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[1]

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Purpose: To evaluate the speciation of gadolinium-containing species after multiple administrations of the gadolinium-based contrast agents (GBCAs) gadodiamide and gadoteridol and to quantify the amount of intact gadolinium complexes and insoluble gadolinium-containing species.

Materials and Methods: A total dose of 13.2 mmol per kilogram of body weight of each GBCA was administered in healthy Wistar rats over a period of 8 weeks. Three days after the final administration, rats were sacrificed, and the brains were excised and divided into three portions. Each portion of brain homogenate was divided into two parts, one for determination of the total gadolinium concentration with inductively coupled plasma mass spectrometry and one for determination of the amount of intact GBCA and gadolinium-containing insoluble species. Relaxometric measurements of gadodiamide and gadolinium trichloride in the presence of polysialic acid were also performed.

Results: The mean total gadolinium concentrations for gadodiamide and gadoteridol, respectively, were 0.317 μg/g ± 0.060 (standard deviation) and 0.048 μg/g ± 0.004 in the cortex, 0.418 μg/g ± 0.078 and 0.051 μg/g ± 0.009 in the subcortical brain, and 0.781 μg/g ± 0.079 and 0.061 μg/g ± 0.012 in the cerebellum. Gadoteridol comprised 100% of the gadolinium species found in rats treated with gadoteridol. In rats treated with gadodiamide, the largest part of gadolinium retained in brain tissue was insoluble species. In the cerebellum, the amount of intact gadodiamide accounts for 18.2% ± 10.6 of the total gadolinium found therein. The mass balance found for gadolinium implies the occurrence of other soluble gadolinium-containing species (approximately 30%). The relaxivity of the gadolinium polysialic acid species formed in vitro was 97.8 mM/sec at 1.5 T and 298 K.

Conclusion: Gadoteridol was far less retained, and the entire detected gadolinium was intact soluble GBCA, while gadodiamide yielded both soluble and insoluble gadolinium-containing species, with insoluble species dominating.

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Gadolinium-based contrast agents (GBCAs) are commonly used in clinical settings, as they add physiologic information to the superb anatomic information provided by magnetic resonance (MR) images (1). Millions of contrast material–enhanced MR images have been acquired in the past 3 decades without any clinical evidence of frequent serious acute or chronic events. Hence, GBCAs are considered to be among the safest parenteral chemicals (2).

Recently, several studies have shown increased signal intensity on unenhanced T1-weighted MR images in some brain regions in patients with normal renal function who had previously received multiple doses of GBCAs (3–14). The observed hyperintense signal has been associated with the retention of small amounts of gadolinium in the brain (4,14). There has been evidence that both linear and macrocyclic GBCAs can yield detectable gadolinium traces in the brain, with linear neutral agents leaving greater quantities (11,13).

When using gadolinium chelates labeled with radioisotopes (gadolinium 153 and carbon 14), about 1% of the administered dose was retained in mice (15).

However, the observation that some gadolinium has been found in patients' brains has created a renewed focus on investigating how GBCA can leak from brain capillaries and accumulate in defined brain structures. One may surmise that GBCA can leak from brain capillaries as a consequence of a temporary blood-brain barrier (16–19) or a cerebrospinal fluid barrier dysfunction (20), for instance, as a consequence of the passage of the GBCA bolus (21).

On this basis, one may assume brain uptake regarding the different types of GBCA to be unspecific. After having entered the brain parenchyma, the amount and chemical form of the retained gadolinium is determined by the thermodynamic and kinetic stability of the GBCA (the latter is strongly affected by the chemical composition of the medium [22]), the washout rate through the lymphatic system, the overall chemical degradation of the GBCA, and other factors.

In this context, it appears to be of fundamental importance to assess the chemical form of the gadolinium-containing species that can be recovered from the brain parenchyma. The intrinsic difficulties associated with the availability of human biopsy samples prompted the development of animal models to investigate gadolinium retention in the brain. It has been found that successive administrations of GBCA to rodents over a few weeks leads to gadolinium retention in brain structures that mimics the pattern observed in patients (23–27).

The purpose of this study was to evaluate the speciation of gadolinium-containing species after multiple administrations of gadodiamide and gadoteridol and to quantify the amount of intact gadolinium complexes and insoluble gadolinium-containing species.

Advances in Knowledge

- The amount of retained gadolinium was 6.6, 8.2, and 12.8 times higher in animals exposed to gadodiamide than in animals exposed to gadoteridol in the cortex, subcortical brain, and cerebellum, respectively.
- After intravenous administration of gadoteridol, 100% of gadolinium recovered in the brain structures corresponded to intact gadoteridol; conversely, for gadodiamide, only 3.6% ± 8.1 (mean ± standard deviation), 5.6% ± 7.7, and 18.2% ± 10.6 corresponded to intact gadodiamide in the cortex, subcortical brain, and cerebellum, respectively.
- In animals treated with gadodiamide, the mean amount of gadolinium found in insoluble fractions was 99.6% ± 31.6, 112.0% ± 18.4, and 53.2% ± 10.4 in the cortex, subcortical brain, and cerebellum, respectively; in the cerebellum, an additional 30% soluble gadolinium-containing fraction that did not correspond to the intact gadolinium complex was found.
- The Nuclear Magnetic Resonance Dispersion profile of gadolinium chloride in the presence of polysialic acid showed the typical relaxivity peak centered in the region of 1.0–1.5 T, which was characteristic of high-molecular-weight paramagnetic species; at 1.5 T and 298 K, the relaxivity of the adduct was close to 100 mM/sec.

Materials and Methods

All procedures involving animals were conducted in accordance with national laws on experimental animals (L.D. no. 26/2014, Directive 2010/63/EU). No validated nonanimal alternatives are known to meet the study objectives.

