Radiology

Signal Enhancement of the Dentate Nucleus at Unenhanced MR Imaging after Very High Cumulative Doses of the Macrocyclic Gadolinium-based Contrast Agent Gadobutrol: An Observational Study[1]

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Purpose: To test for measurable visual enhancement of the dentate nucleus (DN) on unenhanced T1-weighted magnetic resonance (MR) images in a cohort of patients with a primary brain tumor who had not received linear gadolinium-based contrast agents (GBCAs) but had received many injections of macrocyclic GBCAs.

Materials and Methods: Seventeen patients with high-grade gliomas who had received 10–44 administrations of the macrocyclic GBCA gadobutrol (0.1 mmol/kg of body weight) were retrospectively included in this regional ethics committee–approved study. Two neuroradiologists inspected T1-weighted MR images with optimized window settings to visualize small differences in contrast at the baseline and at the last examination for the presence of visual DN signal enhancement. Signal intensity (SI) in the DN was normalized to the SI of the pons, and a one-sample *t* test was used to test for differences between baseline normalized SI (nSI) in the DN (nSI_{DN}) and the average change in nSI_{DN} of all postbaseline MR imaging sessions (ΔnSI_{DNavg}) or the change in nSI_{DN} from baseline to the last MR imaging session (ΔnSI_{DN}). Linear and quadratic correlation analyses were used to examine the association between the number of macrocyclic GBCA administrations and ΔnSI_{DN} or ΔnSI_{DNavg}.

Results: The mean ± standard deviation number of macrocyclic GBCA administrations was 22.2 ± 10.6 administered throughout 706 days ± 454. Visually appreciable signal enhancement was observed in two patients who had received 37 and 44 macrocyclic GBCA injections. Mean ΔnSI_{DN} was greater than zero (0.03 ± 0.05; $P = .016$), and there was a significant linear association between the number of macrocyclic GBCA injections and ΔnSI_{DN} ($r = 0.69$, $P = .002$) and ΔnSI_{DNavg} ($r = 0.77$, $P < .001$).

Conclusion: A small but statistically significant dose-dependent T1-weighted signal enhancement was observed in the DN after multiple macrocyclic GBCA injections. Visually appreciable enhancement in the DN was observed on contrast-optimized images in two patients who had received 37 and 44 standard doses of macrocyclic GBCAs.

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In recent years, there has been increasing focus on the long-term retention of gadolinium-based contrast agents (GBCAs) in the brain. Several independent groups of researchers have reported (1–16) hyperintensity and/or increased normalized signal intensity (SI) (nSI) on unenhanced T1-weighted magnetic resonance (MR) images, indicating that gadolinium is retained in the dentate nucleus (DN) and globus pallidus after repeated administration of GBCAs. Results of confirmatory postmortem analyses (4,17) have shown that gadolinium is indeed responsible for these observed hyperintensities, and results of studies in animal models (18–20) have also confirmed the phenomenon. Authors of a large number of studies (3–5,12,13,20–22) have suggested that observed gadolinium retention only occurs with the use of GBCAs with a linear chelate configuration, whereas macrocyclic chelates are sufficiently stable to avoid gadolinium deposition in the brain. This view was challenged by authors of a recent study (9) who reported an increase in the nSI in the DN normalized to the SI of the pons (nSI_{DN}) and the globus pallidus-to-thalamus nSI after four to six standard-dose injections of a macrocyclic GBCA. However, the study has been criticized because of the lack of visible enhancement on unenhanced MR images and nonconclusive statistical results (21–23). Authors of a recent study (24) in rats suggest that macrocyclic GBCAs are mainly retained in the brain in their chelated (and excretable) configuration, whereas linear GBCAs may, to a much larger extent, dechelate and form insoluble gadolinium molecules with a prolonged retention half-life. However, even the soluble and intact macrocyclic GBCA brain fraction was detectable 24 days after administration, suggesting that macrocyclic GBCAs could exhibit retention in humans detectable with unenhanced MR imaging after a very high number of injections, and especially, if the injection frequency is high relative to the retention half-life.

The purpose of our study was to test for measurable and visual enhancement of the DN on unenhanced T1-weighted MR images in a cohort of patients with primary brain tumors and no history of administration of linear GBCAs but who had received a high number of macrocyclic GBCA injections. Different from those in the study by Stojanov et al (9), the patients for whom we had retrospective data had received variable but much higher numbers of macrocyclic GBCA injections overall, resulting in a large range of accumulated gadolinium doses. Therefore, these data should be valuable to the ongoing discussion of the possible retention properties of macrocyclic GBCAs in the brain.

Advances in Knowledge

- A statistically significant and dose-dependent signal intensity increase in the dentate nucleus on unenhanced T1-weighted images was found in a group of 17 patients who had received 10–44 standard doses of macrocyclic gadolinium-based contrast agents.
- Visually appreciable contrast of the dentate nucleus on unenhanced T1-weighted images was found in two patients who had received 37 and 44 standard doses of macrocyclic gadolinium-based contrast agents.

Implication for Patient Care

- Signal intensity increase, and hence, presumed gadolinium deposition, is indicated in the dentate nucleus after a very high number (> 30) of serial injections of the macrocyclic gadolinium-based contrast agent gadobutrol.

