Clearance of Gadolinium from the Brain with No Pathologic Effect after Repeated Administration of Gadodiamide in Healthy Rats: An Analytical and Histologic Study[1]

Adrian P. L. Smith, PhD
Michael Marino, PhD
Jeanette Roberts, MS
Janell M. Crowder, BS
Jason Castle, MS
Lisa Lowery, BS
Christine Morton, BS
Mark G. Hibberd, MD, PhD
Paul M. Evans, PhD

Purpose: To measure the levels of gadolinium present in the rat brain 1 and 20 weeks after dosing with contrast agent and to determine if there are any histopathologic sequelae.

Materials and Methods: The study was approved by the GE Global Research Center Institutional Animal Care and Use Committee. Absolute gadolinium levels were quantified in the blood and brains of rats 1 week after dosing and 20 weeks after dosing with up to 20 repeat doses of gadodiamide (cumulative dose, 12 mmol per kilogram of body weight) by using inductively coupled plasma–mass spectrometry. Treatment groups (n = 6 rats per group) included low-dosage and high-dosage gadodiamide and osmolality-matched saline controls. Brain sections were submitted (blinded) for standard toxicology assessment per Registry of Industrial Toxicology Animal data guidelines. Analysis of variance and Mann-Whitney U tests with post hoc correction were used to assess differences in absolute gadolinium levels and percentage of injected dose, respectively.

Results: Dose-dependent low levels of gadolinium were detected in the brain, a mean ± standard deviation of 2.49 nmol per gram of brain tissue ± 0.30 or 0.00019% of the injected dose 1 week after dosing. This diminished by approximately 50% (to 1.38 nmol per gram of brain tissue ± 0.10 or 0.00011% of the injected dose) 20 weeks after dosing. As a percentage of injected dose, the levels of gadolinium measured were comparable between different doses, indicating that mechanisms of uptake and elimination were not saturated at the tested doses. There were no histopathologic findings associated with the levels of gadolinium measured.

Conclusion: Low levels of gadolinium are present in the brain after repeat dosing with gadodiamide, which is partially cleared over 20 weeks with no detectable neurotoxicity.

[1] From GE Healthcare, The Grove Centre (GC18), White Lion Rd, Amersham HP7 9LL, England (A.P.L.S., P.M.E.); GE Global Research Centre, Niskayuna, NY (M.M., J.R., J.M.C., J.C., L.L., C.M.); and GE Healthcare, Life Sciences, Marlborough, Mass (M.G.H.). Received May 9, 2016; revision requested June 27; revision received July 5; accepted July 13; final version accepted July 20. Supported by General Electric, Fairfield, Conn. Omniscan was provided by GE Healthcare, Princeton, NJ, and Magnevist was obtained from Bayer Healthcare Pharmaceuticals, Whippany, NJ. **Address correspondence to** P.M.E. (e-mail: *paul.m.evans@ge.com*).

Study supported by GE Healthcare.

A.P.L.S. and M.M. contributed equally to this work.

Gadolinium-based contrast agents (GBCAs) are frequently used to enhance clinical magnetic resonance (MR) imaging examinations, and use of these agents has been standard medical practice for more than 20 years. Recently, hyperintensity on unenhanced T1-weighted MR images in patients with a prior history of GBCA-enhanced examinations was reported in specific brain regions (1–14). Kanda et al (2) linked hyperintensity in the dentate nucleus and globus pallidus to the number of previous GBCA-enhanced examinations. In autopsy samples and in agreement with others (1), Kanda et al went on to show a relationship between the signal intensity on T1-weighted images and direct measurements of gadolinium thought to contribute to the hyperintensity on T1-weighted images (15). Further studies demonstrated that both linear and macrocyclic GBCAs are associated with detectable gadolinium in postmortem brain specimens (13). While these studies demonstrated an association between the amount of GBCA administered and the amount present in the brain tissues of deceased patients, the investigators did not explore the pharmacokinetics of gadolinium presence in the brain, leaving open the question of the potential time dependence of observed gadolinium concentration (1,13,15).

Recent reports demonstrated that enhancement on T1-weighted images could be detected in the healthy rat brain after repeat dosing with GBCA (16–18) and that gadolinium was measurable after acute phase clearance (16,17). However, these nonclinical studies were focused on the enhancement on T1-weighted MR images and the presence of gadolinium and did not include a focus on possible histopathologic sequelae. Several aspects of the association between prior GBCA exposure and unenhanced hyperintensity on T1-weighted images have important implications and remain unexplored. First, despite the presence of gadolinium in the brains of rats and patients that received multiple doses of GBCA, no neurological syndrome has been clearly linked to GBCA use, which prompts the hypothesis that gadolinium presence is clinically silent and below toxic thresholds. Second, to our knowledge, no studies to date have been conducted to investigate whether this material was deposited permanently or was being cleared.

Therefore, the purpose of this study was *(a)* to measure the levels of gadolinium present in the rat brain 1 and 20 weeks after dosing and determine if there were any histopathologic sequelae and *(b)* to test for dose dependency and clearance from the brain, along with any potential long-term toxicity.

Advances in Knowledge

- The small fraction of injected gadolinium (up to 0.00026% of the injected dose) present in the rat brain after multiple gadodiamide doses (up to 20 doses of 0.6 mmol per kilogram of body weight) clears with time.
- There are no acute or chronic histopathologic effects of gadolinium in the rat brain after 6 months.

Implications for Patient Care

- There is clearance of gadolinium from the rat brain over time after multiple gadolinium-based contrast agent (GBCA) administrations, yet it remains to be confirmed whether this also occurs in humans.
- The clinical use of GBCAs remains appropriate when used according to approved product labeling.

