Radiology

Neurologic Effects of Gadolinium Retention in the Brain after Gadolinium-based Contrast Agent Administration

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Background: Concerns over the neurotoxic potential of retained gadolinium in brain tissues after intravenous gadolinium-based contrast agent (GBCA) administration have led to pronounced worldwide use changes, yet the clinical sequelae of gadolinium retention remain undefined.

Purpose: To assess clinical and neurologic effects and potential neurotoxicity of gadolinium retention in rats after administration of various GBCAs.

Materials and Methods: From March 2017 through July 2018, 183 male Wistar rats received 20 intravenous injections of 2.5 mmol per kilogram of body weight (80 human equivalent doses) of various GBCAs (gadodiamide, gadobenate, gadopentetate, gadoxetate, gadobutrol, gadoterate, and gadoteridol) or saline over 4 weeks. Rats were evaluated 6 and 34 weeks after injection with five behavioral tests, and inductively coupled plasma mass spectrometry, transmission electron microscopy, and histopathology were performed on urine, serum, cerebrospinal fluid (CSF), basal ganglia, dentate nucleus, and kidney samples. Dunnett post hoc test and Wilcoxon rank sum test were used to compare differences between treatment groups.

Results: No evidence of differences in any behavioral test was observed between GBCA-exposed rats and control animals at either 6 or 34 weeks ($P = .08$ to $P = .99$). Gadolinium concentrations in both neuroanatomic locations were higher in linear GBCA-exposed rats than macrocyclic GBCA-exposed rats at 6 and 34 weeks ($P < .001$). Gadolinium clearance over time varied among GBCAs, with gadobutrol having the largest clearance (median: 62% for basal ganglia, 70% for dentate) and gadodiamide having no substantial clearance. At 34 weeks, gadolinium was largely cleared from the CSF and serum of gadodiamide-, gadobenate-, gadoterate-, and gadobutrol-exposed rats, especially for the macrocyclic agents (range: 70%–98% removal for CSF, 34%–94% removal for serum), and was nearly completely removed from urine (range: 96%–99% removal). Transmission electron microscopy was used to detect gadolinium foci in linear GBCA-exposed brain tissue, but no histopathologic differences were observed for any GBCA.

Conclusion: In this rat model, no clinical evidence of neurotoxicity was observed after exposure to linear and macrocyclic gadolinium-based contrast agents at supradiagnostic doses.

Online supplemental material is available for this article.

MRI plays an invaluable clinical role in diagnostic clinical medicine. MRI examinations provide superior delineation of soft tissues without exposure to ionizing radiation inherent to other diagnostic imaging modalities. Gadolinium-based contrast agents (GBCAs) further expand the utility of MRI in the detection of a wide variety of disease processes that would otherwise be undetectable with unenhanced MRI or other imaging modalities.

Recent studies have confirmed the retention of gadolinium in tissues after GBCA exposure in patients and preclinical models (1–8). Higher concentrations of gadolinium and slower washout of gadolinium over time have been observed among linear contrast agents compared with macrocyclic contrast agents but with intraclass differences (1,2,5,8). The acute toxicities of free gadolinium are well known and are often due to the ability of the element to disrupt calcium-mediated cellular processes (9–14). Fortunately, acute toxicity from GBCA exposure is exceedingly rare and can be avoided through adherence to standard clinical dosing and administration routes. However, the chronic toxicity of gadolinium and GBCA exposure remain undefined. Long-term retention of GBCAs within tissues provides an opportunity for dechelation, with the potential to form more biologically active forms of gadolinium. Studies of the potential clinical effects of gadolinium retention have focused on neurologic and cognitive effects (15,16), as *(a)* gadolinium is known to cross or circumvent the blood brain barrier and deposit in brain tissue, particularly in the dentate nucleus and basal ganglia (5,6,17), and *(b)* free gadolinium is a documented neurotoxin (18).

Abbreviations

CSF = cerebrospinal fluid, GBCA = gadolinium-based contrast agent

Summary

There was no evidence of differences in clinical or histopathologic neurotoxicity parameters between rats exposed to supradiagnostic human dose equivalents of commercially available gadolinium-based contrast agents and saline-exposed rats.

Key Results

- Male Wistar rats that received intravenous gadolinium-based contrast agents (GBCAs) over 4 weeks (2.5 mmol/kg; 80 human equivalent doses) showed no evidence of differences in either behavioral tests ($P = .08$ to $P = .99$) or histopathologic analysis of brain tissue compared with a control group.
- Gadolinium tissue clearance from 6 to 34 weeks after injection varied among GBCAs and tissue type (basal ganglia: gadobenate = 55%, gadodiamide = negligible; dentate nucleus: gadobenate and gadodiamide = negligible; kidney: gadobutrol = 85%, gadodiamide = 70%, gadobenate = 59%, and gadoterate = 30%).