No industry support was provided for this study. One author (S.A.) is a consultant for Bracco Imaging (Milan, Italy). The other authors had control over the inclusion of any data or information that might have presented a conflict of interest.

Gadoteridol (macrocyclic nonionic, ProHance; Bracco Imaging) and gadodiamide (linear nonionic, Omniscan; GE Healthcare, Chalfont St Giles, England) were the GBCAs used in this work.

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Abbreviations:
aCSF = artificial cerebrospinal fluid
DCN = deep cerebellar nuclei
GBCA = gadolinium-based contrast agent
ICP = inductively coupled plasma
MS = mass spectrometry
SIM = selected ion monitoring
UPLC = ultraperformance liquid chromatography

Author contributions:
Guarantors of integrity of entire study, E.G., P.B., E.D.G., R.I., S.A.; study concepts/study design or data acquisition or data analysis/interpretation, all authors; manuscript drafting or manuscript revision for important intellectual content, all authors; approval of final version of submitted manuscript, all authors; agrees to ensure any questions related to the work are appropriately resolved, all authors; literature research, E.G., P.B., F.A., E.D.G., S.A.; experimental studies, E.G., P.B., F.A., R.S., E.D.G., R.I.; statistical analysis, E.G., E.D.G.; and manuscript editing, all authors

Conflicts of interest are listed at the end of this article.

See also the editorial by Lenkinski in this issue.

Figure 1

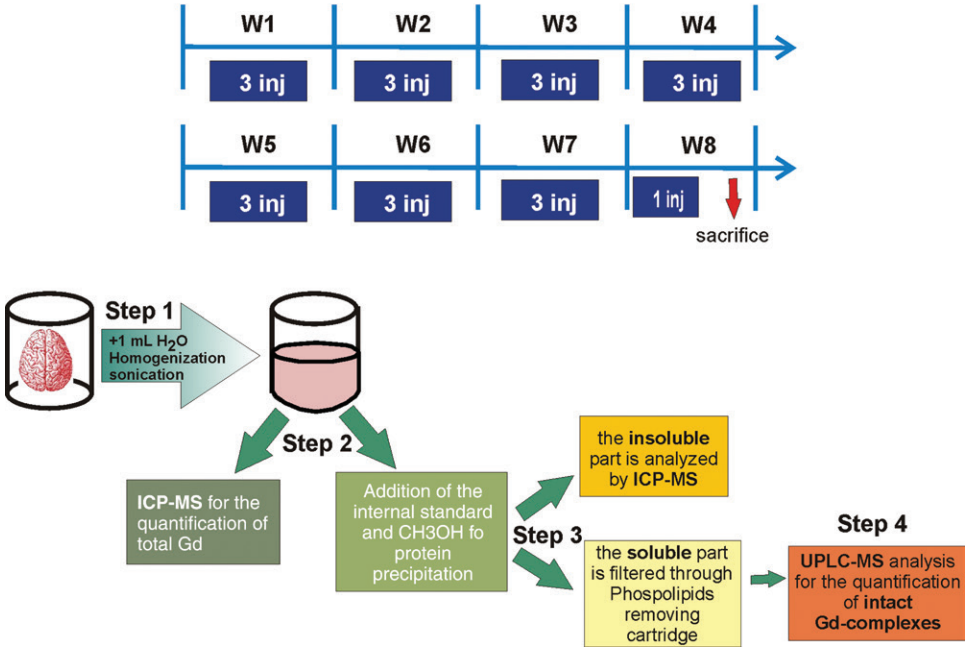

Figure 1: Schematic drawing of the experimental protocol used to assess total gadolinium and the amount of intact GBCA. Top: Outline of the timing of GBCA injections *(inj)*. *W* = week. Bottom: Experimental work-up for preparation of the samples to undergo inductively coupled plasma (ICP) mass spectrometry (MS) and ultraperformance liquid chromatography (UPLC) MS.

The compounds tullium 2-[4,7-bis(carboxylatomethyl)-10-(2-hydroxypropyl)-1,4,7,10-tetrazacyclododec-1-yl]acetate (Tm-HPDO3A) and tullium 2-[bis[2-[carboxymethyl-[2-(methylamino)-2-oxoethyl]amino]ethyl]amino]acetate (Tm-DTPA-BMA) were synthesized by mixing a 1:1 M ratio of thulium trichloride and the aqueous solutions of the HPDO3A or DTPA-BMA ligands, at neutral pH at 50°C overnight. The amount of residual free thulium ions was precipitated at basic pH (pH = 11) and removed via filtration.

Study Set-up

Fifteen male Wistar rats (six in the gadoteridol group, six in the gadodiamide group, three in the control group) with a mean weight of 200 g ± 20 received 22 intravenous injections of 0.6 mmol per kilogram of body weight GBCA over a period of 8 weeks (every 2 days) (Fig 1a). The sample size was calculated by considering a significance level of 5%, a power of 80%, and a minimum difference between groups of 30%. In rats, the 0.6 mmol/kg dose is considered equivalent to the usual human dose of 0.1 mmol/kg after adjusting for body surface area, as recommended by the Food and Drug Administration (28).