Materials and Methods

Ethical Statement

Author A.B. acts as a consultant for NordicNeuroLab. Appropriate ethical approval was obtained from the regional ethics committee (reference number 2009/1867b) and the hospital local ethics committee, and all procedures were consistent with the guidelines of the Declaration of Helsinki (25). All patients had given signed informed consent at the time of each examination for the use of their imaging data in research.

Subjects, Inclusion Criteria, and GBCA Injections

A retrospective analysis was performed with a data set from a prospective high-grade glioma treatment monitoring study. Patients were asked to be included in our prospective treatment study if they had histologically confirmed high-grade gliomas and were undergoing treatment with the protocol proposed by Stupp et al (25). The Stupp treatment protocol consisted of tumor-specific radiation therapy (2 Gy given 5 days per week for 6 weeks) plus concomitant administration of daily temozolomide (75 mg per square meter of body surface area per day, 7 days per week, from the 1st to the last day of radiation therapy), followed by six cycles of adjuvant temozolomide (150–200 mg per square meter for 5 days during each 28-day cycle). Between 2009 and 2015, a convenience series of 27 patients were

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Abbreviations:

ΔnSI_{DN} = change in nSI_{DN} from baseline to the last MR imaging session

ΔnSI_{DNavg} = average change in nSI_{DN} of all postbaseline MR imaging sessions

DN = dentate nucleus

GBCA = gadolinium-based contrast agent

nSI = normalized SI

nSI_{DN} = nSI in the DN normalized to the SI in the pons

ROI = region of interest

SI = signal intensity

Author contributions:

Guarantors of integrity of entire study, A.B., C.L., I.R.G.; 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, all authors; clinical studies, all authors; statistical analysis, A.B., S.A.S.V., C.L., P.K.H.; and manuscript editing, all authors

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

included in our treatment monitoring study, and they were imaged with a multimodal MR imaging protocol immediately before radiation therapy and chemotherapy and thereafter every 2nd week for 8 weeks (a total of five imaging sessions in the treatment phase) and subsequently every 3rd month until the patient died or withdrew from the study. At each examination, patients received two separate injections of 0.1 mmol of gadolinium per kilogram of body weight of the macrocyclic GBCA gadobutrol (Gadovist; Bayer Schering Pharma, Berlin, Germany) for two separate dynamic perfusion-weighted image acquisitions performed approximately 6 minutes apart, resulting in a total dose of gadobutrol of 0.2 mmol of gadolinium per kilogram of body weight at each imaging session. Gadobutrol was only given if the patients' preimaging glomerular filtration rate was higher than 60 mL/min per 1.73 m^2.

In addition to gadobutrol injections in our study protocol, most patients had received GBCA injections as part of initial diagnosis of high-grade glioma and for other diagnostic purposes before enrollment into our treatment monitoring study. Some patients also received GBCA outside of our protocol during the treatment study. All GBCA injections in all patients were traced from their medical records and noted for exclusion purposes and for estimation of total macrocyclic GBCA exposure.

All patients who were adherent to the protocol of our prospective study were eligible for inclusion in the gadolinium retention assessment study described here. A total of 306 MR imaging examinations in the 27 patients of the treatment study were evaluated for inclusion in our study. Exclusion criteria were the following: *(a)* radiologically confirmed abnormality of the cerebellum and pons at any time; *(b)* any injection of linear GBCA before entrance in our high-grade glioma treatment monitoring study; *(c)* any injection of linear GBCA during our high-grade glioma treatment monitoring study; *(d)* any injection of GBCA for which the injected agent was undocumented or information was unavailable; *(e)* less than five consecutive, exclusive administrations of 0.2 mmol of gadolinium per kilogram of body weight of gadobutrol; and *(f)* radiologically unacceptable image quality at unenhanced T1-weighted MR imaging. The GBCA history of all patients was determined by first identifying all MR imaging examinations performed as registered in our hospital radiology information system. On the basis of this information, the GBCA injection history of each individual patient was obtained by means of telephone contact with the local hospital for all MR imaging examinations performed outside our hospital. Of the 27 eligible patients, two patients were excluded because of the presence of abnormality in the cerebellopontine area, six were excluded because they had received linear GBCAs before entering the study, one patient was excluded because of undocumented GBCA injections before entering the study, and one patient was excluded for having less than five double-dose injections of gadobutrol. No patients were excluded because of insufficient image quality or injection of a linear or unknown GBCA during the study period. This left 17 patients with a total of 210 T1-weighted MR imaging series for further analysis.

MR Imaging

Our treatment monitoring study was conducted with a 3-T MR imaging unit (Achieva and Ingenia; Philips Healthcare, Best, the Netherlands). The study was a multimodal imaging protocol including structural imaging with high-spatial-resolution two-dimensional and three-dimensional T2-weighted imaging, unenhanced and gadobutrol-enhanced three-dimensional T1-weighted imaging, diffusion-tensor imaging, and gadobutrol contrast material–enhanced T1- and T2-weighted dynamic perfusion measurements. The full-brain unenhanced T1-weighted structural imaging was performed with a sagittal three-dimensional fast field-echo sequence with the following imaging parameters: repetition time msec/echo time msec, 5.2/2.3; field of view, 256 × 256; matrix, 256 × 232; section thickness, 1 mm; flip angle, 8°; and number of sections, 190. T1-weighted MR images were acquired in 22 patients with an eight-channel head coil (InVivo, Gainesville, Fla) with the Achieva (Philips) platform, and after a system upgrade to the dStream Ingenia (Philips) platform, a total of 12 examinations in four patients were performed with a 32-channel head coil (Philips). The imaging protocol and all imaging parameters were kept identical before and after system upgrade. No images for which other imaging units or imaging parameters were used were evaluated.