The purpose of the current study was to assess the clinical and neurologic effects and potential neurotoxicity of gadolinium retention in rats after administration of various GBCAs. Effects were assessed by behavioral studies, histologic characteristics, transmission electron microscopy, and inductively coupled plasma mass spectrometry.

Materials and Methods

Research reported in our study was financially supported by an investigator-initiated research grant from GE Healthcare. None of the authors are or have been employed by GE Healthcare. The authors maintained control of the study data and the submitted manuscript at all times.

The design and execution of this single-center study were subject to institutional animal care and use committee oversight.

Study Design and Animals

Healthy male Wistar rats (Charles River Laboratories) (mean weight, 200 g; age range, 5–7 weeks at first injection) received intravenous injections of GBCA ($n = 133$) or saline ($n = 50$) from March 2017 through July 2018. Animals that died over the course of the study were excluded from final analyses. Behavioral studies were performed, and urine, serum, and cerebrospinal fluid (CSF) were collected at 6 and 34 weeks after the final injection (Table 1) to simulate 3 and 20 years after chronic GBCA exposure in humans, respectively (19). Animals were then humanely euthanized and perfused with ice-cold saline followed by fixative, and the brain and kidneys were harvested.

GBCA Administration

Animals in each treatment group were given tail vein injections of their respective GBCA once a day for 5 days for 4 consecutive weeks (20 injections total) or twice per day for 5 days for 4 consecutive weeks (40 injections total) for gadoxetate, equivalent to a total dose of 50 mmol/kg (80 human equivalent doses), or saline (Baxter) (control group) (C.R.F., 1 year of experience; S.H., 3 years of experience; D.R.J., 5 years of experience; B.S., 1 year of experience; and G.T., 1 year of experience). Seven GBCAs were administered: gadodiamide (Omniscan, GE Healthcare), gadobenate dimeglumine (MultiHance, Bracco), gadopentetate dimeglumine (Magnevist, Bayer), gadoxetate disodium (Eovist, Bayer), gadoterate meglumine (Clariscan, GE Healthcare), gadobutrol (Gadavist, Bayer), and gadoteridol (ProHance, Bracco). Percutaneous tail vein injections were performed using vaporized isoflurane (1%–3%, inhalation).

Behavioral Studies

Rats underwent behavioral testing at 6 and 34 weeks after final injection at our institutional rodent behavioral facility. Behavioral testing was designed to assess neurologic and cognitive function, particularly of the dentate nucleus and basal ganglia. Tests included open field (assessing locomotor function and anxiety), novel object recognition (memory, cognitive function), Y maze (spatial working memory, short-term memory, locomotor), social interaction (social anxiety), and horizontal ladder rung walking (motor coordination, balance, planning). All testing was performed by experienced personnel (J.A., 6 years of animal experience; M.A.C., 1 year of animal experience; C.R.F., 1 year of animal experience; D.R.J., 5 years of animal experience; B.S., 1 year of animal experience; and K.M.W., 5 years of animal experience), who were blinded to contrast agent exposure. Detailed study protocols are provided in Figures E1–E6 (online) and Table E1 (online).

Table 1: Animal Treatment Groups (133 Experimental Rats vs 50 Control Rats) with Procedure or Sample Time Points

Group	No. of Animals	Urine and Serum Collection	CSF Collection	TEM	Behavioral Studies	Tissue Collection
Saline control	50	6 and 34 weeks	6 and 34 weeks	6 and 34 weeks	6 and 34 weeks	6 and 34 weeks
Gadopentetate	5	6 weeks	6 weeks	6 weeks	NP	6 weeks
Gadoteridol	22	6 weeks	6 weeks	6 weeks	6 and 34 weeks	6 weeks
Gadoxetate	6	6 weeks	6 weeks	6 weeks	NP	6 weeks
Gadobenate	9	6 and 34 weeks	6 and 34 weeks	6 weeks	NP	6 and 34 weeks
Gadobutrol	44	6 and 34 weeks	6 and 34 weeks	6 and 34 weeks	6 and 34 weeks	6 and 34 weeks
Gadodiamide	27	6 and 34 weeks	6 and 34 weeks	6 and 34 weeks	6 and 34 weeks	6 and 34 weeks
Gadoterate	20	6 and 34 weeks	6 and 34 weeks	6 and 34 weeks	6 and 34 weeks	6 and 34 weeks

Note.—CSF = cerebrospinal fluid, NP = not performed, TEM = transmission electron microscopy.