Rats were sacrificed 3 days after the last GBCA administration, and their brains were excised and dissected into parts corresponding to the cortex, subcortical brain, and cerebellum. Each brain portion was immersed in 1 mL of water, homogenized (Homogenizer OV5; VELP Scientifica, Usmate Velate, Italy), and subjected to ultrasonic vibration to induce complete cell lysis. As shown in Figure 1, the homogenate was divided into two equal volumes: one was lyophilized for subsequent analysis with ICP MS to quantify total gadolinium, and the other was processed to quantify the amounts of insoluble gadolinium-containing species and intact gadolinium complexes. To the latter portion, 0.25 nmol of the appropriate internal standard (Tm-HPDO3A or Tm-DTPA-BMA) were added. Then, the homogenate was treated with methanol (CH_3OH) containing 0.1% formic acid (HCOOH) (four volumes of organic solvent per volume of homogenate) to precipitate proteins together with other insoluble species. The solid residue was removed via centrifugation at 4000 rpm for 10 minutes. The supernatant was filtered three times through Phree Phospholipid Removal Tabbed 1-mL tubes (Phenomenex, Torrance, Calif) to completely remove phospholipids and proteins not precipitated with the previous treatment. The resulting solutions were dried overnight in a vacuum desiccator to remove CH_3OH and were concentrated to a final volume of 50 μL. The supernatant was analyzed with an UPLC tandem mass spectrometer to identify and quantify intact gadolinium complexes. The solid residue was mineralized and analyzed with ICP MS to determine gadolinium (III) content. The complete recovery of gadoteridol and gadodiamide after treatment with CH_3OH containing 0.1% HCOOH and filtration through the aforementioned tubes was checked with UPLC MS analysis. Four volumes of organic solvent (CH_3OH +

0.1% HCOOH) were added per volume of water solutions of gadoteridol or gadodiamide to a final concentration of 20 μM. The obtained solutions were then analyzed with UPLC MS before and after filtration, and the corresponding peaks of the two gadolinium complexes were integrated and compared with those of the relative solutions in water (20-μM concentration).

The procedure was further validated by assessing the total gadolinium recovery after addition of GBCA aliquots to brain samples from healthy rats. After running the spiked samples through the entire separation process, UPLC MS analysis was performed.

ICP MS Analysis

The gadolinium content in brain homogenates was measured with ICP MS analysis (Element-2; Thermo-Finnigan, Rodano, Italy), and the results were expressed as milligrams per gram of wet tissue weight. Preparation of the samples for ICP MS analysis was performed, as follows: *(a)* One milliliter of concentrated nitric acid (70%) was added to each lyophilized homogenate, and the sample was mineralized by microwave heating at 160°C for 40 minutes in a microwave laboratory station (MicroSynth; Milestone, Bergamo, Italy) equipped with an optical fiber temperature control and an HPR-1000/6M six-position high-pressure reactor. *(b)* After mineralization, the volume of each sample was brought to 2 mL with ultrapure water, and the sample was analyzed with ICP MS. The calibration curve was obtained by using four gadolinium absorption standard solutions (Sigma-Aldrich, Milan, Italy) of 0.005–0.1 μg/mL.

UPLC MS Analysis

Separation and quantification of the intact GBCAs was performed by using UPLC MS or ESI MS with an Acquity H-class UPLC system coupled to an Acquity QDa detector (Waters, Vimodrone, Italy). An Acquity UPLC ethylene bridged hybrid hydrophilic interaction lIquid chromatography (BEH HILIC) column (2.1 × 100 mm; 1.7-mm particle size) with a VanGuard precolumn was used in isocratic elution with mobile phase A (ammonium formate, 12.5 mM; formic acid, 12.5 mM; 3.75 pH) set at 76% and mobile phase B (acetonitrile) set at 24%. Flow rate was 0.6 mL/min for a total high-performance liquid chromatography MS analysis time of 5 minutes (for each sample). The column was kept at 40°C. The injection volume was 2 or 4 mL. The chromatographic conditions were chosen on the basis of a previously reported high-performance liquid chromatography method used to separate GBCAs (29,30).

Electrospray ionization with or without MS was performed either in the full scan mode (range, 250–800 m/z) or in the selected ion monitoring (SIM) mode. Instrumental MS conditions were as follows: capillary voltage, 0.8 kV; cone voltage, 20 V; source temperature, 120°C; and probe temperature, 600°C.

The signal of gadoteridol was acquired in the SIM mode at $[MH]^+$ equal to 557–558–559–560–562 and in its adduct with K^+ at $[MK]^+$ equal to 595–596–597–598–600. The selected reference standard was Tm-HPDO3A with $[MH]^+$ equal to 571–572 and $[MK]^+$ equal to 609–610, respectively. Analogously, gadodiamide was analyzed with SIM at $[MH]^+$ equal to 572–573–574–575–577 and in its adduct with K^+ at $[MK]^+$ equal to 610–611–612–613–615, respectively. The selected reference standard was Tm-DTPA-BMA with [MH]+ equal to 586–587 and [MK]+ equal to 624–625, respectively. A representative example of the acquired chromatograms and MS spectra is shown in Figure 2.

The set-up of this method involved acquisition of calibration curves obtained by adding aliquots of an appropriate internal standard (Tm-HPDO3A or Tm-DTPA-BMA) to brain homogenates at a final concentration of 0.5–10 μM and aliquots of the gadolinium complexes to be analyzed in the same concentration range (in triplicate).

To quantify the amount of intact gadolinium complexes in the brain samples, 0.25 nmol of the internal standard (ie, the selected thulium complex) was added to brain homogenates. SIM chromatographic peaks of the intact gadolinium complexes were integrated with respect to the corresponding peaks of the internal standard. Samples were analyzed in triplicate. The amount of gadolinium complexes was obtained by using the following formula:

$$[\mathrm{Gd-complex}] = \frac{K \times A(\mathrm{Gd}) \times [\mathrm{Tm-complex}]}{A(\mathrm{Tm})},$$

where $A(\mathrm{Gd})$ and $A(\mathrm{Tm})$ are the areas of the peaks that correspond to gadolinium and thulium complexes, respectively, and K is the experimentally derived conversion factor (Figs E1-E3 [online]).