Materials and Methods

Study Design

This study was supported by a financial contribution from GE Healthcare. M.M., J.R., J.M.C., J.C., L.L., and C.M. had control of inclusion of any data and information that might present a conflict of interest for the authors who are employees of GE Healthcare. The study was designed to investigate clinically relevant dose dependency, kinetics, and potential neurotoxicity of gadolinium presence in the healthy rat brain by using high and low cumulative dosage groups (6 and 12 mmol per kilogram of body weight) and two time points (1 and 20 weeks after dosing). Gadolinium levels in the brain were determined by using inductively coupled plasma–mass spectrometry (ICP-MS), and a blinded, independent, standard histopathologic toxicology assessment of the brain was performed. A gadopentetate dimeglumine group was included as a GBCA class comparator. A schematic of the study groups and timelines is presented in Figure 1.

Animals

The study was approved by the GE Global Research Center Institutional Animal Care and Use Committee. Female Sprague-Dawley CD International Genetic Standard rats (150–200 g; Charles River Laboratories, Wilmington, Mass) were housed two rats per cage (a microisolator) with water and food ad libitum under a 12:12 light-dark cycle in an Association for Assessment and Accreditation of Laboratory Animal Care International–accredited vivarium. All animals were

Published online before print
10.1148/radiol.2016160905 **Content code:** NR

Radiology 2017; 282:743–751

Abbreviations:
ANOVA = analysis of variance
GBCA = gadolinium-based contrast agent
ICP-MS = inductively coupled plasma–mass spectrometry

Author contributions:
Guarantors of integrity of entire study, A.P.L.S., M.M., M.G.H., P.M.E.; 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, A.P.L.S., M.M., J.R., M.G.H., P.M.E.; experimental studies, A.P.L.S., M.M., J.R., J.M.C., J.C., L.L., C.M., M.G.H.; statistical analysis, A.P.L.S., M.M.; and manuscript editing, A.P.L.S., M.M., J.R., J.M.C., J.C., L.L., M.G.H., P.M.E.

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

Figure 1

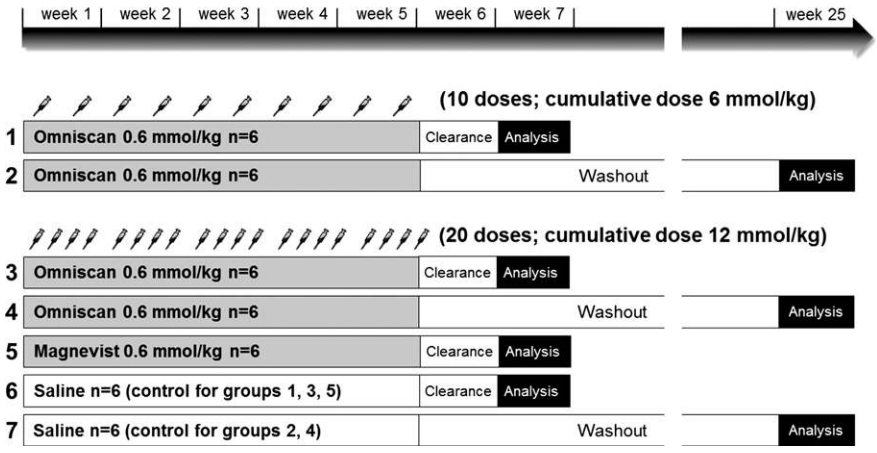

Figure 1: Schematic of the study design, including all dosing regimens and the two time points, at week 7 (1 week after dosing) and week 25 (20 weeks after dosing). Note that the high and low cumulative dose groups were administered identical daily doses but at different frequencies per week. Each dose administered (0.6 mmol per kilogram of body weight) is equivalent to a human dose of 0.1 mmol per kilogram of body weight, adjusted for allometric scaling (19).

allowed 6 days to acclimate before the study. Health monitoring was performed daily to assess any visible signs of distress and was performed weekly for body weight. Rats were randomly assigned to seven treatment groups (n = 6 per group; Fig 1). GBCAs were administered at daily doses of 0.6 mmol per kilogram of body weight (Fig 1), equivalent to the typical human dose of 0.1 mmol per kilogram of body weight, after adjustment for body surface area as recommended by the U.S. Food and Drug Administration (19) and as comparable to doses used by others (16,17). Note that although each dose was equivalent to a clinical dose (adjusted for species differences in rates of distribution and/or clearance), repeated dosing represents supraclinical exposure.

Gadodiamide (Omniscan; GE Healthcare, Princeton, NJ) or gadopentetate dimeglumine (Magnevist; Bayer, Whippany, NJ) was administered intravenously via the lateral tail vein. Dose quantities were confirmed by weighing syringes before and after administration. Low-dosage groups received two doses per week for 5 weeks. High-dosage groups received four doses per week for 5 weeks (Fig 1). With these regimens, we attempted to model potential repeat total cumulative dose exposure that might occur in the clinic, albeit over a period of 5 weeks in this study, compared with a period of months to years in a realistic clinical time frame. Doses for each animal were calculated once weekly on the basis of body weight. Controls (n = 6) received an equivalent volume of osmolality-matched saline (789 mOsm per kilogram of body weight; a 9.15:1 mixture of sterile United States Pharmacopeia, or USP, water and sterile USP 23.4% NaCl) (Fig 1).

Blood samples (>500 μL) were collected into dry anticoagulant tubes prior to terminal procedures. Euthanasia was performed via transcardiac perfusion fixation with deep isoflurane anesthesia to ensure the best possible tissue morphologic appearance and to remove blood from the brain. After initiating anesthesia, the abdominal and chest cavities were opened, the descending vena cava was transected, and a needle was inserted into the heart. Saline (60 mL) was pumped through the heart (10 mL per minute via a syringe pump), followed by 100 mL of either formalin fixative (n = 3 per treatment group) or Karnovsky's fixative (n = 3 per treatment group) before the heart was transected to ensure death. The fixed brain was isolated and bisected along the sagittal midline.