Biologic Fluid Collection

Urine and blood samples were collected from rats at baseline (pre-GBCA injection), 6 weeks, and 34 weeks after final injection. Rats undergoing urine sample collection were housed individually in metabolic cages. Uncontaminated urine was collected over a 14-hour period. Blood was drawn via jugular venipuncture from sedated rats, allowed to clot, centrifuged for serum isolation, and stored at –80°C until analysis.

CSF was collected at 6 or 34 weeks after injection immediately before sacrifice for tissue collection (D.D., 22 years of experience). After anesthetization, a needle was inserted through the dura mater and into the cisterna magna, and CSF was collected, as previously described (20). The sample was stored at –80°C until testing.

Tissue Processing and Histopathologic Analysis

Tissue processing was performed as described in Appendix E1 (online). All slides were independently reviewed by a veterinary pathologist (D.D., 22 years of experience) and a board-certified pathologist blinded to contrast agent exposure.

Mass Spectrometry

Gadolinium levels were quantified by blinded technologists using a fully validated inductively coupled plasma mass spectrometry assay, as previously described (5,6). The limits of detection of this assay for biologic fluid samples and tissue samples are 0.1 ng/mL and 0.1 μg/g, respectively.

Transmission Electron Microscopy

Transmission electron microscopy with energy dispersive x-ray spectroscopy (Tecnai 12 transmission electron microscopy from FEI [Thermo Fisher] equipped with an Oxford X-mat EDX detector) was performed by blinded technologists to characterize the distribution of gadolinium deposits in tissues, as previously described (5,6).

Statistical Analyses

All statistical analyses were performed using JMP, version 14 (SAS Institute) (21). Continuous variables were presented as medians with interquartile ranges due to nonnormal data distributions, unless otherwise noted. Dunnett post hoc test (behavioral test results) and Wilcoxon rank sum test with post hoc analysis (tissue and biologic fluid gadolinium results) were used to compare differences between treatment groups. Details of these analyses are described in Appendix E1 (online). Significance was assigned when $P \leq .05$.

Results

Animal Population

A total of 183 healthy rats were included in this study (Table 1). Six rats per group were used for biologic fluids and tissue samples (6 weeks after injection for all contrast agents and saline; 34 weeks after injection for gadodiamide, gadoterate meglumine, gadobutrol, gadobenate dimeglumine, and saline). Six rats per group underwent urine and serum analysis at both time points (6 and 34 weeks after injection of gadodiamide, gadoterate meglumine, gadobutrol, gadobenate dimeglumine, and saline). Sixteen GBCA-exposed rats per group underwent behavior testing (gadodiamide, gadoterate meglumine, gadobutrol, and gadoteridol). Forty saline-exposed rats were used to provide a control for each behavior group.

Gadolinium Quantification in Tissues

All GBCA-exposed groups had higher median gadolinium concentrations in the dentate nucleus than did the control group at 6 weeks ($P = .005$) and 34 weeks ($P = .007$ to $P = .004$) (Table 2; Table E2, Fig E8 [online]), with higher concentrations observed when comparing linear and macrocyclic GBCAs ($P < .001$). Both GBCA class and ionicity were associated with gadolinium concentrations (Table E3 [online]). Similar results were observed within the basal ganglia (6 weeks: $P = .005$ to $P = .02$; 34 weeks:

Table 2: Gadolinium Levels in Tissue at 6 and 34 Weeks after Injection

Tissue and Treatment Group	6-Week Gd Level (μg/g)	34-Week Gd Level (μg/g)	Median 6- to 34-Week Washout (%)*
Dentate nucleus			
Saline	0 (0–0)	0 (0–0)	…
Gadodiamide	6.8 (6.2–7.3)	7.4 (5.5–8.2)	–8.8
Gadobenate	2.9 (2.3–4.9)	4.1 (3.0–4.9)	–41
Gadopentetate	5.1 (4.4–5.9)	NP	…
Gadoxetate	1.2 (0.5–2.7)	NP	…
Gadoterate	0 (0–0.1)	0 (0–0.1)	…
Gadobutrol	0.1 (0.1–0.2)	0 (0–0.1)	…
Gadoteridol	0.1 (0.1–0.1)	NP	…
Basal ganglia			
Saline	0 (0–0)	0 (0–0)	…
Gadodiamide	5.6 (1.9–7.7)	6.8 (3.3–8.8)	–21
Gadobenate	4.4 (2.6–5.1)	2.0 (1.4–4.0)	55
Gadopentetate	6.5 (4.4–7.7)	NP	…
Gadoxetate	0.9 (0.4–1.4)	NP	…
Gadoterate	0 (0–0.2)	0 (0–0)	…
Gadobutrol	0.1 (0–0.1)	0 (0–0.1)	…
Gadoteridol	0.1 (0–0.1)	NP	…
Kidney			
Saline	0 (0–0)	0 (0–0)	…
Gadodiamide	524 (478–588)	157 (130–179)	70
Gadobenate	161 (98–188)	66 (52–97)	59
Gadopentetate	143 (107–210)	NP	…
Gadoxetate	41 (21–87)	NP	…
Gadoterate	12 (9.8–24)	8.4 (5.3–9.4)	30
Gadobutrol	64 (41–97)	9.8 (8.8–18)	85
Gadoteridol	24 (9.6–29)	NP	…