MR Imaging

MR imaging was performed with general anesthesia (2.0%–2.5% isofluorane) before the first GBCA administration and after the 22nd injection. MR imaging was performed 3 days after the last injection, corresponding to a minimal 72-hour clearance period. MR images were acquired with a dedicated quadrature body coil and a 3-T preclinical imager (Biospec 30/40; Bruker, Ettlingen, Germany). After scout image acquisition, a T2-weighted anatomic image was acquired by using the rapid acquisition with relaxation enhancement sequence (repetition time msec/echo time msec, 4400/46; 16 sections; section thickness, 1.0 mm; field of view, 50 mm; matrix, 128 × 128; two signal averages; acquisition time, 2 minutes 20 seconds).

T1-weighted images were acquired with a section package centered on the deep cerebellar nuclei (DCN), hippocampus, and cerebral cortex by using a multisection multiecho sequence (400/10.2; seven sections; section thickness, 1.0 mm; field of view, 50 mm; matrix, 192 × 192; 64 signal averages; acquisition time, 20 minutes 28 seconds) (Fig E2 [online]).

Image Analysis

Quantitative evaluation of enhanced signal intensity in the brain was performed by drawing a region of interest over the more visible of the two DCN zones and over a reference zone in the

Figure 2

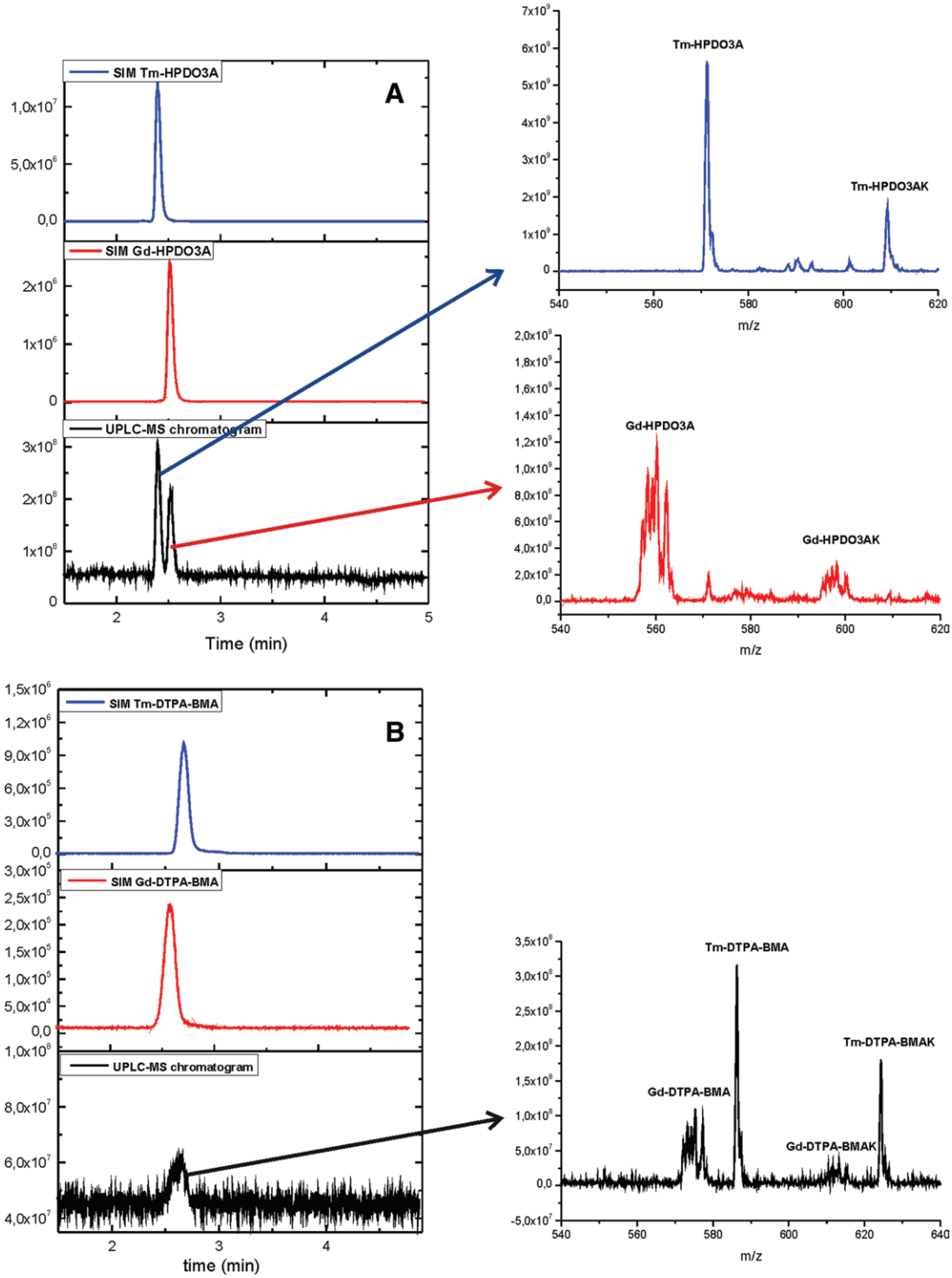

Figure 2: *A,* Gadoteridol and Tm-HPDO3A. *B,* Gadodiamide and Tm-DTPA-BMA. Left: Full scan mode (black) and selected ion mode (red and blue) chromatograms of brain homogenates containing equal concentrations (5 μM) of GBCA and the internal reference standard. Right: Corresponding mass spectra.