Visual Assessment of Unenhanced T1-weighted Images

Radiologic image evaluation was conducted by two experienced neuroradiologists (P.K.H. and P.D.T., both with more than 20 years of experience in radiology) who were blinded to the administered dose data. All assessments were made with transverse unenhanced T1-weighted images. In the assessments, the level of DN was identified, and DN enhancement (separately for left and right DN) was classified in consensus as 0, no DN enhancement; 1, slight DN enhancement; or 2, strong DN enhancement. DN enhancement was here defined as the DN having visibly higher SI than that of background tissue. In addition, the images were rated for whether DN enhancement was 1, confined to the DN only, or 2, extending beyond the DN. Baseline examinations and last-time-point examinations were scored separately. Imaging parameters were identical for all images evaluated, but for three patients, baseline imaging had been performed with the Achieva platform and an eight-channel head coil and the last-time-point examination was performed with the Ingenia platform with a 32-channel head coil. The range of SI levels displayed (window width) that were used for the visual assessment was optimized to highlight subtle contrast differences by reducing the window width by a factor of approximately five. Figure 1 shows an example of *(a)* standard window level and width settings and *(b)* contrast-optimized settings used for the radiologic assessment.

ROI Placement and SI Normalization

Image analysis of unenhanced T1-weighted images was conducted as defined and described in detail previously by Kanda

Figure 1

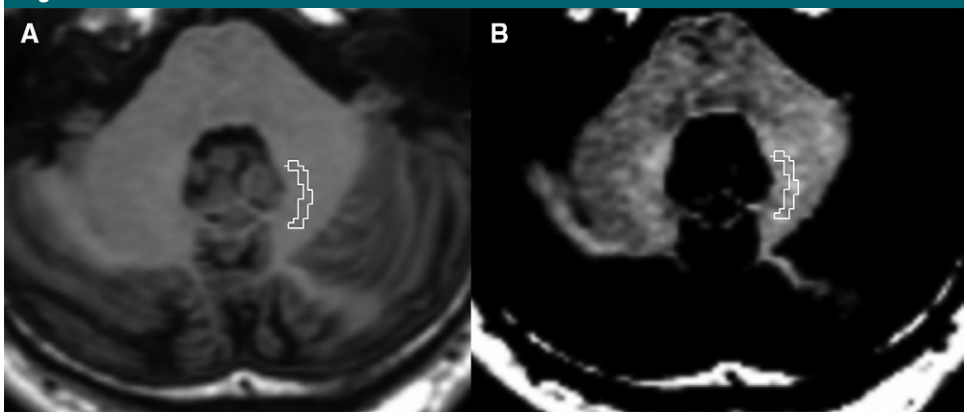

Figure 1: MR images show effect of optimizing image contrast for visualization of subtle intensity differences. *A*, Standard radiologic image window level and width (level = 800, width = 1600) and, *B*, optimized level and width (level = 800, width = 300), where level = median displayed intensity level and width = range of levels centered around median level (intensities in arbitrary display units). Placement of region of interest (ROI) in DN is outlined with white line.

et al (1,3,17) and replicated in other reports on cerebral gadolinium retention in humans. Placement of ROIs was conducted by a senior radiologist (P.K.H.) who was blinded to the clinical data. All structural data were reformatted to axial sections before ROI analysis and radiologic assessment for easier identification of the relevant anatomic structures.

ROI placement and subsequent image-based analysis was performed by using software (nordicICE; NordicNeuroLab, Bergen, Norway). Bean-shaped ROIs were drawn freehand around the DN on the contralateral side to the neoplasm, and an elliptical ROI was placed in the central pons on the first unenhanced T1-weighted image of the time series (baseline image acquired the day before the patient started radiation therapy and chemotherapy) by using two-dimensional and three-dimensional T2-weighted images for additional guidance. All successive T1-weighted images and corresponding ROIs were then coregistered to the first time point by using the rigid body normalized mutual information method with software (SPM 12, *fil.ion.ucl.ac.uk/spm*), running in Matlab (version 2014 B; MathWorks, Natick, Mass). Finally, all ROIs were visually inspected for correctness to adjust for possible structural displacement from baseline due to time-evolving structural deformation or suboptimal coregistration. T1-weighted images for each subject were displayed as dynamic time series, and the baseline ROI placement was manually edited per time point as required. In accordance with the bulk of previous work (1–3,6–9, 13,14,17,26,27), nSIs were obtained by normalizing the mean SI of the ROI in the DN to the mean SI of the ROI in the pons.