Note.—Data are medians, and data in parentheses are the interquartile range. Six-and 34-week samples were obtained from different animals. Percentage washout may be negative because of variations in retention between animals. Percentage washout was not calculated when median gadolinium (Gd) concentration was at the inductively coupled plasma mass spectrometry assay limit of detection (0.1 μg/g). NP = not performed.

P = .008 to P = .03; linear vs macrocyclic contrast agent, $P <$ .001) and in renal tissues (6 weeks: P = .005; 34 weeks P = .008 to P = .01; linear vs macrocyclic contrast agent, $P <$ .001).

Differences in median gadolinium tissue washout between 6 and 34 weeks after injection were observed between some GBCAs (Table 2). Gadobenate showed a 55% median decrease in gadolinium in the basal ganglia at 34 weeks and no decrease in the dentate nucleus, whereas gadodiamide showed no decrease in either region. In renal tissue, gadolinium washout between 6 and 34 weeks was 85% for gadobutrol, 70% for gadodiamide, 59% for gadobenate, and 30% for gadoterate.

Gadolinium Quantification in Biologic fluids

In urine samples, all GBCA-exposed groups had higher median urine gadolinium concentrations compared with the control group at 6 weeks after exposure ($P <$ .001) and persisted at 34 weeks (P = .03 to P = .003) (Table 3, Fig E9 [online]). However, gadolinium was almost completely eliminated (range, 95%–99%) from urine by 34 weeks for the four GBCAs examined (gadodiamide, gadobenate, gadoterate, gadobutrol).

Table 3: Gadolinium Levels in Biologic Fluid at 6 and 34 Weeks after Injection

Biologic Fluid and Treatment Group	6-Week Gd (ng/mL)	34-Week Gd (ng/mL)	6- to 34-Week Washout (%)
Urine*			
Saline	3.9 (1.1–7.7)	0.6 (0.5–1.2)	85
Gadodiamide	1969 (1329–2722)	42 (26–53)	98
Gadobenate	336 (255–534)	18 (12–21)	95
Gadopentetate	318 (160–640)	NP	…
Gadoxetate	386 (171–586)	NP	…
Gadoterate	510 (417–1281)	14 (11–38)	97
Gadobutrol	1624 (1148–2361)	11 (7.2–19)	99
Gadoteridol	273 (145–544)	NP	…
Serum			
Saline	0.8 (0.3–1.2)	0.3 (0–0.5)	63
Gadodiamide	5.6 (4.5–9.0)	3.7 (2.3–5.3)	34
Gadobenate	4.1 (3.6–5.2)	1.1 (1.0–1.4)	73
Gadopentetate	3.8 (3.0–5.6)	NP	…
Gadoxetate	2.2 (2.0–2.6)	NP	…
Gadoterate	3.6 (2.4–4.2)	0.3 (0.2–1.4)	92
Gadobutrol	7.4 (5.8–16)	0.5 (0.3–0.6)	93
Gadoteridol	6.3 (2.5–8.7)	NP	…
CSF			
Saline	0 (0–0.2)	0 (0–0.1)	…
Gadodiamide	0.5 (0.4–0.8)	0.2 (0.1–0.4)	60
Gadobenate	0.3 (0.2–0.6)	0.1 (0.1–0.1)	67
Gadopentetate	1.0 (0.5–1.5)	NP	…
Gadoxetate	0.4 (0.3–0.5)	NP	…
Gadoterate	1.6 (0.8–2.7)	0 (0–0.1)	100
Gadobutrol	1.4 (1.3–2.5)	0.1 (0–0.1)	93
Gadoteridol	0.5 (0.3–2.3)	NP	…

Note.— Data are medians, and data in parentheses are the interquartile range. Six-and 34-week samples were obtained from the same animal for urine and serum and from different animals for cerebrospinal fluid (CSF). GD = gadolinium, NP = not performed.

* Urine results not normalized to urine creatinine.