Figure 3

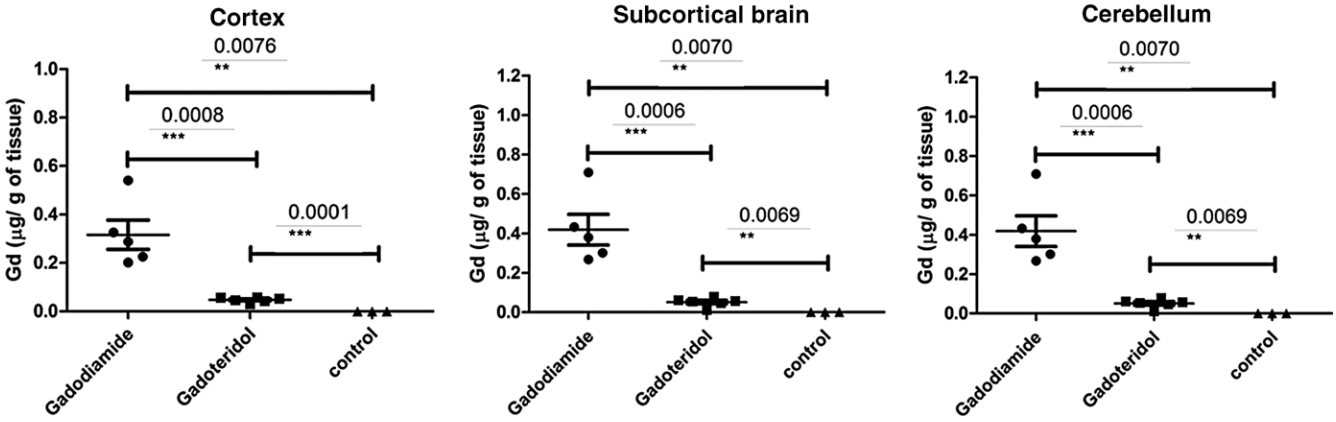

Figure 3: Total amount of gadolinium, as determined with ICP MS in the cortex, subcortical brain, and cerebellum 3 days after final administration of gadodiamide and gadoteridol. There were five animals in the gadodiamide and gadoteridol groups and three in the control group. Error bars show standard deviation.

cerebellar cortex. The variation of signal intensity was calculated as a ratio of maximum DCN signal intensity to maximum cerebellar cortex signal intensity and was expressed as DCN-to–cerebellar cortex signal intensity ratio (Fig E2 [online]).

One-way analysis of variance was used to compare the DCN-to–cerebellar cortex signal intensity ratio between rats treated with gadodiamide, those treated with gadoteridol, and those given saline at the end of treatment period. All statistical analyses were performed by using statistical software (Prism 6; *https://graphpad.com*). Values are presented as mean ± standard deviation, and $P < .05$ indicated a significant difference.

Water Proton Relaxation Measurements

The longitudinal water proton relaxation rates of gadodiamide in an artificial cerebrospinal fluid (aCSF) medium (with and without the addition of polysialic acid–colominic acid sodium salt from *Escherichia coli* [Sigma-Aldrich] at a concentration of 1 mg/mL) were measured at 25°C by using a Spinmaster (Stelar, Mede, Pavia, Italy) spectrometer operating at 0.5 T (21.5-MHz proton Larmor frequency), by means of the standard inversion recovery technique. The aCSF medium was prepared according to the published protocol (31) with the following composition: sodium chloride, 121 mM; sodium bicabonate, 23.1 mM; potassium chloride, 2.8 mM; magnesium chloride, 2.2 mM, sodium dihydrogen phosphate, 1.1 mM; and glucose, 0.61 g/L.

The proton $1/T_1$ nuclear MR dispersion profiles (field dependence of the longitudinal water proton relaxation rates) of gadolinium chloride (0.1 mM) with and without the addition of polysialic acid at 1 mg/mL concentration were measured at 25°C on a fast-field-cycling relaxometer (Stelar) over a continuum of magnetic field strengths from 0.00024 to 0.47 T (corresponding to 0.01–20.0-MHz proton Larmor frequencies). The relaxometer is controlled via computer with an absolute uncertainty in 1/T1 of ±1%. Additional data points in the range of 21.5–70.0 MHz were obtained with the Spinmaster spectrometer (Stelar). The concentration of solutions used for relaxometric characterization was determined according to a previously reported relaxometric method (32).

Statistical Analysis

All data are expressed as mean ± standard deviation. Between-group differences with respect to the mean gadolinium concentration of a GBCA were assessed by using the unpaired *t* test. The aforementioned statistical software was used for data analysis. $P < .05$ indicated statistical significance.

Results

Total Gadolinium Determination with ICP MS

The amount of gadolinium found in the cerebellum, subcortical brain, and cortex was significantly higher in rats treated with gadodiamide or gadoteridol than in control rats (Fig 3).

The absolute mean amounts for gadodiamide and gadoteridol, respectively, were 0.317 μg/g ± 0.060 and 0.048 μg/g ± 0.004 in the cortex, 0.418 μg/g ± 0.078 and 0.051 μg/g ± 0.009 in the subcortical brain, and 0.781 μg/g ± 0.079 and 0.061 μg/g ± 0.012 in the cerebellum. The amount of retained gadolinium was 6.6, 8.2, and 12.8 times higher in animals treated with gadodiamide than in those treated with gadoteridol in the cortex, subcortical brain, and cerebellum, respectively.