Gadolinium Measurement with ICP-MS

The left hemisphere of each brain and 200 μL of blood were analyzed with ICP-MS (J.M.C., with 18 years of experience). Each weighed sample was predigested in a polytetrafluoroethylene microwave liner (CEM, Buckingham, United Kingdom) at room temperature for 30 minutes in 2.0 mL of concentrated HNO_3, 1.5 mL of concentrated H_2O_2, and 3.0 mL of deionized water. Complete digestion was achieved in a microwave digester at 180°C for 30 minutes. Samples were diluted to 20 g with deionized water, and a twofold secondary dilution to 10 mL included internal standards of indium, niobium, rhodium, and terbium (final concentrations of 2 ng/mL each). Samples were examined with an ICP-MS unit (Elan 6000; PerkinElmer, Waltham, Mass) (with gadolinium 158, gadolinium 160, indium 115, and terbium 159). Stock osmolality-matched saline, saline for perfusion, and fixatives were analyzed to confirm the absence of gadolinium. Twenty gadolinium calibration standards were prepared as solutions in 5% HNO_3 that ranged from 5 pg/mL to 13 ng/mL. Raw data were presented as a report (M.M., J.R., J.M.C., J.C., L.L., and C.M., with 10–25 years of experience) with gadolinium levels in tissue expressed as nanomoles per gram of tissue. Percentage of injected dose was calculated as the ratio of the amount of gadolinium measured in the whole brain (2 times the hemispheric measure) divided by the cumulative dose administered.

Histopathologic Toxicology Assessment

The right hemisphere was trimmed to provide coronal tissue blocks according to Registry of Industrial Toxicology Animal data (20) and North American Control Animal Database (21–23) guidelines (L.L., with 13 years of experience), which corresponded to levels 1–3 as defined by *(a)* cerebrum at the

optic chiasm, *(b)* cerebrum at the base of the posterior hypothalamus, and *(c)* midcerebellum and medulla oblongata. This slide set contains multiple anatomic structures that include both the globus pallidus and the deep cerebellar nuclei (of which part is the equivalent of the human dentate nucleus). Tissues were postfixed overnight in formalin and processed to paraffin blocks, which were shipped to Pathology Associates International (a division of Charles River Laboratories) for slide preparation and independent histopathologic toxicology assessment. Toxicology assessments (24) were performed on hematoxylin-eosin–stained sections by a board-certified toxicology pathologist who was blinded to the treatment groups until all individual animal assessments had been performed. Assessment included presence or absence of, and any appropriate grading of extent or severity of, any neoplastic and nonneoplastic morphologic changes in numerous anatomic regions present, as per best-practice guidelines (25).

Statistical Analysis

Statistical analysis and graphing were performed by A.P.L.S. (with 17 years of experience) by using Statistica software (version 8; StatSoft, Tulsa, Okla). Parametric tests (analysis of variance [ANOVA]) were performed for continuous variables (eg, gadolinium measurements), and nonparametric tests were used for normalized data (eg, percentage of injected dose). A level of significance of .05 was assumed. Results of all pairwise tests presented in the Figures remained significant after Bonferroni post hoc correction. The elimination half-life was calculated by using the equation $T_{1/2} = (T_t - T_0)\ln(2)/[\ln(N_0) - \ln(N_t)]$, where $T_{1/2}$ is the elimination half-life, N_0 is the level measured at time T_0, and N_t is the amount measured at time T_t.

Results

All animals completed the study successfully, and there were no adverse health monitoring reports. There were no significant differences in body weight increase between groups either during the treatment period or during the period after dosing, which was indicative of general good health.

Figure 2

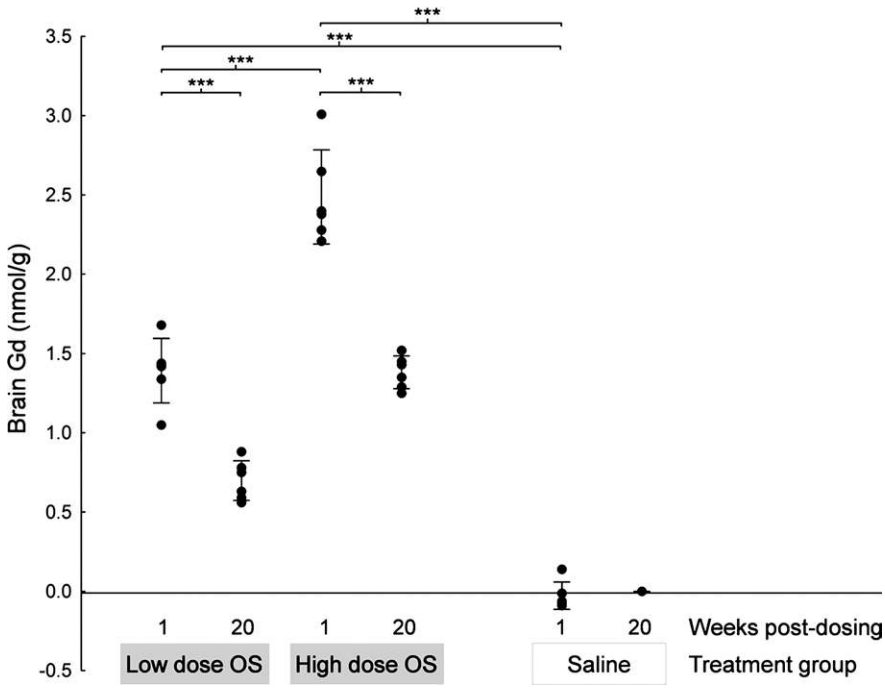

Figure 2: Plot shows that gadolinium levels measured in the brain with ICP-MS were dose dependent (compare high- and low-dosage gadodiamide groups) and decreased with time (compare levels 1 week after dosing with those 20 weeks after dosing). Error bars represent ±1 standard deviation of the mean. *** = $P < .0001$ according to ANOVA for pairwise comparison. *OS* = Omniscan.