In serum samples, at 6 weeks, all GBCA-exposed groups had a higher median gadolinium concentration than the saline concentration in the control group ($P <$ .001 to P = .01). At 34 weeks, gadodiamide (P = .003) and gadobenate (P = .01) had a higher median gadolinium concentration than saline but not gadobutrol (P = .24) or gadoterate (P = .47). Gadolinium was largely cleared from serum by 34 weeks after macrocyclic exposure (92%–93% removal), with less removal after linear GBCA exposure (34%–73% removal).

In CSF samples at 6 weeks, all GBCA-exposed groups had higher median concentrations compared with the control group (P = .002 to P = .006). At 34 weeks, only gadodiamide was higher than in the control group (P = .02) but not gadobutrol (P = .41), gadobenate (P = .14), or gadoterate (P = .65). gadolinium was largely cleared from CSF by 34 weeks after macrocyclic exposure (93%–100% removal), with less clearance after linear exposure (60%–67% removal).

Gadolinium-based contrast agent ionicity, but not class, was typically associated with gadolinium concentrations in biologic fluids (Table E3 [online]). Some macrocyclic GBCAs demonstrated higher urine, serum, and CSF gadolinium concentrations at 6 weeks than some linear GBCAs (ie, gadobutrol- and gadoterate-exposed rats had higher urine gadolinium concentration than did gadobenate-exposed rats (median, 1624 and 510 ng/mL, respectively vs 336 ng/mL). However, at 34 weeks, linear GBCAs demonstrated consistently higher biologic fluid gadolinium concentrations than macrocyclic GBCAs.

Behavioral Tests

Comprehensively, no GBCA-exposed group performed better or worse than the saline control group in any of the five behavioral tests at either 6 or 34 weeks (P = .08 to P = .99; Tables E4–E8, Figs E9–E13 [online]). Details of the results of these tests are in Appendix E1 (online). We found no evidence of differences between the GBCA-exposed groups and the control group for the open field test (overall locomotion or percentage distance traveled in the center zone), Y maze test (number of arm entries or percentage alternation), novel object recognition test (percentage time exploring the novel objects), social interaction test (percentage time with stranger rat), or horizontal ladder rung walking test (average foot fault score on test ladder).

Transmission Electron Microscopy

At 6 weeks, all samples exposed to gadodiamide, gadobenate, and gadopentetate and six of nine samples exposed to gadoxetate (two dentate nucleus, one basal ganglia, three kidney) demonstrated foci of gadolinium (Table 4, representative spectra, Fig E14 [online]). More foci were observed in samples exposed to gadodiamide and gadobenate compared with samples exposed to gadopentetate and gadoxetate. None of the samples exposed to macrocyclic GBCAs demonstrated gadolinium foci at 6 weeks. At 34 weeks, seven of nine samples exposed to gadodiamide (all dentate nucleus and basal ganglia, one kidney) and one of nine samples exposed to gadobutrol (basal ganglia) were positive for gadolinium.

In brain tissues, gadolinium was predominantly endothelial in distribution in the dentate nucleus and basal ganglia, but a smaller amount was also observed to be within the neuropil (Fig 1). In renal tissues, foci were predominantly localized to the endothelial and subendothelial regions (Fig 1). Foci were rarely observed in the glomerulus.

Histologic Characteristics

We found no evidence of histopathologic abnormality in the cerebellar roof nuclei (including the dentate nucleus) or basal ganglia at either 6 or 34 weeks in any GBCA-exposed sample (Fig 2).

Table 4: Number and Type of Tissues with Gadolinium Foci Confirmed with Transmission Electron Microscopy with Energy Dispersive X-ray Spectroscopy

Group and Time Point	Dentate Nucleus	Basal Ganglia	Kidney
Gadodiamide			
6 weeks	3 of 3	3 of 3	3 of 3
34 weeks	3 of 3	3 of 3	1 of 3
Gadoterate			
6 weeks	0 of 3	0 of 3	0 of 3
34 weeks	0 of 3	0 of 3	0 of 3
Gadobutrol			
6 weeks	0 of 3	0 of 3	0 of 3
34 weeks	0 of 3	1 of 3	0 of 3
Gadobenate			
6 weeks	3 of 3	3 of 3	3 of 3
Gadopentetate			
6 weeks	3 of 3	3 of 3	3 of 3
Gadoteridol			
6 weeks	0 of 3	0 of 3	0 of 3
Gadoxetate			
6 weeks	2 of 3	1 of 3	3 of 3