Intact GBCA Determination with UPLC MS

The method to assess how much of the total gadolinium determined with ICP MS corresponds to the intact GBCA and how much corresponds to the insoluble gadolinium-containing species showed good linearity within the range of investigated concentrations (0.5–10.0 μM), with a conversion factor *(K)* between the areas under the thulium complex peaks and those relative to the corresponding gadolinium complex

Figure 4

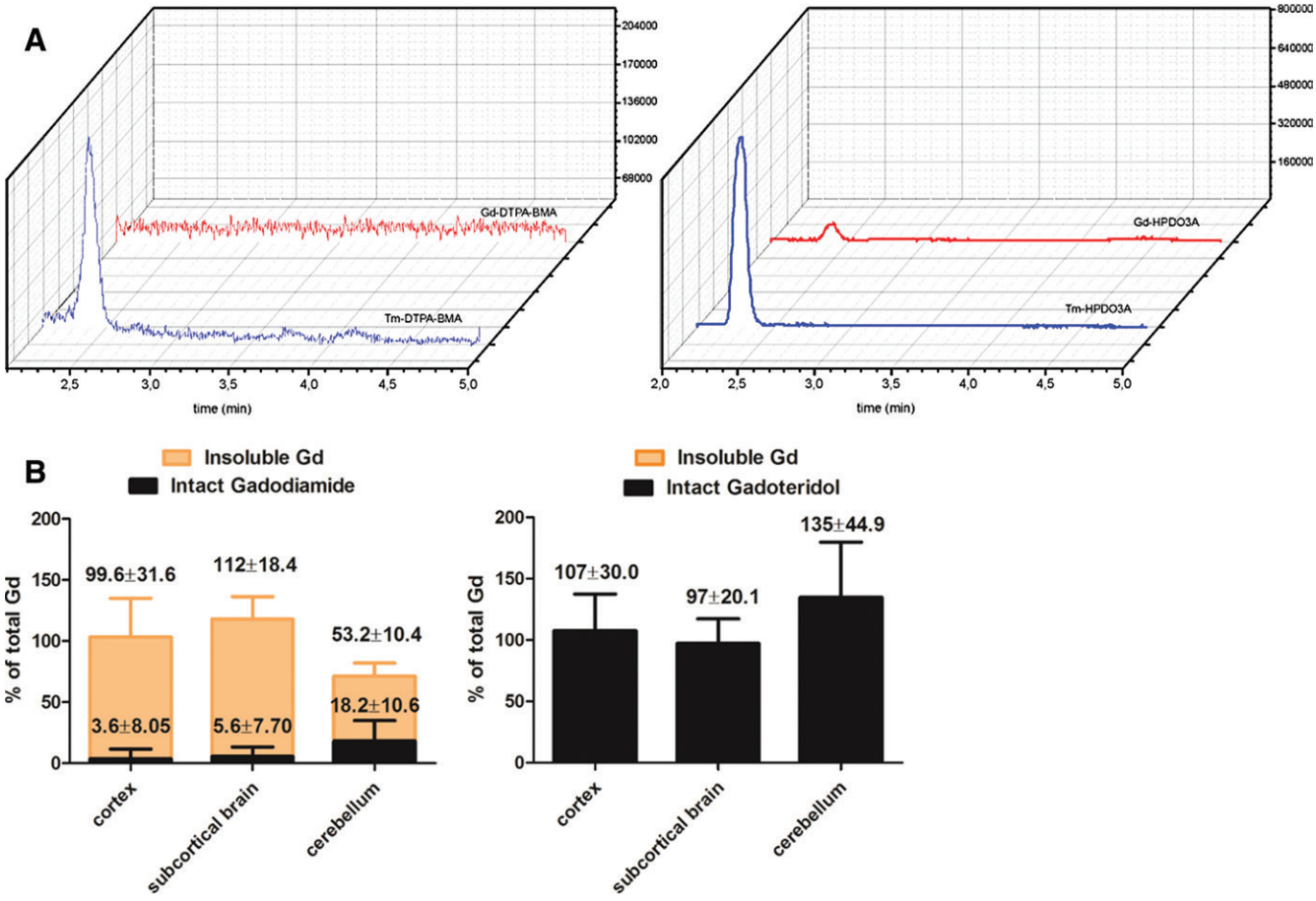

Figure 4: *A,* Example SIM chromatograms obtained for brain samples spiked with Tm-DTPA-BMA (5 μM) and Tm-HPDO3A (5 μM) from rats treated with gadodiamide (left) and gadoteridol (right). *B,* Graphs show mean percentages of intact GBCA (determined with UPLC MS analysis) and insoluble gadolinium *(Gd)*-containing species (determined with ICP MS analysis) in the cortex, subcortical brain, and cerebellum of rats ($n = 5$) treated with gadodiamide (left) and gadoteridol (right).

peaks of 1.6 for both complexes (Fig E1 [online]). The method enabled quantification of intact gadoteridol down to 0.2 μM and of gadodiamide down to 1.0 μM. The difference in the observed limit of quantification between the two complexes depends on the lower sensitivity observed for gadodiamide. The observed limit of detection is in agreement with previously reported values (29).

Two examples of SIM chromatograms of brain samples spiked with 5 μM of Tm-DTPA-BMA in rats treated with gadodiamide and 5 μM of Tm-HP-DO_3A in those treated with gadoteridol are reported in Figure 4, *A*. Most of the analyzed samples obtained in the brains of rats treated with gadodiamide did not show a detectable peak corresponding to the intact gadodiamide complex.

Another experiment was performed to evaluate whether treatment with CH_3OH or HCOOH for protein precipitation and the phospholipid removal steps could affect recovery of the amount of intact GBCA. Neither procedure resulted in the removal of either gadolinium complex, as 100% of gadoteridol and gadodiamide was recovered after the addition of CH_3OH and HCOOH and after three passages through phospholipid removal tubes (Fig E3 [online]).

Figure 4, *B*, reports the mean percentages of intact gadolinium complexes, as determined with UPLC MS analysis of the soluble extracts, and of the gadolinium-containing species, as determined with ICP MS, in the fraction containing insoluble species in rats treated with gadodiamide or gadoteridol.