Determination of Brain and Blood Gadolinium Concentrations with ICP-MS

Gadolinium levels detected in the brain—up to 0.489 μg of gadolinium per gram of brain tissue (equivalent to approximately 3 nmol per gram of brain tissue in the high-dosage gadodiamide treatment group 1 week after dosing, $n = 6$)—were a small fraction of the injected dose, maximally 0.00026% of the injected dose (3.38 nmol/1.29 mmol). The levels of gadolinium per gram of brain tissue as a percentage of injected dose agreed with those in previous (26) and recent (16) rat studies and in most subjects in autopsy studies (1,13,15).

One week after dosing, levels of gadolinium in the brain showed a dose response between the low-dosage and high-dosage gadodiamide groups (mean ± standard deviation, 1.39 nmol per gram of brain tissue ± 0.20 vs 2.49 nmol per gram of brain tissue ± 0.30, respectively; $P < .0001$ according to ANOVA; $n = 6$ per group; Fig 2). Levels of gadolinium detected in the brains of animals treated with gadopentetate dimeglumine were lower than those in the equivalent gadodiamide group (2.49 nmol per gram of brain tissue ± 0.3 vs 1.40 nmol per gram of brain tissue ± 0.14, respectively; $P < .0001$ according to ANOVA; $n = 6$ per group) in agreement with others (17). Levels of gadolinium in whole blood were detectable in all treated animals (Fig 3) (low-dosage gadodiamide, 0.50 nmol per gram of blood ± 0.08 [$n = 6$]; high-dosage gadodiamide, 0.70 nmol per gram of blood ± 0.07 [$n = 6$]; high-dosage gadopentetate dimeglumine, 0.16 nmol per gram of blood ± 0.02 [$n = 6$]; saline, 0.00 nmol per gram of blood ± 0.02 [$n = 6$]). Blood gadolinium levels

Figure 3

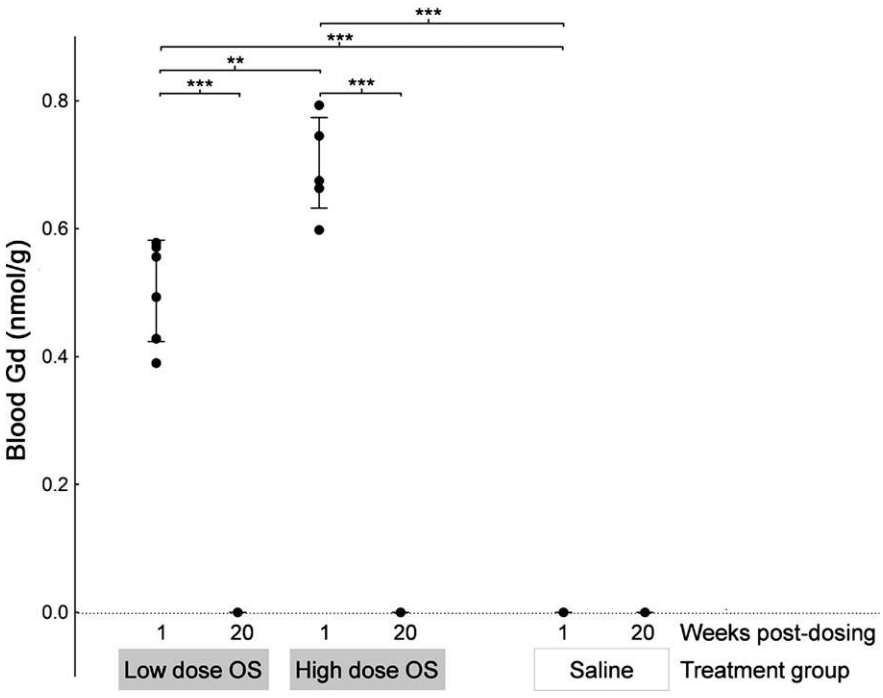

Figure 3: Plot shows that gadolinium levels measured in the blood with ICP-MS were dose dependent 1 week after dosing but were below detection limits 20 weeks after dosing. Error bars represent ±1 standard deviation of the mean. ** = $P < .001$ according to ANOVA, *** = $P < .0001$ according to ANOVA for pairwise comparison. *OS* = Omniscan.

were significantly lower in the animals treated with gadopentetate dimeglumine when compared with the equivalent animals treated with a high dosage of gadodiamide ($P < .0001$ according to ANOVA; $n = 6$ per group). The fraction of gadolinium in the brain measured as a percentage of injected dose was comparable between the low- and high-dosage gadodiamide groups (0.000216% ± 0.000032 of injected dose [mean, 2.78 nmol/1.29 mmol; $n = 6$] vs 0.000193% ± 0.000023 of injected dose [mean, 4.97 nmol/2.58 mmol; $n = 6$], respectively; $P = .18$ according to the Mann-Whitney *U* test; Fig 4).