Statistical Analysis

The average change in patient nSI_{DN} throughout all postbaseline MR imaging sessions (ΔnSI_{DNavg}) and change in nSI_{DN} from baseline to the last MR imaging session (ΔnSI_{DN}) were calculated. The null hypotheses, ΔnSI_{DNavg} is equal to 0 and ΔnSI_{DN} is equal to 0, were tested by using one-sample tests. The strength of the null hypotheses versus the alternative hypotheses (different from 0) was tested with Jeffreys-Zellner-Siow Bayes factors (28). For visualization of dose response throughout patients, the within-subject mean change in nSI_{DN} from baseline was calculated in bins of five macrocyclic GBCA injections (between one and 45 injections) and was averaged for each bin throughout all subjects. Linear and nonlinear (quadratic) regression analyses were performed to examine whether ΔnSI_{DN} and ΔnSI_{DNavg} were related to the cumulative administered dose of macrocyclic GBCA. The analysis was performed by both including and excluding the MR imaging sessions performed after the system upgrade to investigate potential bias introduced by the upgrade on measured nSI_{DN}. Additional multivariate stepwise linear regression was performed to assess the effect of the covariates of sex, age, and MR system upgrade status on measured ΔnSI_{DN}. Finally, the association between total macrocyclic GBCA administration before the start of the study and nSI_{DN} at baseline was also tested by using linear regression analysis. Data were tested for normality by using the Shapiro-Wilk test, and the choice of parametric versus nonparametric test statistics was made on the basis of the outcome of this test.

A *P* value of .05 was considered to indicate a significant difference for all statistical tests. Statistical analysis was performed with software (SPSS Version 24; IBM, Armonk, NY), except for the Jeffreys-Zellner-Siow Bayes factor, which was calculated by using the Bayes Factor package in R software (Version 3.3.2; R Foundation for Statistical Computing, Vienna, Austria).

Results

Patient Data and Contrast Agent Administration Statistics

The mean number of single doses ± standard deviation (0.1 mmol of

gadolinium per kilogram of body weight) of all macrocyclic GBCAs administered was 22.2 ± 10.6, and they were administered throughout 706 days ± 454. The Table summarizes the characteristics of the 17 included patients. Note that only one dose of gadobutrol was administered at some time points, resulting in a noninteger number of double-dose–equivalent doses for some patients. All patients had at some point received macrocyclic GBCAs other than gadobutrol. The total number of macrocyclic GBCA single-dose administrations before the treatment monitoring study was 36, of which 27 were gadoterate dimeglumine, five were gadoteridol, and four were gadobutrol. The total number of macrocyclic GBCA single-dose administrations during the study was 377, of which 371 were gadobutrol and six were gadoterate dimeglumine. For the nSI analyses, ROI data were obtained from all time points for all patients.

Visual Assessment

The radiologic assessment of DN hyperintensity revealed two patients in whom the end point images were rated to show visible bilateral DN enhancement, with no enhancement on baseline images. These two patients had received 37 and 44 single-dose macrocyclic GBCA injections during the study period. Two additional patients had unilateral enhancement with findings that were less clear due to a variable amount of enhancement also in baseline examinations. Figure 2 shows the images obtained at baseline and those from the last time point in the two patients who were judged to exhibit bilateral DN enhancement.

Quantitative Analysis of nSI Values

ΔnSI_{DN} and ΔnSI_{DNavg} were normally distributed, both by including and excluding postupgrade examinations (Shapiro-Wilk test, $P > .1$), and therefore, parametric test statistics were used. Figure 3 shows a box plot of the mean change in nSI_{DN} from baseline as a function of the number of single-dose macrocyclic GBCA injections. The dose response appears distinctly nonlinear, with a step increase in nSI_{DN} appearing at approximately 30 macrocyclic GBCA injections. In the comparison of baseline with last-time-point images within each patient, a significant increase in nSI_{DN} was observed ($\Delta nSI_{DN} = 0.03 \pm 0.05$; 95% confidence interval: 0.002, 0.05; $P = .016$; Bayes factor = 2.9; supporting $\Delta nSI_{DN} > 0$). The ΔnSI_{DNavg} throughout all postbaseline time points was not different from zero ($\Delta nSI_{DNavg} = 0.007 \pm 0.03$; 95% confidence interval: −0.006, 0.021; $P = .13$; Bayes factor = 1.6; supporting $\Delta nSI_{DNavg} = 0$). There was a significant linear association between ΔnSI_{DNavg} and number of macrocyclic GBCA injections ($r = 0.77$, $P < .001$) and also a significant linear correlation between ΔnSI_{DN} and number of macrocyclic GBCA administrations ($r = 0.69$, $P = .002$). Given the apparent nonlinear dose response from Figure 3, the regression analysis was also performed by using a quadratic model function, resulting in a slightly improved curve fit compared with that of linear regression for both ΔnSI_{DN} (R^2 linear = 0.57, R^2 quadratic = 0.634) and ΔnSI_{DNavg} (R^2 linear = 0.642, R^2 quadratic = 0.687). There was no association between the number of prestudy macrocyclic GBCA administrations and baseline nSI_{DN} values ($r = 0.21$, $P = .34$). The results of the linear and quadratic regression analysis are shown in Figures 4 and 5.

