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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

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Objectives: In this prospective preclinical study, we evaluated T1-weighted signal intensity in the deep cerebellar nuclei (CN) and globus pallidus (GP) up to 24 days after repeated administration of linear and macrocyclic gadolinium-based contrast agents (GBCAs) using homologous imaging and evaluation methods as in the recently published retrospective clinical studies. In a second part of the study, cerebrospinal fluid (CSF) spaces were evaluated for contrast enhancement by fluid-attenuated magnetic resonance imaging (MRI).

Materials and Methods: Sixty adult male Wistar-Han rats were randomly divided into a control and 5 GBCA groups (n = 10 per group). The administered GBCAs were gadodiamide, gadopentetate dimeglumine, and gadobenate dimeglumine (linear GBCAs) as well as gadobutrol and gadoterate meglumine (macrocyclic GBCAs) and saline (control). Over a period of 2 weeks, the animals received 10 intravenous injections at a dose of 2.5 mmol Gd/kg body weight, each on 5 consecutive days per week. Before GBCA administration, as well as 3 and 24 days after the last injection, a whole-brain MRI was performed using a standard T1-weighted 3-dimensional turbo spin echo sequence on a clinical 1.5 T scanner. The ratios of signal intensities in deep CN to pons (CN/Po) and GP to thalamus (GP/Th) were determined. For the evaluation of the CSF spaces, 18 additional rats were randomly divided into 6 groups (n = 3 per group) that received the same GBCAs as in the first part of the study. After MR cisternography for anatomical reference, a fluid-attenuated inversion recovery sequence was performed before and 1 minute after intravenous injection of a dose of 1 mmol Gd/kg body weight GBCA or saline.

Results: A significantly increased signal intensity ratio of CN/Po was observed 3 and 24 days after the last injection of gadodiamide and gadobenate dimeglumine. No significant changes were observed between the 2 time points. Gadopentetate dimeglumine injection led to a moderately elevated but statistically not significant CN/Po signal intensity ratio. No increased CN/Po signal intensity ratios were determined in the MRI scans of rats that received macrocyclic GBCAs gadobutrol and gadoterate meglumine or saline. The ratio of signal intensity in GP/Th was not elevated in any group injected with GBCAs or saline. Enhanced signal intensities of CSF spaces were observed in the postcontrast fluid-attenuated inversion recovery images of all animals receiving GBCAs but not for saline.

Conclusions: In this animal study in rats, increased signal intensity in the CN was found up to 24 days after multiple, extended doses of linear GBCAs.

However, in contrast to clinical reports, the signal enhancement in the GP was not reproduced, demonstrating the limitations of this animal experiment. The elevated signal intensities remained persistent over the entire observation period. In contrast, no changes of signal intensities in either the CN or the GP were observed for macrocyclic GBCAs. However, all GBCAs investigated were able to pass the blood-CSF barrier in rats to a certain, not yet quantified extent.

Key Words: GBCA, contrast agent, MRI, gadolinium, pons, retention, animal experiment, cerebrospinal fluid

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Gadolinium-based contrast agents (GBCAs) are frequently used in various magnetic resonance imaging (MRI) examinations and are generally considered to have an excellent safety profile.¹ Based on the chemical structure, GBCAs can be divided into 2 classes—linear and macrocyclic agents. Both classes comprise ionic or nonionic compounds.² The different GBCAs exhibit different thermodynamic and kinetic complex stabilities *in vivo*,^{3–5} imposing a higher risk of release of Gd³⁺ at physiological conditions upon linear GBCAs than upon macrocyclic agents.⁶

Recent publications have reported increased signal intensities on unenhanced T1-weighted (T1w) images in the dentate nucleus and globus pallidus (GP) regions of the brain of patients who received repeated contrast-enhanced MRI examination with linear GBCA gadodiamide,^{7–10} gadopentetate dimeglumine,^{7,11–13} and gadobenate dimeglumine.¹⁴ A correlation between these signal hyperintensities and the number of contrast-enhanced MRI examinations was first described by Kanda et al⁷ in 2014. Subsequent autopsy studies verified the accumulation of gadolinium in the enhanced brain structures.^{9,12} In 2 retrospective clinical studies, linear gadopentetate dimeglumine was directly compared with macrocyclic gadoterate meglumine¹¹ and gadoteridol.¹² Therein, a signal increase in the dentate nucleus was found only in patients receiving multiple administrations of the linear gadopentetate dimeglumine. This observation has been reproduced in a rat animal model after repeated administration of gadodiamide, but not after gadoterate meglumine.¹⁵ In addition, Radbruch et al¹⁶ showed in a recent clinical study no signal intensity increase in dentate nucleus and GP on unenhanced T1w images after multiple applications of the macrocyclic GBCA gadobutrol. The results of Radbruch et al are contrary to a prior published study of Stojanov et al,¹⁷ reporting that gadobutrol causes significant SI enhancement of these brain regions. However, the study design of this publication was critically discussed and does not convincingly support this conclusion.^{18,19}