When compared with values 1 week after dosing, 20 weeks after dosing, the gadolinium measured was significantly decreased both in absolute terms (low-dosage gadodiamide, 1.39 nmol per gram of brain tissue ± 0.20 vs 0.70 nmol per gram of brain tissue ± 0.12, respectively [$P < .0001$]; high-dosage gadodiamide, 2.49 nmol per gram of brain tissue ± 0.30 vs 1.38 nmol per gram of brain tissue ± 0.10, respectively [$P < .0001$]; *P* values obtained with ANOVA; $n = 6$ per group) and as a fraction of injected dose (low dosage, 0.000216% ± 0.000032 of injected dose [mean, 2.78 nmol/1.29 mmol; $n = 6$] vs 0.000108% ± 0.000019 of injected dose [mean, 1.40 nmol/1.29 mmol; $n = 6$], respectively [$P = .005$]; high dosage, 0.000193% ± 0.000023 of injected dose [mean, 4.97 nmol/2.58 mmol; $n = 6$] vs 0.000107% ± 0.000008 of injected dose [mean, 2.76 nmol/2.58 mmol; $n = 6$], respectively [$P = .005$ according to the Mann-Whitney test]). The levels 20 weeks after dosing represent approximately 1 000 000th of the injected cumulative dose. Gadolinium was below detection limits in the blood 20 weeks after dosing (Fig 3). A brain elimination half-life of 19.08–22.40 weeks was calculated (on the basis of either percentage of injected dose or absolute measures, respectively).

Histologic Toxicology Findings

Histologic specimens from three anatomic levels of the brain were stained with hematoxylin-eosin and examined (Fig 5). One week after dosing, a total of 72 sections from four treatment groups (three sections per animal and six rats per group) were examined, and all were described as having findings within normal limits. These sections included six animals treated with saline and 18 animals treated with GBCA (six rats in each of three groups: low-dosage gadodiamide, high-dosage gadodiamide, and gadopentetate dimeglumine groups) with known gadolinium levels ranging from 1.049 to 3.009 nmol per gram of tissue present for 1 week (both low-dosage and high-dosage gadodiamide groups and high-dosage gadopentetate dimeglumine group; $n = 6$ per group). Twenty weeks after dosing, a total of 54 sections from two gadodiamide treatment groups (low dosage and high dosage; $n = 6$ per group) and saline controls ($n = 6$) were examined, and all were also described as having findings within normal limits. These included 18 sections from animals with known measured gadolinium levels higher than 0.56 nmol per gram of brain tissue that had been present in the tissue for 20 weeks ($n = 6$ rats in the low-dosage gadodiamide group and $n = 6$ rats in the high-dosage gadodiamide group).

Discussion

This study confirms the presence of small amounts of gadolinium (approximately 1/1 000 000th of the injected dose) in the brain after repeated dosing of gadodiamide in healthy rats and demonstrates partial clearance over 20 weeks. Toxicology evaluation showed no evidence of treatment-related histopathologic findings.

For most studies to date, some differentiation has been reported between the classes of GBCA in both the clinical (5,10,27) and nonclinical (16–18) setting with respect to hyperintensity on T1-weighted images, but there is evidence of low levels of gadolinium in the brain from all GBCAs tested with ICP-MS in rats (16,17) and humans

Figure 4

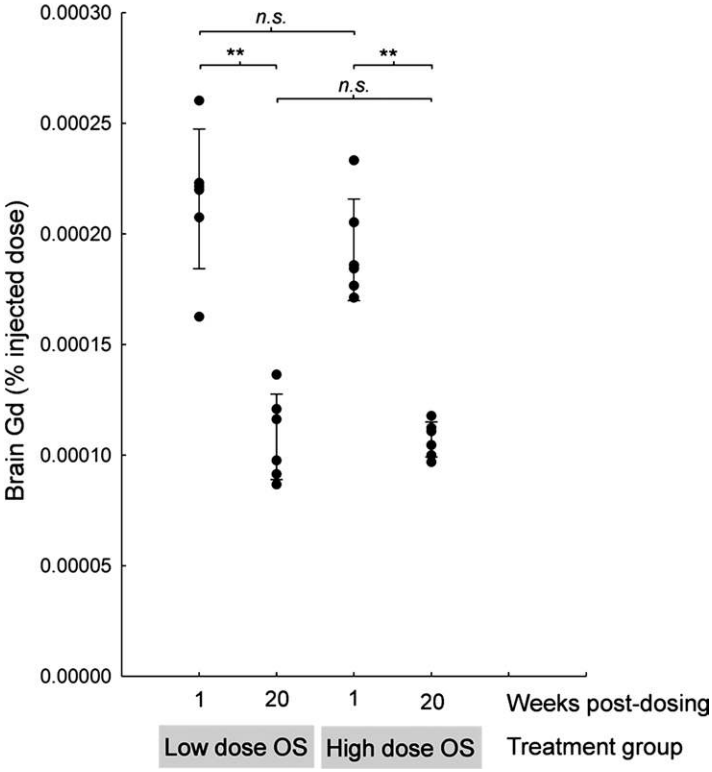

Figure 4: Plot shows that gadolinium measured in the brain as a percentage of injected dose shows equivalency with dose, which indicates that uptake mechanisms are not saturated, and equivalency in elimination shows that elimination mechanisms are not saturated. Error bars represent ±1 standard deviation of the mean. *n.s.* = not significant, ** = $P = .005$ according to the Mann-Whitney *U* test.

(1,13,15). In the rat, there is a fourfold to 14-fold range of gadolinium concentration in the brain between linear and macrocyclic agents at equivalent doses (17), and even within the linear agents, we see twofold lower initial brain levels with gadopentetate dimeglumine than with gadodiamide, in agreement with others (17). These observed gadolinium levels of 0.2–4.0 nmol per gram of brain tissue result from a supraclinical dosing regimen in healthy rats *(a)* with a presumably intact blood-brain barrier and *(b)* within a few weeks of dosing. In the clinical setting, gadolinium levels in autopsy samples are typically similar, on the order of 0.1–6.0 nmol per gram of brain tissue across a range of linear and macrocyclic GBCAs (1,13,15), but may be as high as 370 nmol per gram of brain tissue in exceptional cases (1). However, in the clinical setting, these levels are in the context of a highly variable period of time between the last GBCA dose and autopsy, ranging from a few days to years. Indeed, the highest levels of gadolinium seen in the autopsy cases may be associated with the shortest time between dosing and autopsy (13,15), so it is difficult to determine any clearance rates from the human data.