Effects of Confounding Variables

The exclusion of all MR imaging sessions performed after system upgrade resulted in a smaller and only borderline significant increase in nSI_{DN} between the baseline and last preupgrade MR imaging sessions ($\Delta nSI_{DN} = 0.016 \pm 0.03$; 95% confidence interval: −0.001, 0.03; $P = .034$; Bayes factor = 1.6; supporting $\Delta nSI_{DN} > 0$). There was also a significant linear association between the number of macrocyclic GBCA administrations and ΔnSI_{DNavg} ($r = 0.66$, $P = .004$), but not ΔnSI_{DN} ($r = 0.44$, $P = .081$). Use of a quadratic model function did not improve the curve fit compared with linear regression for the preupgrade dose response. Figure 6 shows scatterplots of ΔnSI_{DN} and ΔnSI_{DNavg} versus the number of single-dose macrocyclic GBCA administrations and resulting linear regression analysis, excluding postupgrade examinations.

The stepwise multiple linear regression model revealed the number of macrocyclic GBCA injections to be the most significant single predictor of increase in ΔnSI_{DNavg} ($r = 0.77$). Adding patient age as a covariate increased the model fit ($r = 0.84$) with both total macrocyclic GBCA dose and patient age being significant predictors. However, age was also correlated with the total number of GBCA injections ($r = 0.543$, $P = .024$), reflecting the fact that younger patients, on average, underwent more MR imaging sessions than did older patients. Patient sex and system upgrade status did not significantly contribute to the linear regression model.

Figure E1 (online) shows the effect of system upgrade on unenhanced T1-weighted images (optimized image window level and width settings) from a sample patient in whom the baseline MR imaging was performed before the system upgrade and the last MR imaging examination was performed after upgrade. There is a visible difference in image quality and appearance before and after system upgrade, but no apparent difference in contrast between the DN and the background, which suggests that the system upgrade alone did not cause a systematic change in DN-to-background T1-weighted contrast.

Discussion

In an assessment of 17 patients with high-grade gliomas who had received 10–44 standard doses of the macrocyclic GBCA gadobutrol in addition to a variable amount of other macrocyclic GBCAs, we measured a statistically significant and dose-dependent increase in the nSI_{DN} when we compared precontrast MR images from the last time point with those from the baseline examination. These results are in agreement with those from the recent study by Stojanov et al (9), but are in contradiction to results of most published data (1,12,13,29), in which authors concluded that multiple injections of macrocyclic GBCAs do not result in

Patient Characteristics and Contrast Agent Administration Statistics

Patient ID	Age (y)	Sex	No. of MR Imaging Sessions	Interval between First and Last MR Imaging Session (d)	No. of Within-protocol Single-dose Gadobutrol Injections	Outside-protocol Macrocyclic GBCA Single Injections	Prestudy Macrocyclic GBCA Single Injections	Total Macrocyclic GBCA Dose* (mmol/kg)
1	69	F	6	104	10	0	1	1
6	69	M	16	1022	30	1	1	3.1
7	58	M	14	791	26	0	2	2.6
8	67	M	7	252	12	0	3	1.2
9	52	M	8	298	14	0	2	1.4
12	61	F	10	518	19	0	3	1.9
15	36	M	23	1576	44	0	2	4.4
16	56	F	9	442	16	0	2	1.6
17	57	M	6	133	10	0	3	1
18	56	M	9	329	11	0	2	1.1
20	60	M	14	912	22	2	2	2.4
21	36	F	19	1359	35	2	2	3.7
22	68	M	9	322	10	1	2	1.1
23	58	M	19	1344	36	0	3	3.6
24	60	M	13	735	24	0	2	2.4
25	45	F	14	937	24	0	2	2.4
26	65	M	14	924	26	2	2	2.8
Mean†	57.2 ± 10.2 (36–69)		12.4 ± 5.0 (6–23)	705.8 ± 454.1 (104–1576)	21.8 ± 10.3 (10–44)	0.4 ± 0.8 (0–2)	2.1 ± 0.6 (1–3)	2.2 ± 1.1 (1.0–4.4)

* Excluding prestudy contrast agent administrations.

† Data are means ± standard deviation, with the range in parentheses.

measurable gadolinium retention. The hypothesis that macrocyclic GBCAs do not result in gadolinium retention detectable at imaging was substantiated in a large retrospective study (7) in which patients who had received exclusively linear GBCAs were compared with those who received exclusively macrocyclic GBCAs (7). The only study (9) to date in which the authors found measurable enhancement in the DN and globus pallidus after repeated macrocyclic GBCA administrations was criticized because of a lack of evidence of visible MR imaging enhancement to support their findings and a problematic statistical inference (22,23,30). In our study, radiologic assessment of contrast-optimized images revealed visible DN enhancement in two patients who had received 37 and 44 single-dose equivalents of macrocyclic GBCA. These results raise the question whether visible enhancement based on standard radiologic reading is an absolute criterion to confirm gadolinium deposition in the DN or whether optimized image contrast or ROI measurements may reveal subtle enhancement effects not evident with standard visual inspection.