Discussion

Despite concerns about gadolinium retention in brain tissue after exposure to gadolinium-based contrast agents (GBCAs), the potential neurologic effects of retained gadolinium have not been fully assessed. Our study found no evidence of clinical or histopathologic neurotoxicity due to chronic gadolinium retention within a well-established rat model exposed to supradiagnostic human dose equivalents of various commercially available GBCAs when compared with control rats given saline. General mobility, spatial memory, short-term memory, social interactions, and balance and coordination all appeared to be unaffected by the GBCAs in our study (P = .08 to P = .99). Linear GBCA exposure was associated with higher gadolinium concentrations in the dentate nucleus and basal ganglia compared with rats exposed to macrocyclic GBCAs (dentate nucleus median gadolinium concentrations were 1.2–6.8 μg/g gadolinium for linear GBCAs and 0–0.1 μg/g gadolinium for macrocyclic GBCAs at 6 weeks, 4.1–7.4 μg/g gadolinium for linear GBCAs and 0 μg/g gadolinium for macrocyclic GBCAs at 34 weeks; basal ganglia median gadolinium concentrations were 0.9–6.5 μg/g gadolinium for linear GBCAs and 0–0.1 μg/g gadolinium for macrocyclic GBCAs at 6 weeks, 2.0–6.8 μg/G gadolinium for linear GBCAs and 0 μg/g gadolinium for macrocyclic GBCAs at 34 weeks; $P < .001$ for all comparisons). Gadolinium clearance from tissues varied among GBCAs and between brain and renal tissues (median percentage decrease in gadolinium from 6 weeks to 34 weeks after injection—basal ganglia: gadobenate = 55%, gadodiamide = negligible; dentate nucleus: gadobenate and gadodiamide = negligible; kidney: gadobutrol = 85%, gadodiamide = 70%, gadobenate = 59%, and gadoterate = 30%).

Our findings correlate with previous preclinical and clinical studies (2,5,6,8,17,22–24). However, our study was able to better compare the washout kinetics of various linear and macrocyclic GBCAs in various tissues and biologic fluids and demonstrate that macrocyclic GBCAs appear to have more complete washout versus linear agents. Intraclass differences in these washout property biodistributions were also observed between

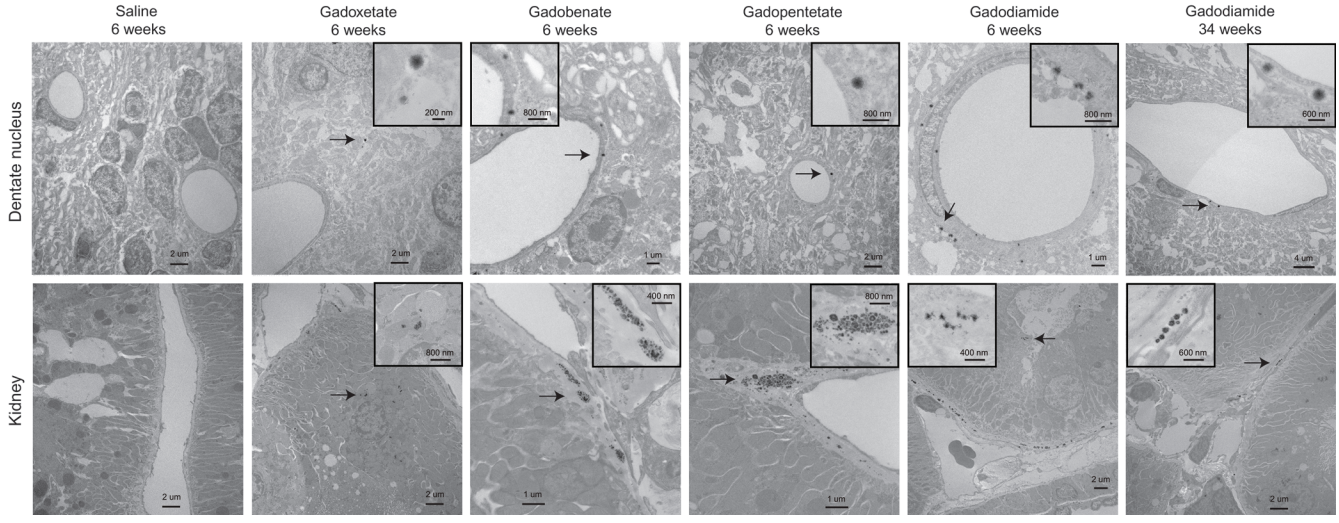

Figure 1: Tissue localization of gadolinium deposits. Cellular localization of gadolinium deposits (arrows) using transmission electron microscopy are shown for dentate nuclei (top row) and kidney (bottom row) tissues of control and gadolinium-based contrast agent–exposed rats harvested at indicated postinjection time points.