The mechanism of GBCA accumulation in these specific brain regions is yet unknown and raises several questions; these are as follows: (1) “By which pathway does gadolinium enter the brain?” (2) “How does it distribute itself within the brain to reach the 2 areas GP and CN, which are located widely apart from each other and from any potential BBB disruption?” and (3) “What is the chemical species of Gd?” One possible pathway might be the infiltration of GBCAs from

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blood into the cerebrospinal fluid (CSF), which is supported by a study on patients suspected of Ménière disease. This study described contrast enhancement of the subarachnoid space, the anterior eye segment, and the CSF in the internal auditory canal after intravenous administration of GBCA.²⁰

The aim of this preclinical study was to reproduce the observed T1w signal intensity in humans in a rat model after repeated administration of linear and macrocyclic GBCAs. Comparable to the clinical observations, we performed MRI with a clinical 1.5 T scanner and evaluated the data with similar methods. Furthermore, the influence of both GBCA classes on T1w signal hyperintensity was determined by applying 3 linear (ionic, nonionic, protein-binding) and 2 macrocyclic (ionic, nonionic) GBCAs. To evaluate the potential pathway of GBCA infiltration into CSF, we performed fluid-attenuated MR cisternography before and after intravenous GBCA application.

MATERIALS AND METHODS

Animals

Seventy-eight healthy Han-Wistar rats (CrI:WI; males; 200–225 g) were obtained from Charles River (Sulzfeld, Germany). The animals were kept under standard laboratory conditions at a temperature of 22°C and a dark/light rhythm of 12 hours. Standard rat chow and water were provided ad libitum. The animals were handled and treated according to German animal regulations. The GBCA administration and the MRI were performed in anesthesia initiated with 4% isoflurane and maintained with 1.5% isoflurane (Baxter GmbH, Unterschleißheim, Germany). During the MRI, the body temperature was maintained with a circulating warm water pad.

Study Setup

Signal Enhancement in the Brain

Sixty rats were randomly divided into a control and 5 GBCA groups (n = 10 per group). The animals received the linear GBCAs

gadodiamide (Omniscan; GE Healthcare Buchler GmbH & Co KG, Braunschweig, Germany), gadopentetate dimeglumine (Magnevist; Bayer Vital GmbH, Leverkusen, Germany), gadobenate dimeglumine (Multihance; Bracco Imaging Deutschland GmbH, Konstanz, Germany), or the macrocyclic GBCAs gadobutrol (Gadovist; Bayer Vital GmbH) and gadoterate meglumine (Dotarem; Guerbet GmbH, Sulzbach/Taunus, Germany) or saline (control). Over a period of 2 weeks, the animals received 10 intravenous injections at a dose of 2.5 mmol Gd/kg body weight (b.w.), each on 5 consecutive days per week. All investigated GBCAs were applied by slow hand injection (~0.8 mL/min) using their marketed formulation. The extended dose of 2.5 mmol Gd/kg b.w. per injection approximates about 4 times the human standard dose based upon body surface area normalization between rats and humans.²¹ One day before the first GBCA administration, as well as 3 days after the last injection (p.i.), a whole-brain MRI was performed for all animals. Subsequently, 5 randomly chosen animals per group were euthanized by exsanguination for dissection. The other 5 animals per group received a second brain MRI before dissection at 24 days p.i. All tissue samples were frozen for further detailed analysis (ongoing). An overview of the study setup is shown in Figure 1A.

Evaluation of CSF Spaces

Eighteen rats were randomly divided into 6 groups (n = 3 per group), which received the same GBCAs as in the first part of the study. After an MRC for anatomical reference and a fluid attenuated baseline brain scan, the animals received an intravenous injection of GBCA at a dosage of 1 mmol Gd/kg b.w. or volume adjusted saline. One minute after injection, a fluid attenuated sequence was started.

MRI Protocol

Magnetic resonance imaging was performed in a clinical 1.5 T MRI (Avanto; Siemens Healthcare, Erlangen, Germany) using a dedicated 2-channel rat brain coil (Rapid Biomedical GmbH, Rimpf, Germany).