Our study results differ from those of previous work in the large total dose of macrocyclic GBCA administered to the patients throughout a relatively short time. In their 2016 study, Radbruch et al (14) investigated the highest number of repeated macrocyclic GBCA administrations in humans to date, to our knowledge, with patients who received an average of 23 macrocyclic GBCA-enhanced examinations but with only two patients who received more than 26 examinations and an average of 12 weeks between successive administrations. In comparison, we investigated a wider range of total macrocyclic GBCA injections, including six patients who had received 26 or more single-dose macrocyclic GBCA injections. Our protocol also differed from that of Radbruch et al (14) in that the patients in our study were subject to frequent double-dose macrocyclic GBCA administrations (every 2 weeks) for the first five MR imaging examinations. Authors of a recent study (31)

Figure 2

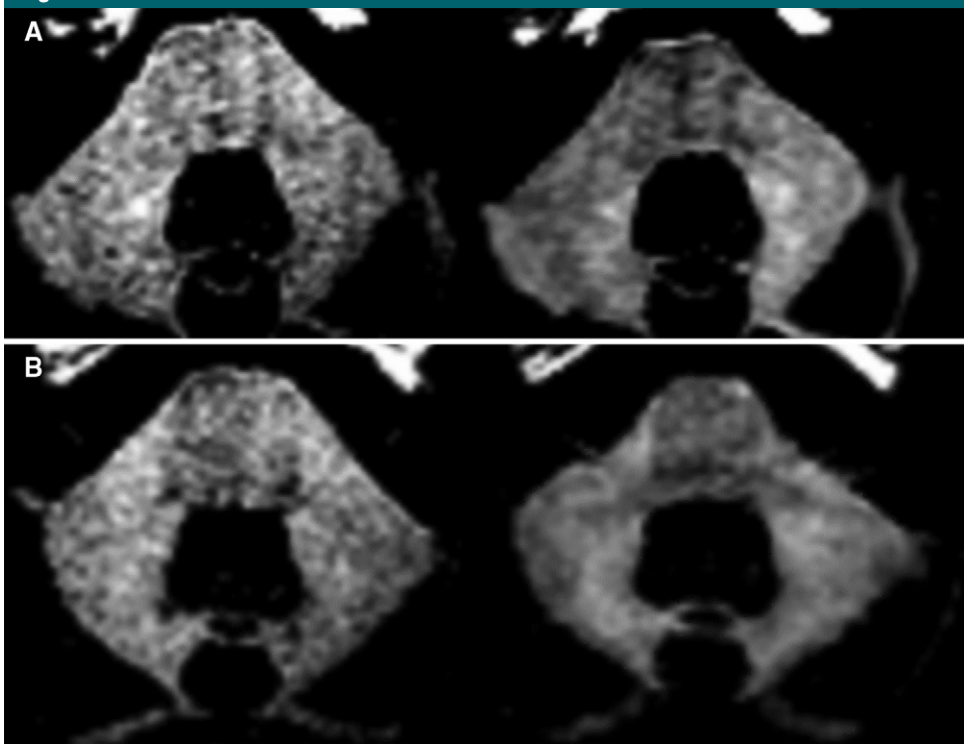

Figure 2: Unenhanced T1-weighted images from the two patients who were radiologically scored to have visible or strong DN enhancement at last time point (right column) and no DN enhancement at baseline examinations (left column). Two patients are, *A*, patient 5 and, *B*, patient 21 in the Table.

in mice concluded that gadolinium retained in the brain after repeated high doses of linear GBCAs was reduced by approximately 50% at 20 weeks after dosing, which could indicate that the rate of repeated administration could be important to the steady-state gadolinium retention profile. This hypothesis is further supported by results of a recent study (24) in rats in which the authors concluded that macrocyclic GBCAs are mainly retained in the brain in a soluble form (as intact chelate or soluble small molecules), whereas linear GBCAs, to a much larger extent, are retained as macromolecules or in an insoluble form. Whereas the soluble small molecular fraction of GBCA showed a significant clearance between day 3 and day 24 after administration, the macromolecular and insoluble fractions retained in the brain where largely constant throughout the same time interval. The results of this study in rodents, therefore, provide some support to the hypothesis that frequency of repeated injections could be important in the observed retention of macrocyclic GBCAs, given the higher fraction retained as intact chelate or soluble

Figure 3

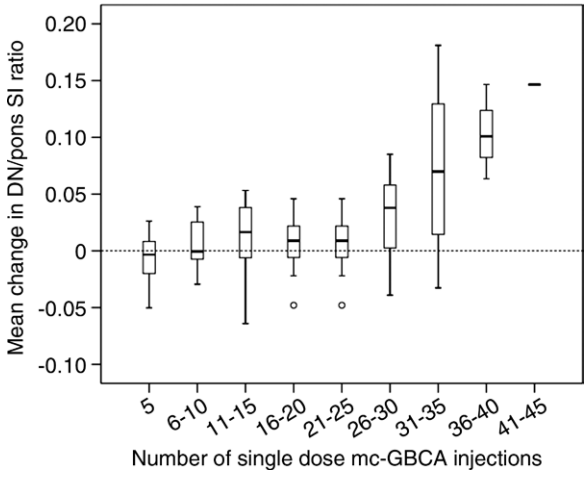

Figure 3: Boxplot shows mean change in DN-to-pons unenhanced T1-weighted SI ratio from baseline MR imaging examination as function of number of macrocyclic *(mc)* GBCA injections.