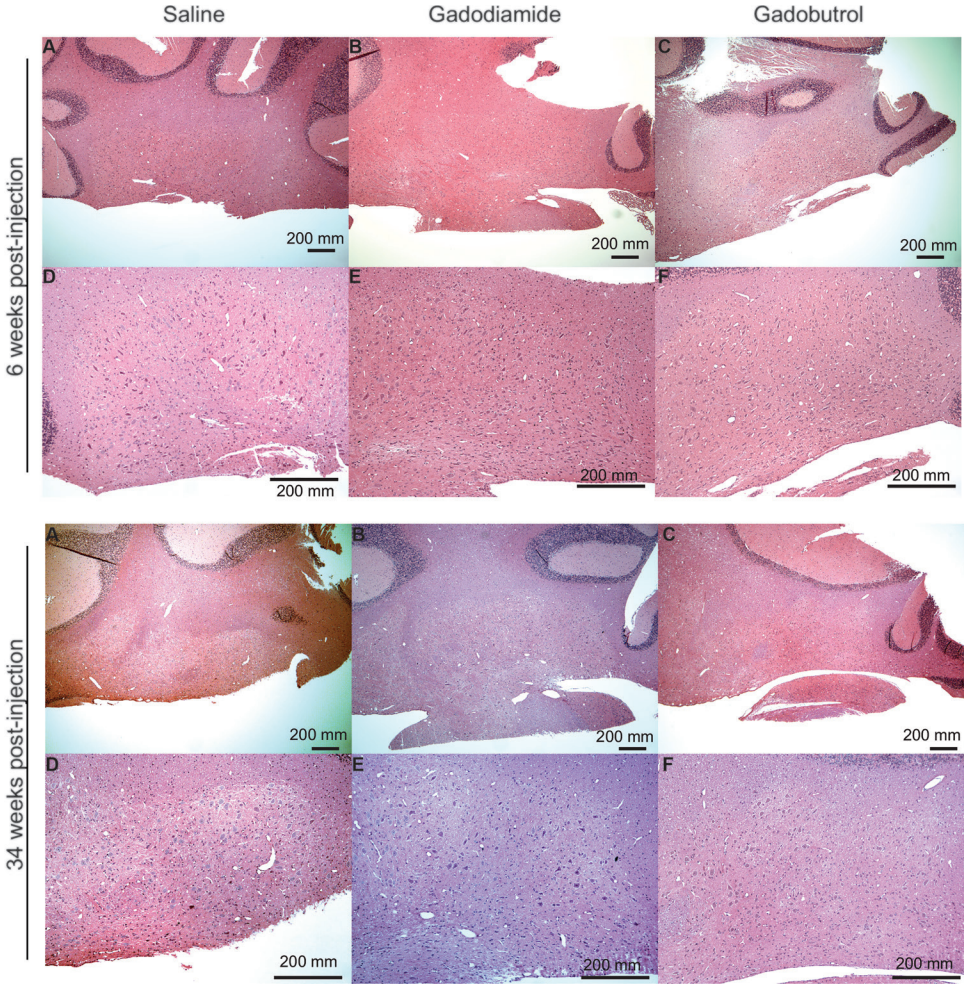

Figure 2: Histopathologic analysis. Representative light microscopy images of the dentate nucleus are shown for **(A, D)** saline (control), **(B, E)** gadodiamide, and **(C, F)** gadobutrol-exposed animals at 6 and 34 weeks after injection. (Originial magnification, ×40 in **A**–**C** and ×100 in **D**–**F**; hematoxylin-eosin stain.)

GBCAs, as higher gadolinium biologic fluid concentrations at 6 weeks after injection were observed among some macrocyclic agents compared with linear agents (ie, gadobutrol- and gadoteridol-exposed rats had higher serum gadolinium concentrations than gadodiamide and gadobenate-exposed rats [median 7.4 ng/mL and 6.3 ng/mL vs 5.6 ng/mL and 4.1 ng/mL]). Such findings underscore the complex biodistribution of retained GBCAs that demand a more holistic understanding of retained Gd in animals and humans before GBCA class alone is used as a differentiator of potential GBCA safety as it relates to retained forms of gadolinium.

To date, gadolinium retention within brain tissue has yet to be correlated with histopathologic findings of injury or toxicity. Behavior tests can detect perturbations due to toxicologic sequalae before or in the absence of any histologic changes. Thus, we used a battery of behavioral tests to evaluate the clinical neurotoxic potential of chronic exposure to supradiagnostic concentrations of GBCAs. The behavior tests we performed were chosen to target the dentate nucleus and basal ganglia—the brain regions showing the highest levels of MRI enhancement (6,17). The dentate nucleus, although important in skeletal motor functions, is also involved in spatial learning, exploration, and cognition (25–29). The basal ganglia are involved in many different functions, including motivation, memory, volitional fine motor control, impulse control, and anxiety-related disorders (30–38). The behavior tests we performed revealed no significant differences between treatment groups and the control group at 6 or 34 weeks after the final GBCA injection, consistent with findings from other studies (39,40).