Previous pharmacokinetic studies of gadodiamide have been described as fitting an open two-compartment model with a rapid extracellular distribution phase (on the order of minutes) and longer elimination phase (with a half-life on the order of hours) and with recovery of GBCAs on the order of 94%–99% in the urine within 24–48 hours (28). After a single dose of labeled gadodiamide, levels of either radiolabeled gadolinium (29) or the radiolabeled chelate (30) in the brain are below the limit of detection within 24 hours. With inductively coupled plasma atomic emission spectroscopy, levels in the brain 24 hours after dosing were 0.000344% of the injected dose after a single dose of 1 mmol per kilogram of body weight and 0.000191% of the injected dose 48 hours after six repeat doses of 1 mmol per kilogram of body weight (26), the same order of magnitude as our findings. We interpret the detection of gadolinium in the blood 1 week after dosing and persistence in the brain 1 week and 20 weeks after dosing as both a function of the repeated dose regimen with a higher cumulative dose (26) and the higher sensitivity of ICP-MS over previously used techniques (29,30). We also provide evidence that gadolinium is slowly clearing from the brain, albeit at slower rates than seen in the first 24–48 hours (26,28,31), which suggests that the simple two-compartment model used to describe previous GBCA pharmacokinetic data is an oversimplification.

Importantly, we saw no overt clinical sequelae from these supraclinical treatment regimens. This is in agreement with previous studies in rats at lower (26) or equivalent doses (16,17) in accordance with comparable body weights between the treatment and control groups in our study. A lack of acute histopathologic findings in the brain agrees with findings in a previous study (26), while our study included animals with a higher cumulative dose of 12 mmol per kilogram of body weight and extended the exposure to 20 weeks after dosing. We also included perfusion fixation to optimize morphologic preservation and a standardized toxicology assessment of the brain, which conforms to methods used in safety toxicology studies. We considered it important to conduct histopathologic analysis after both acute (1 week after dosing) and chronic (20 weeks after dosing) exposure to eliminate the possibility of both acute toxicity and delayed toxicity that might only be apparent after prolonged periods of exposure. The lack of histopathologic

Figure 5

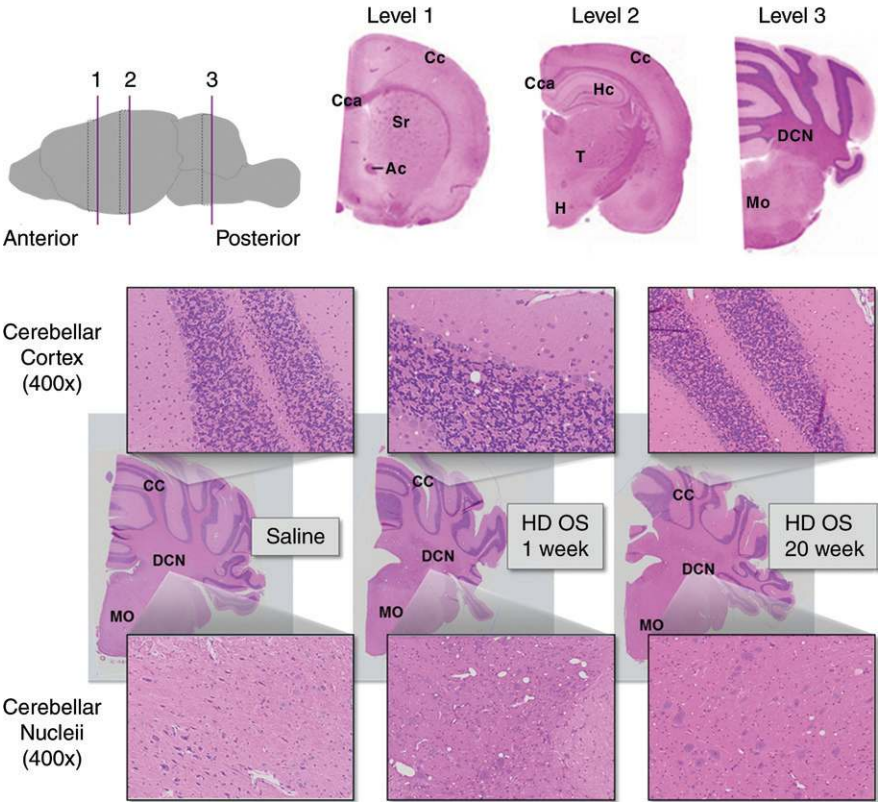

Figure 5: No histopathologic abnormalities were observed in any treatment group at any time point. Histopathologic assessment was conducted on specimens from three anatomic levels (upper panels). The cerebrum at the optic chiasm, the cerebrum at the base of the posterior hypothalamus, and the midcerebellum and medulla oblongsata are shown (low-power middle panels). High-power representative photomicrographs (hematoxylin-eosin stain; original magnification, ×400) from the deep cerebellar nuclei (*DCN*; lower expanded panels) and cerebellar cortex (upper expanded panels) of animals treated with saline and those treated with a high dosage of gadodiamide 1 week and 20 weeks after dosing are shown. *Ac* = anterior commisure, *Cc* = cerebral cortex, *CC* = cerebellar cortex, *Cca* = corpus callosum, *H* = hypothalamus, *Hc* = hippocampus, *HD* = high-dose, *Mo* = medulla oblongata, *MO* = medulla oblongata, *OS* = Omniscan, *SR* = striatum, *T* = thalamus.

findings in the brain is consistent with a lack of in-life sequelae in this study or similar rat studies (16–18,26). To date, there remain no definitive clinical findings associated with hyperintensity on T1-weighted images or the presence of gadolinium in the brain (1–14), and it is important to rationally evaluate the potential risk of gadolinium present in the brain against the considerable diagnostic benefits of contrast material–enhanced MR imaging examinations.