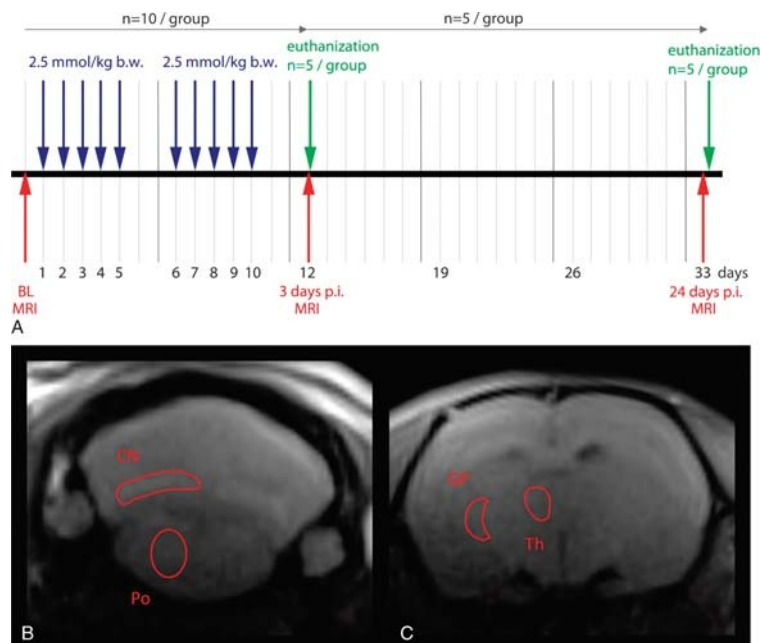


FIGURE 1. A, Study setup and application scheme for GBCAs. Five consecutive daily intravenous injections (blue arrows) per week of 2.5 mmol GBCA/kg b.w. for 2 weeks were given to 10 animals per GBCA group. MRI scans (red arrows) were performed before (BL) GBCA application, and on day 15 (3 days p.i.) for 10 rats per group and on day 36 (24 days p.i.) of study initiation on 5 animals per group. On day 15 and on day 36, 5 rats each per GBCA group were euthanized for dissection. B and C, Examples of the positioning of the ROI for the signal intensity evaluation of deep CN and pons (Po) in B and of GP and thalamus (Th) in C.

For the evaluation of the signal enhancement, a 3-dimensional T1-weighted turbo spin echo sequence (repetition time (TR), 500 milliseconds; echo time 19 milliseconds) was adjusted to cover the whole rat brain with a resolution of $0.3 \times 0.3 \times 0.8$ mm (field of view, 80×32.5 mm; 30 transversal slices). A turbo factor of 3 was used, the number of signal excitations was 1, and the acquisition time was 9:55 minutes.

For the evaluation of the CSF space, heavily T2-weighted MRC for anatomical references of the fluid space was followed by a pre-contrast and post-contrast heavily T2-weighted, fluid-attenuated inversion recovery (FLAIR) sequence. For the MRC, a variable flip angle 3-dimensional turbo spin echo sequence (TR, 4400 milliseconds; echo time, 553 milliseconds) with an initial refocusing flip angle of 180 degrees (decreased to a constant angle of 120 degrees for the refocusing echo train), a turbo factor of 79, and a magnetization restore pulse was used. The spatial resolution was $0.3 \times 0.3 \times 0.6$ mm (field of view, 100×48 mm; 30 transversal slices), the number of signal excitations was 1.8, and the acquisition time was 7:57 minutes. Identical scan parameters were used for the FLAIR sequence. However, a nonselective inversion recovery pulse with an inversion time of 2250 milliseconds was included, and the TR was extended to 9000 milliseconds. The acquisition time was 16:14 minutes.

Image Evaluation

The image analysis was performed by 2 experienced readers, blinded to the experimental groups and time points, on an external workstation (MMWP; Siemens Healthcare, Erlangen, Germany). Manually defined region of interests (ROIs) were drawn around the deep cerebellar nuclei (CN), the pons, the GP, and the thalamus, respectively. The slice selection as well as the ROI positioning was guided by anatomical landmarks taken from a stereotactic rat brain atlas.²² Examples of the ROI placement are shown in Figure 1, B and C. The signal intensities of the left and right hemispheres were averaged for each region. The deep cerebellar nucleus-to-pons ratio (CN/Po) was calculated by dividing the mean signal intensity of the CN by that of the pons. The GP-to-thalamus signal intensity ratio (GP/Th) was calculated by dividing the mean signal intensity of the GP by that of the thalamus. The CN/Po and GP/Th signal intensity ratios of the 2 readers were averaged. The difference to baseline (% change) was calculated for both signal intensity ratios.