Figure 4

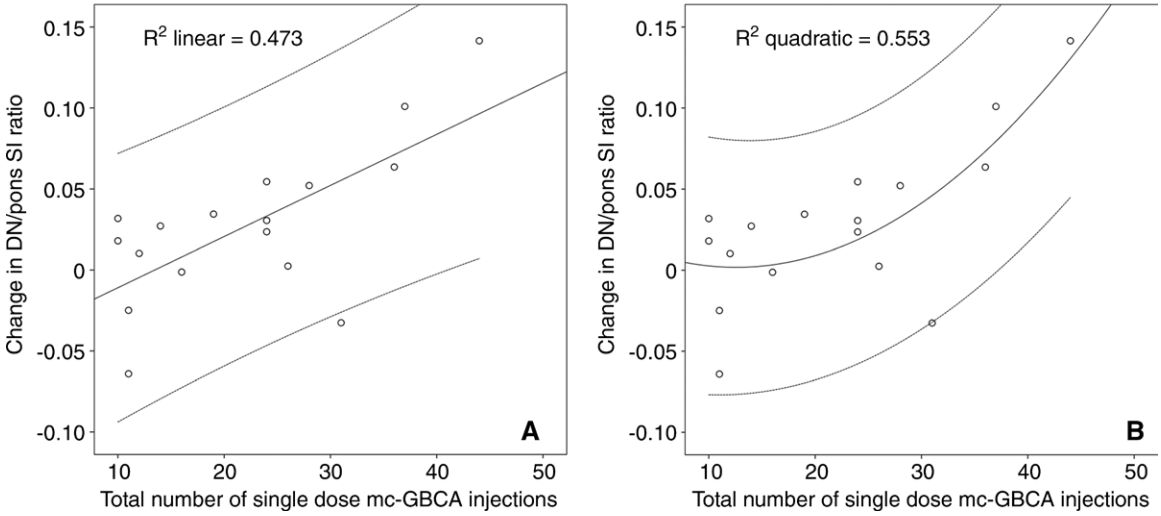

Figure 4: Scatterplots show change in DN-to-pons unenhanced T1-weighted SI ratio from baseline to last MR imaging examination as function of number of single-dose macrocyclic *(mc)* GBCA injections. Corresponding, *A*, linear and, *B*, quadratic regression lines (center lines) and 95% confidence intervals (outer lines) are shown. Best fit was obtained with quadratic function.

Figure 5

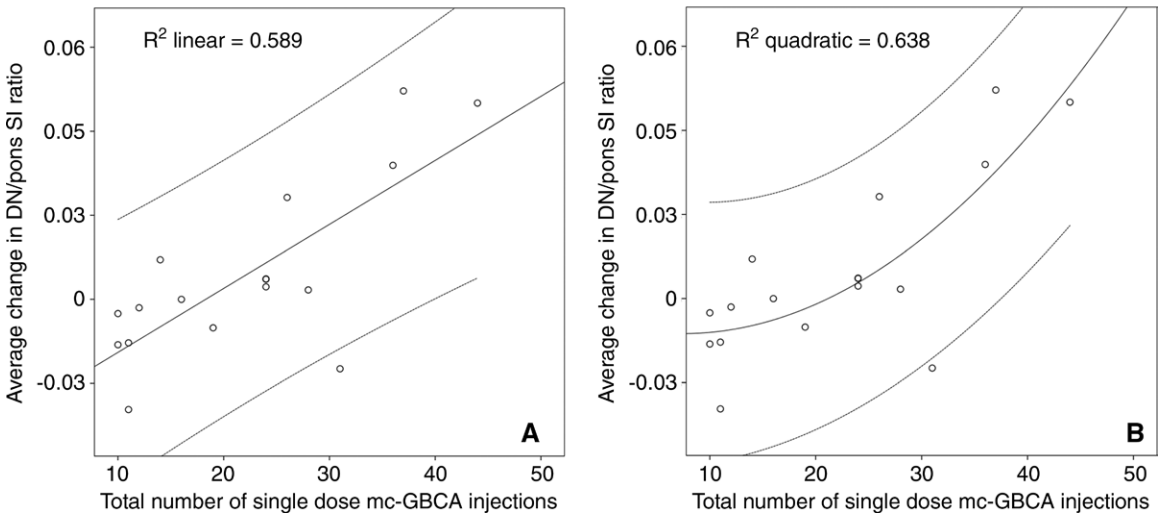

Figure 5: Scatterplots show change in DN-to-pons unenhanced T1-weighted SI ratio from baseline MR imaging examination to mean intensity ratio throughout all postbaseline examinations as function of number of single-dose macrocyclic *(mc)* GBCA injections. The corresponding, *A*, linear and, *B*, quadratic regression lines (center lines) and 95% confidence intervals (outer lines) are shown. Best fit was obtained with quadratic function.

small molecules that exhibit a shorter retention half-life compared with those of linear GBCAs.