Limitations of our study include the assessment of only two contrast agents, and further work needs to be performed to investigate the kinetics of gadolinium in the brain and the histopathologic findings across the various approved GBCAs. We did not include a 20-week assessment for gadopentetate dimeglumine. The methods used in the current study did not address the questions of the gadolinium species present, although the absence of neurotoxicity suggests that gadolinium remains chelated or in a nontoxic form. In our study, gadolinium was not localized within the brain. We measured gadolinium levels in whole hemispheric samples, and this may dilute the potential concentration in the deep cerebellar nuclei. Although others report only modestly lower gadolinium levels in cerebral cortex and subcortical structures when compared with cerebellum, local concentrations are likely to be heterogeneous and focally higher than the values reported here and by others (16). It is important to note that to date, in only a single study has gadolinium been localized, measured with respect to the blood-brain barrier at the microscopic level (1). In autopsy samples, most gadolinium was present in the endothelial wall of the blood-brain barrier, with a variable level apparently within the brain parenchyma. Localization should be further investigated in a nonclinical model without potentially confounding comorbidities and with the better tissue morphologic appearance over cadaveric specimens afforded by perfusion fixation.

The mechanism by which gadolinium reaches the brain remains unknown, as most GBCAs were considered not to cross an intact blood-brain barrier. A possible route of brain exposure to all GBCAs via the cerebrospinal fluid observed in rats (18) and some humans (32), thereby bypassing the blood-brain barrier, needs to be explored further in both nonclinical and clinical settings.

In conclusion, results of the present study confirm the presence of low levels of gadolinium in the rat brain after repeated GBCA exposure up to a cumulative dose of 12 mmol per kilogram of body weight (approximately 0.0002% of the injected dose). This gadolinium is in a form that is cleared over time without histopathologic consequence in the rat.

Acknowledgment: We acknowledge James P Morrison, DVM, DACVP, at Charles River Laboratories, who performed the histopathologic assessment.

Disclosures of Conflicts of Interest: A.P.L.S. Activities related to the present article: disclosed no relevant relationships. Activities not related to the present article: disclosed no relevant relationships. Other relationships: author is a full-time employee of GE Healthcare. **M.M.** Activities related to the present article: institution received money and nonfinancial support from GE Healthcare. Activities not related to the present article: disclosed no relevant re-

lationships. Other relationships: disclosed no relevant relationships. **J.R.** Activities related to the present article: institution received money and nonfinancial support from GE Healthcare. Activities not related to the present article: disclosed no relevant relationships. Other relationships: disclosed no relevant relationships. **J.M.C.** Activities related to the present article: institution received money and nonfinancial support from GE Healthcare. Activities not related to the present article: disclosed no relevant relationships. Other relationships: disclosed no relevant relationships. **J.C.** Activities related to the present article: institution received money and nonfinancial support from GE Healthcare. Activities not related to the present article: disclosed no relevant relationships. Other relationships: disclosed no relevant relationships. **L.L.** Activities related to the present article: institution received money and nonfinancial support from GE Healthcare. Activities not related to the present article: disclosed no relevant relationships. Other relationships: disclosed no relevant relationships. **C.M.** Activities related to the present article: institution received money and nonfinancial support from GE Healthcare. Activities not related to the present article: disclosed no relevant relationships. Other relationships: disclosed no relevant relationships. **M.G.H.** Activities related to the present article: disclosed no relevant relationships. Activities not related to the present article: disclosed no relevant relationships. Other relationships: author is an employee of GE Healthcare. **P.M.E.** Activities related to the present article: disclosed no relevant relationships. Activities not related to the present article: disclosed no relevant relationships. Other relationships: author is an employee of GE Healthcare.