The total amount of gadolinium recovered in the brain structures after intravenous administration of gadoteridol corresponded to intact gadoteridol. Conversely, only 3.6% ± 8.1, 5.6% ± 7.7, and 18.2% ± 10.6 corresponded to the intact complex in the cortex, subcortical brain, and cerebellum, respectively, in the corresponding experiment with gadodiamide. At ICP MS analysis, it was found that in the cortex and subcortical brain structures, 99.6% ± 31.6 and 112.0% ± 18.4, respectively, of the

Figure 5

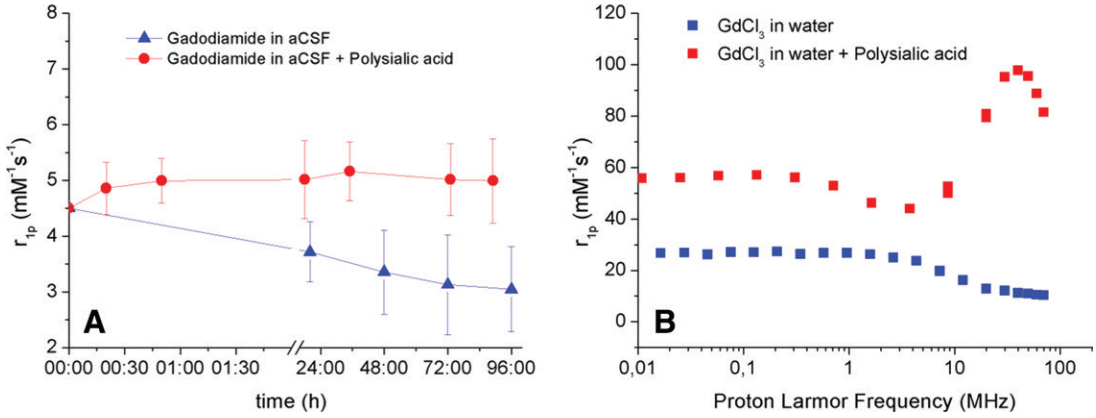

Figure 5: *A,* Time dependence of the relaxivity of gadodiamide in aCSF (blue) and in aCSF added to polysialic acid (red) measured at 298 K and 0.5 T. *B,* NMRD profiles of solutions of gadolinium chloride in water with (red) and without polysialic acid (blue). Data are normalized to 1 mM concentration of gadolinium (298 K and pH = 6).

administered gadolinium was found in the insoluble part of the specimen (obtained at treatment for protein precipitation), while 53.0% ± 10.4 of the total gadolinium was present in the precipitate in the cerebellum. Another gadolinium aliquot in the cerebellum, corresponding to approximately 30% of total gadolinium, was present in the soluble portion but did not correspond to intact gadodiamide.

Relaxometric Investigations

To attain further insight into the speciation of the dechelated gadolinium, in vitro relaxometric experiments were performed. In Figure 5, *A*, the observed relaxation rates measured at 0.5 T and 298 K, once subtracted of the diamagnetic contribution (0.37 sec^{-1} at 0.47 T, 298 K), were normalized to a 1-mM gadolinium concentration to represent the experimental data in terms of millimolar paramagnetic relaxivity (r1p). When gadodiamide was dissolved in an aCSF medium, its relaxivity progressively decreased with time. After 4 days, relaxivity was approximately 30% lower than the starting value.

Conversely, when polysialic acid was added to gadodiamide in aCSF, relaxivity slightly increased to reach a plateau value that was approximately 10% higher than the original relaxivity of gadodiamide. The observed behavior may be accounted for in terms of partial sequestration of the gadolinium ions released from gadodiamide by the added sugar to yield a high relaxivity adduct. The occurrence of the formation of a high-relaxivity system when gadolinium ions are in the presence of polysialic acid was checked by measuring the nuclear MR dispersion profile of an aqueous solution containing 0.1-mM gadolinium chloride and 1 mg/mL polysialic acid (Fig 5, *B*). When the nuclear MR dispersion profile in the presence of polysialic acid was compared with the nuclear MR dispersion profile of gadolinium chloride in water, it showed the typical relaxivity peak centered in the region of approximately 1.0–1.5 T, which is characteristic of high-molecular-weight paramagnetic species. At 1.5 T and 298 K, relaxivity of the adduct was close to 100 mM/sec.

T1-weighted MR imaging showed a mean increase of signal intensity in the dentate nuclei of 11.4% ± 1.41 at the end of treatment in the case of gadodiamide. In animals treated with gadoteridol, signal enhancement (4.02% ± 0.94) in the dentate nuclei was not significantly higher than that in control rats treated with saline. A representative example of pre- and posttreatment images and quantification of the respective signal enhancements in rats treated with gadodiamide or gadoteridol or given saline is reported in Figures E1-E3 (online).

Discussion

In agreement with previously reported investigations (6,8,23–27), the amount of retained gadolinium in our study was much higher in animals exposed to the linear neutral GBCA gadodiamide than in those exposed to the macrocyclic neutral GBCA gadoteridol. Nevertheless, the amount of retained total gadolinium in animals treated with gadoteridol was significantly higher than that in the control group in all three brain regions evaluated.

