodistribution of radiolabeled, formulated gadopentetate, gadoteridol, gadoterate, and gadodiamide in mice and rats. *Invest Radiol*. 1995;30:372–380.
- Di Gregorio E, Ferrauto G, Furlan C, et al. The issue of gadolinium retained in tissues: insights on the role of metal complex stability by comparing metal uptake in murine tissues upon the concomitant administration of lanthanum- and gadolinium-diethylenetriaminopentaacetate. *Invest Radiol*. 2018;53:167–172.
- Radbruch A, Richter H, Fingerhut S, et al. Gadolinium deposition in the brain in a large animal model Comparison of linear and macrocyclic gadolinium-based contrast agents. *Invest Radiol*. 2019;54:531–536.
- US Food and Drug Administration; 2005. USFDA. Guidance for Industry: Estimating the Maximum Safe Starting Dose in Adult Healthy Volunteer. Available at: <https://www.fda.gov/downloads/Drugs/Guidances/UCM078932.pdf>. Accessed March 5, 2019.
- Robert P, Frenzel T, Factor C, et al. Methodological aspects for preclinical evaluation of gadolinium presence in brain tissue: critical appraisal and suggestions for harmonization—a joint initiative. *Invest Radiol*. 2018;53:499–517.
- US Food and Drug Administration; 2018. USFDA. Bioanalytical Method Validation Guidance for Industry. Available at: <https://www.fda.gov/files/drugs/published/Bioanalytical-Method-Validation-Guidance-for-Industry.pdf>. Accessed March 5, 2019.
- D'Agostino R, Pearson ES. Tests for departure from normality. Empirical results for the distributions of  $b_2$  and  $\sqrt{b_1}$ . *Biometrika*. 1973;60:613–622.
- Motulsky HM, Brown RE. Detecting outliers when fitting data with nonlinear regression—a new method based on robust nonlinear regression and the false discovery rate. *BMC Bioinformatics*. 2006;7:123.
- Kahakachchi CL, Moore DA. Identification and characterization of gadolinium (III) complexes in biological tissue extracts. *Metallomics*. 2010;2:490–497.