References

1. McDonald RJ, McDonald JS, Kallmes DF, et al. Intracranial gadolinium deposition after contrast-enhanced MR imaging. Radiology 2015;275(3):772–782.
2. Kanda T, Ishii K, Kawaguchi H, Kitajima K, Takenaka D. 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(3):834–841.
3. Kanda T, Osawa M, Oba H, et al. High signal intensity in dentate nucleus on unenhanced T1-weighted MR images: association with linear versus macrocyclic gadolinium chelate administration. Radiology 2015;275(3):803–809.
4. Quattrocchi CC, Mallio CA, Errante Y, et al. Gadodiamide and dentate nucleus T1 hyperintensity in patients with meningioma evaluated by multiple follow-up contrast-enhanced magnetic resonance examinations with no systemic interval therapy. Invest Radiol 2015;50(7):470–472.
5. 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(3):783–791.
6. Errante Y, Cirimele V, Mallio CA, Di Lazzaro V, Zobel BB, Quattrocchi CC. Progressive increase of T1 signal intensity of the dentate nucleus on unenhanced magnetic resonance images is associated with cumulative doses of intravenously administered gadodiamide in patients with normal renal function, suggesting dechelation. Invest Radiol 2014;49(10):685–690.
7. Ramalho J, Castillo M, AlObaidy M, et al. High signal intensity in globus pallidus and dentate nucleus on unenhanced T1-weighted MR images: evaluation of two linear gadolinium-based contrast agents. Radiology 2015;276(3):836–844.
8. Stojanov DA, Aracki-Trenkic A, Vojinovic S, Benedeto-Stojanov D, Ljubisavljevic S. Increasing signal intensity within the dentate nucleus and globus pallidus on unenhanced T1W magnetic resonance images in patients with relapsing-remitting multiple sclerosis: correlation with cumulative dose of a macrocyclic gadolinium-based contrast agent, gadobutrol. Eur Radiol 2016;26(3):807–815.
9. Adin ME, Kleinberg L, Vaidya D, Zan E, Mirbagheri S, Yousem DM. Hyperintense dentate nuclei on T1-weighted MRI: relation to repeat gadolinium administration. AJNR Am J Neuroradiol 2015;36(10):1859–1865.
10. Weberling LD, Kieslich PJ, Kickingereder P, et al. Increased signal intensity in the dentate nucleus on unenhanced T1-weighted images after gadobenate dimeglumine administration. Invest Radiol 2015;50(11):743–748.
11. Roberts DR, Holden KR. Progressive increase of T1 signal intensity in the dentate nucleus and globus pallidus on unenhanced T1-weighted MR images in the pediatric brain exposed to multiple doses of gadolinium contrast. Brain Dev 2016;38(3):331–336.
12. Miller JH, Hu HH, Pokorney A, Cornejo P, Towbin R. MRI brain signal intensity changes of a child during the course of 35 gadolinium contrast examinations. Pediatrics 2015;136(6):e1637–e1640.
13. Murata N, Gonzalez-Cuyar LF, Murata K, et al. Macrocyclic and other non-group 1 gadolinium contrast agents deposit low levels of gadolinium in brain and bone tissue: preliminary results from 9 patients with normal renal function. Invest Radiol 2016;51(7):447–453.
14. Cao Y, Huang DQ, Shih G, Prince MR. Signal change in the dentate nucleus on T1-weighted MR images after multiple administrations of gadopentetate dimeglumine versus gadobutrol. AJR Am J Roentgenol 2016;206(2):414–419.
15. Kanda T, Fukusato T, Matsuda M, et al. Gadolinium-based contrast agent accumulates in the brain even in subjects without severe renal dysfunction: evaluation of autopsy brain specimens with inductively coupled plasma mass spectroscopy. Radiology 2015;276(1):228–232.
16. 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(8):473–480.
17. Robert P, Violas X, Grand S, et al. Linear gadolinium-based contrast agents are associated with brain gadolinium retention in healthy rats. Invest Radiol 2016;51(2):73–82.
18. Jost G, Lenhard DC, Sieber MA, Lohrke J, Frenzel T, Pietsch H. Signal increase on unenhanced T1-weighted images in the rat brain after repeated, extended doses of gadolinium-based contrast agents: comparison of linear and macrocyclic agents. Invest Radiol 2016;51(2):83–89.
19. Center for Drug Evaluation and Research, U.S. Food and Drug Administration. Guidance for industry. Estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM078932.pdf. Published July 2005. Accessed September 8, 2016.
20. Bahnemann R, Jacobs M, Karbe E, et al. RITA—Registry of Industrial Toxicology Animal-data—guides for organ sampling and trimming procedures in rats. Exp Toxicol Pathol 1995;47(4):247–266.
21. Ruehl-Fehlert C, Kittel B, Morawietz G, et al. Revised guides for organ sampling and trimming in rats and mice—part 1. Exp Toxicol Pathol 2003;55(2-3):91–106.
22. Kittel B, Ruehl-Fehlert C, Morawietz G, et al. Revised guides for organ sampling and trimming in rats and mice—part 2. A joint publication of the RITA and NACAD groups. Exp Toxicol Pathol 2004;55(6):413–431.
23. Morawietz G, Ruehl-Fehlert C, Kittel B, et al. Revised guides for organ sampling and trimming in rats and mice—part 3. A joint publication of the RITA and NACAD groups. Exp Toxicol Pathol 2004;55(6):433–449.

24. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline: guidance on nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals: M3(R2). Step 4 version. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Multidisciplinary/M3_R2/Step4/M3_R2__Guideline.pdf. Published June 11, 2009. Accessed September 8, 2016.

25. Crissman JW, Goodman DG, Hildebrandt PK, et al. Best practices guideline: toxicologic histopathology. Toxicol Pathol 2004;32(1):126–131.

26. Bussi S, Fouillet X, Morisetti A. Toxicological assessment of gadolinium release from contrast media. Exp Toxicol Pathol 2007;58(5):323–330.

27. Huang D, Cao Y, Prince M. Signal changes in dentate nuclei with gadolinium-based contrast administrations: comparison of linear versus 1 macrocyclic contrast agent [abstr]. In: Proceedings of the Twenty-Third Meeting of the International Society for Magnetic Resonance in Medicine. Berkeley, Calif: International Society for Magnetic Resonance in Medicine, 2015; 3229.

28. VanWagoner M, O'Toole M, Worah D, Leese PT, Quay SC. A phase I clinical trial with gadodiamide injection, a nonionic magnetic resonance imaging enhancement agent. Invest Radiol 1991;26(11):980–986.

29. Tweedle MF, Wedeking P, Kumar K. Biodistribution of radiolabeled, formulated gadopentetate, gadoteridol, gadoterate, and gadodiamide in mice and rats. Invest Radiol 1995;30(6):372–380.

30. Kindberg GM, Uran S, Friisk G, Martinsen I, Skotland T. The fate of Gd and chelate following intravenous injection of gadodiamide in rats. Eur Radiol 2010;20(7):1636–1643.

31. Harpur ES, Worah D, Hals PA, Holtz E, Furuhama K, Nomura H. Preclinical safety assessment and pharmacokinetics of gadodiamide injection, a new magnetic resonance imaging contrast agent. Invest Radiol 1993; 28(Suppl 1):S28–S43.

32. Mamourian AC, Hoopes PJ, Lewis LD. Visualization of intravenously administered contrast material in the CSF on fluid-attenuated inversion-recovery MR images: an in vitro and animal-model investigation. AJNR Am J Neuroradiol 2000;21(1):105–111.