The total amount of gadolinium recovered in the brain structures after administration of gadoteridol corresponded entirely to intact gadoteridol. Conversely, only a small amount of the intact complex was found after administration of gadodiamide. This indicates that a large fraction of the administered gadodiamide is transformed into insoluble species. This finding is consistent with the early observation made by McDonald et al (4), who reported the occurrence of gadolinium-containing deposits observed in transmission electron microscopy images obtained at postmortem biopsy of human brain tissues. These results also agree with those

recently reported by Frenzel et al (33), in which the chemical form of residual gadolinium in the brain was investigated. They found that in animals treated with macrocyclic GBCAs, gadolinium was found only in the soluble brain fraction and solely as low-molecular-weight molecules, whereas, in rats treated with linear GBCAs, gadolinium was found to a large extent in the insoluble tissue fraction. In our study, the low-molecular-weight molecules (as defined by Frenzel et al) found in the soluble fraction corresponded to the intact gadolinium complex. Although the chemical form of the gadolinium-containing insoluble species has not yet been established, it is reasonable to assume that any solid form of gadolinium would contribute only marginally to the generation of high signal intensity on T1-weighted MR images because water access to paramagnetic centers in solids is limited. The separation method used in this experimental set-up does not exclude that part of the membrane or protein-bound gadolinium, which may contribute to the high signal intensity seen on MR images, that would possibly become part of the insoluble fraction.

In animals treated with gadodiamide, the sum of insoluble gadolinium species (53%) and intact GBCA (18%) found in the cerebellar tissue supports the view that an additional approximately 30% must have been present in the soluble fraction. This finding is relevant to account for hyperintensity detected on T1-weighted images in the cerebellar region estimated at approximately 10% signal enhancement, which is not dissimilar from what was reported for the analogous experimental set-up (5,6,24,26). The detected amount of intact gadodiamide yielded a concentration of about 1 μM that appears to be insufficient to account for the observed signal enhancement on the T1-weighted MR images. Thus, the observed hyperintensity must result from contributions of the intact gadodiamide (minor) and the additional soluble gadolinium-containing species. Likely, the latter species may be characterized by relatively high relaxivity values to account for the observed signal intensity, to which the insoluble gadolinium-containing materials likely contribute little, if anything. One possibility is to think of species endowed with a reduced rotational mobility formed on the transmetalation of the administered gadodiamide. This hypothesis is supported by the findings of Frenzel et al (33), who observed the presence of soluble macromolecular (250–300-KDa) gadolinium-containing species in the cerebellum homogenate of rats treated with gadodiamide.

One may surmise that the high concentration of sugars bound to phospholipids of brain cells (34) may offer coordination sites for free gadolinium ions and may act as an anchoring site for oligomeric gadolinium-containing structures or to opened systems that contain DTPA-BMA. Some support for this suggestion has been gained from in vitro relaxometric measurements. When gadodiamide (0.1 mM) is added to aCSF, a decrease in observed relaxivity is seen. Tentatively, one may suggest that this decrease in relaxivity might be ascribable to anion (carbonate and phosphate are present in aCSF at concentrations of 23.1 and 1.1 mM, respectively)-assisted dechelation of the gadolinium complex, with consequent precipitation of gadolinium carbonate (solubility product constant, approximately 10^{-32}) and, to a minor extent, gadolinium phosphate (solubility product constant, approximately 10^{-22}) (35) salts.

Conversely, a slight increase in relaxivity is observed when aCSF is added to polysialic acid (1 mg/mL) (Fig 5, *A*). The observed behavior can be accounted for by the formation of gadolinium-containing polysialic species that behave as a high relaxivity adduct. The nuclear MR dispersion profile of gadolinium chloride (0.1 mM) added to polysialic acid (1 mg/mL) displays a markedly higher relaxivity in respect to that of gadolinium chloride in water (pH = 6), with a relaxivity hump centered at approximately 1 T, typical of slowly tumbling molecules.

Signal intensity enhancement (approximately 10%) observed on T1-weighted images in the DCN region in rats treated with gadodiamide could correspond to a local concentration in the range of 1–2 μM for a gadolinium system endowed with the relaxivity detected in the polysialic gadolinium model (approximately 100 $mM^{-1} \cdot sec^{-1}$).

Overall, the results reported herein are consistent with the view that a GBCA starts a journey after it crosses the blood-brain barrier or the cerebrospinal fluid barrier and that it seems to be strongly affected by its kinetic and thermodynamic stability, as well as by the composition of the tissue regions experienced by the GBCA. Gadodiamide is less stable than gadoteridol, and this is consistent with the observation that 3 days after the last GBCA administration only 3.6%–18.2% of the agent was recovered intact in three different brain tissue regions.

On the basis of the available data, not much can be said about the chemical forms of the insoluble and highly relaxing soluble gadolinium-containing species. We can only establish the amount of intact GBCA over the total gadolinium. We cannot say whether the dechelation corresponds to the complete separation between the DTPA-BMA ligand and the gadolinium ion or whether there are ternary complexes still containing the ligand (with a lower denticity) in association with biomolecules. The potential role of sialic acid containing macromolecules is reasonable, given the large amount of this and similar classes of molecules on the surface of glial cells.

The experimental design described herein does not enable us to establish whether the lower amount of the total gadolinium retained in the case of gadoteridol is the result of a faster wash-out of this species or if it reflects decreased penetrance of this complex into brain tissues.

One of the main limitations of this study is the relatively short time to sacrifice (3 days) after GBCA delivery. This time was chosen because it appeared to represent a good compromise to cope with the wash-out process that would have led to lower gadolinium levels in the investigated tissues. Obviously, the

differences in the speciation process of gadodiamide and gadoteridol affect the excretion process, which will need to be properly addressed by collecting brain tissue specimens at longer times after the end of treatment.

In conclusion, our study showed the quantification of intact GBCA in brain tissue. While the entire fraction of retained gadolinium was in the form of the intact parent complex in animals treated with gadoteridol, the majority of retained gadolinium was in the form of insoluble species and, in part, of a macromolecular system endowed with very high relaxivity in animals treated with gadodiamide.

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