The macrocyclic GBCA agent gadobutrol was used exclusively in the prospective study on which our analysis was based. In addition, a small number of gadoterate dimeglumine doses were administered outside of our protocol during the study period, but these accounted for less than 2% of the total administered macrocyclic GBCA dose. Preclinical studies have revealed differences in brain gadolinium retention, even among different macrocyclic GBCAs. In early work, Wedeking et al (32) used radiolabeled gadolinium in mice and revealed measurable whole-body gadolinium retention, even with macrocyclic GBCA, albeit at much lower levels than those for linear GBCA. They also showed that the degree of retention was associated with the measured conditional stability constants of

Figure 6

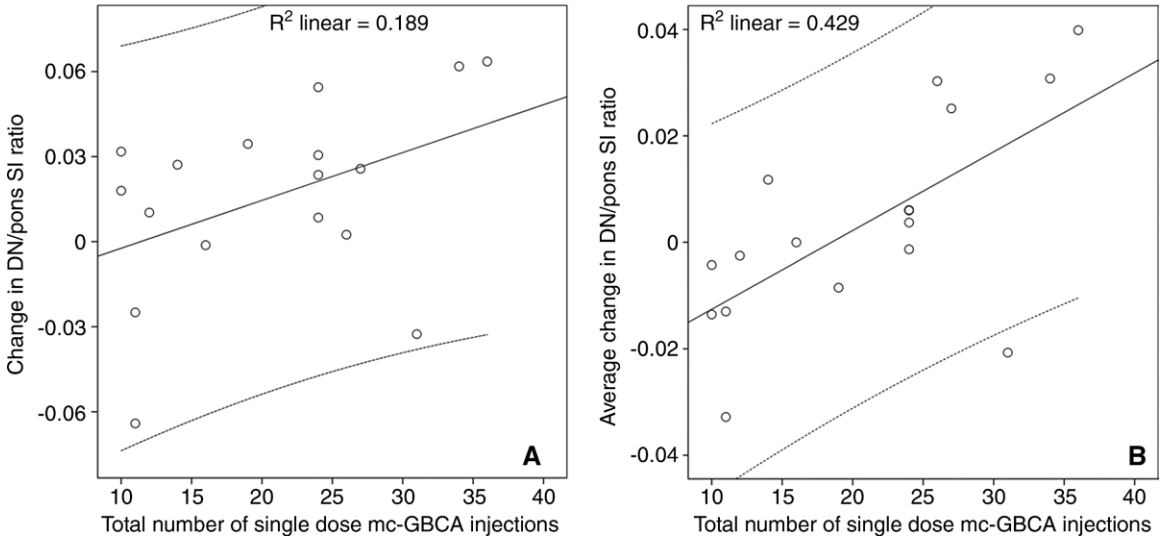

Figure 6: Scatterplots show, *A*, change in DN-to-pons unenhanced T1-weighted SI ratio from baseline to last MR imaging examination and, *B*, mean SI ratio throughout all postbaseline examinations as function of number of single-dose macrocyclic *(mc)* GBCA injections before system upgrade. Corresponding linear regression lines (center lines) and 95% confidence intervals (outer lines) are shown.

the gadolinium chelates investigated. Gadolinium stability and dissociation constants for a large range of gadolinium chelates have been studied in detail and have revealed variations also among macrocyclic complexes (33). Murata et al (34) recently showed measurable gadolinium concentrations higher than control levels in autopsy samples from multiple brain regions (including the pons, globus pallidus, and the DN) in patients who received different macrocyclic GBCAs at various total doses. Although the sample size was too small to draw firm conclusions, their results provided some evidence in support of our findings that gadolinium retention may be detectable in human studies as a result of macrocyclic GBCA injections.

On the basis of this discussion, our results were unexpected and are likely controversial. Therefore, possible sources of errors and study limitations must be considered carefully. Our study had several limitations. First, this was a retrospective study that included only 17 patients and in which MR imaging data from patients with high-grade gliomas who were recruited for a longitudinal radiation therapy and chemotherapy study were used. Therefore, all patients had known brain neoplasms that could have influenced the time evolution of the MR imaging SI in different parts of the brain. For this reason, we did not include measurements in the commonly investigated globus pallidus, because high-grade gliomas frequently affect this region of the brain. The effect of chemotherapy and radiation therapy on MR imaging SI changes has been addressed previously in studies including patients with glioma, and no correlation between chemotherapy or radiation therapy and DN enhancement was found (7). A second potential limitation was that the radiologists who performed the visual assessment of the precontrast T1-weighted images were not blinded to the order of the images (baseline vs last-time-point images), although they were blinded to the actual dose administered to each patient evaluated. This could have introduced a bias toward observing less enhancement in the baseline examinations, but it was unlikely to have influenced the relative enhancement scoring of the last-time-point images among patients.

A third limitation of our study was that an MR imaging system upgrade took place during the latter part of the study. Changes were made to the MR imaging hardware and software that had the potential to cause subtle differences in image contrast in the relevant regions because of generally improved image quality after the system upgrade. However, even when all postupgrade examinations were excluded from the analysis, DN enhancement and dose dependence remained significant. There were also patient image sets with no visible DN enhancement after the system upgrade, which supports the conclusion that the observed dose response was not due to the system upgrade alone.

The confounding factors of MR imaging system and protocol changes are likely to be general challenges in these types of analyses, because long-term longitudinal analysis is inherently required. Previous studies have commonly included imaging data from different MR imaging systems (6,7,10,34), sequence types (7,16,35), and field strengths (4,7,10,11,35). The occurrence of system upgrades and changes during the course of the studies is rarely documented.

In conclusion, we measured a small but statistically significant dose-dependent T1-weighted nSI enhancement in the DN after multiple administrations of the macrocyclic GBCA gadobutrol. The effect could only be visualized in two patients who received a high cumulative dose of gadobutrol. Our results must be confirmed in larger and better-controlled prospective studies, but they suggest that both linear and macrocyclic GBCAs can cause gadolinium retention, albeit at very different levels of total gadolinium exposure.

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