The lack of histopathologic injury in rat brain tissues exposed to GBCAs, where considerable gadolinium deposits are detected via transmission electron microscopy energy dispersive x-ray spectroscopy, reinforces preliminary findings from our group and others (4–6,41–44). We previously observed gadolinium foci in the brain tissue of gadobutrol-exposed rats 1week after injection (5), suggesting that gadolinium foci are present early after GBCA exposure for all agents, but the more favorable washout of macrocyclic GBCAs eliminates these deposits compared with linear GBCAs. Interestingly, gadoxetate-exposed rats had basal ganglia gadolinium levels comparable with macrocyclic GBCA-exposed rats and dentate nucleus gadolinium levels intermediate to levels observed in rats exposed to other linear and macrocyclic GBCAs, a finding also observed in the recent study from Jost et al (45). This may be explained by the difference in dosing

(50 mmol/kg total dose of gadoxetate = 333 human equivalent doses, whereas 50 mmol/kg total dose of other GBCAs = 80 human equivalent doses) or by inherent in vivo differences in biodistribution and bioavailability between gadoxetate and other GBCAs.

Our findings of greater washout of gadolinium between 6 and 34 weeks after injection with macrocyclic GBCAs compared with linear GBCAs concur with findings of other studies (2,8,44). Our findings showed kidney and brain tissue had similar gadolinium washout for rats exposed to gadoterate, gadobutrol, and gadobenate, but rats exposed to gadodiamide had a much higher amount of washout in the kidney (median 70%) compared with the brain (zero washout). Biologic fluid (serum, urine, CSF) washout kinetics did not appear to correlate with the type of GBCA administered and mirrored findings from other preclinical studies (23,24,46–49). Although GBCAs have very similar early elimination kinetics in biofluids, there is a growing body of evidence that a smaller amount of gadolinium is sequestered into one or more tissue compartments during this rapid elimination, where it is slowly released again over time, reequilibrating back into these same biologic fluids and tissues. The propensity for GBCAs to sequester into these tissues and the rates of reequilibration appear to differ considerably between agents and classes and appear to be associated with the kinetic lability of each chelate. Furthermore, as our data show that linear GBCAs circulate in the blood and CSF for a longer time than macrocyclic GBCAs, it remains possible that this longer "dwell time" in these biologic fluids may be a result of greater amounts of sequestered GBCA or may even reflect slower clearance of different chemical forms of gadolinium circulating in the blood or CSF after initial dechelation. This latter theory requires further evaluation with speciation analysis.

Our study had several limitations. First, comparative anatomy indicates that rodent brains are more primitive in structure and complexity when compared with the brains of larger mammals. This potentially makes them less susceptible to the neurotoxic effects of gadolinium exposure or less likely to manifest subtle symptoms when compared with humans and other mammals with more highly evolved neuroanatomy. Second, the life span of a rodent is much shorter than the life span of humans, so the chronic effects of toxicity may not have manifested during the life span of this model, despite the simulated 30-plus years of GBCA exposure. Third, as the clinical manifestations of gadolinium-mediated neural cell toxicity remain largely unknown, we assumed that injury is most likely to occur where gadolinium retention is highest, and we tailored our behavioral testing accordingly. Certain regions of the brain that do not substantially accumulate gadolinium may be uniquely susceptible to injury that was not assessed in our testing paradigms. Fourth, we did not include positive controls for our behavioral tests. We attempted to perform focused dentate lesioning on a subgroup of rats to serve as a positive control; however, these attempts were unsuccessful because of a high mortality rate. Fifth, urine gadolinium results were not normalized to urine creatinine because of budgetary constraints. Sixth, the rats were subjected to high doses of GBCAs with multiple administrations over a short time. The effects of these high doses may be attenuated or absent in patients exposed to these agents at clinically relevant doses and schedules. Seventh, because of study budgetary constraints, not all commercially available GBCAs were assessed at all time points. Eighth, only a limited number of organs and regions of the brain were assessed with inductively coupled plasma mass spectrometry, transmission electron microscopy, and histology in this study, and no speciation analyses were performed.

In conclusion, no clinical or histopathologic evidence of neurotoxicity was observed after exposure of rats to various linear and macrocyclic gadolinium-based contrast agent (GBCAs) when compared with saline-exposed rat controls. Additional studies should be performed to address these limitations and further assess the safety of GBCAs.

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