Statistical Evaluation

Statistical comparisons between the experimental groups and control group were performed at each time point with 2-way analysis of variance followed by the post hoc Bonferroni test for multiple comparison. The statistical evaluation of the signal ratios within experimental groups (ie, the time course) was performed with a paired *t* test after confirming Gaussian distribution with the Kolmogorov-Smirnov test. The calculations were performed with GraphPad Prism (GraphPad Software, La Jolla, CA) using a significance level of 5%.

RESULTS

Signal Enhancement in the Brain

All animals successfully completed the entire study. Increased signal intensities in the CN were observed 3 and 24 days p.i. of gadodiamide, gadobenate dimeglumine, and to a lesser extent of gadopentetate dimeglumine. No signal changes were visible after administration of gadobutrol, gadoterate meglumine, or saline at any time point. Representative images for each experimental group and time point are shown in Figure 2.

The results of the quantitative analysis of the CN/Po signal intensity ratio are summarized in Figure 3, showing the absolute CN/Po ratios for the 3 time points (Fig. 3A) and the respective percent change

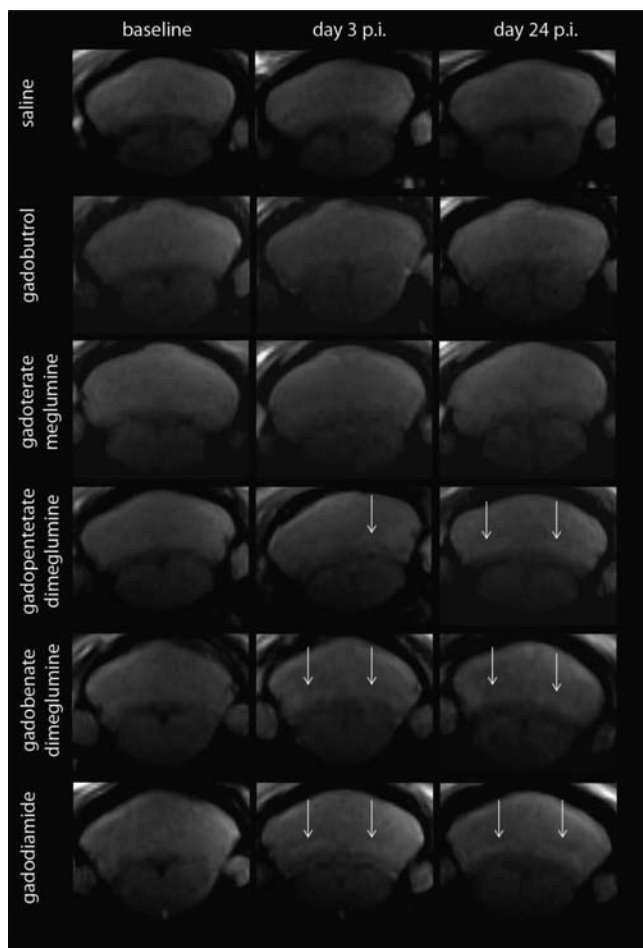


FIGURE 2. Representative T1w MRI scans of CN in the cerebellum before (baseline, left column), at day 3 p.i. (middle column) and at day 24 p.i. (right column) with saline, gadobutrol, gadoterate meglumine, gadopentetate dimeglumine, gadobenate dimeglumine, and gadodiamide. Increased signal intensities of the CN are indicated by white arrows.

to baseline (Fig. 3B). Statistical comparison within the experimental groups revealed significantly increased CN/Po ratios for gadodiamide and gadobenate dimeglumine on day 3 and 24 p.i. compared with baseline. On day 24 p.i., gadodiamide exhibited the highest CN/Po ratio increase in 9.6% compared with baseline. No significant differences were found between 3 and 24 days p.i. in these groups. A moderately elevated, but not significant, increase in the CN/Po ratios was observed for gadopentetate dimeglumine only on day 24 p.i. No increased CN/Po ratios compared with baseline were observed in the MRI scans of rats that received gadobutrol, gadoterate meglumine, or saline at any time point. Instead, a slight decrease was determined 3 days p.i. with mean reductions of 1.8%, 2.6%, and 2.2% (% change to baseline) for gadobutrol, gadoterate meglumine, and saline, respectively (Fig. 3B). This reduction returned to the baseline CN/Po ratios at day 24 p.i.

For the GP, no signal increase was visible in the MRI scans for any group. The results of the quantitative evaluation of the GP/Th signal intensity ratio are shown in Figure 4. No significant changes over time or between any experimental and the control group were found.

Evaluation of CSF Spaces

To examine a potential pathway for the infiltration of GBCAs into the brain, the penetration of the GBCA into the CSF was studied.

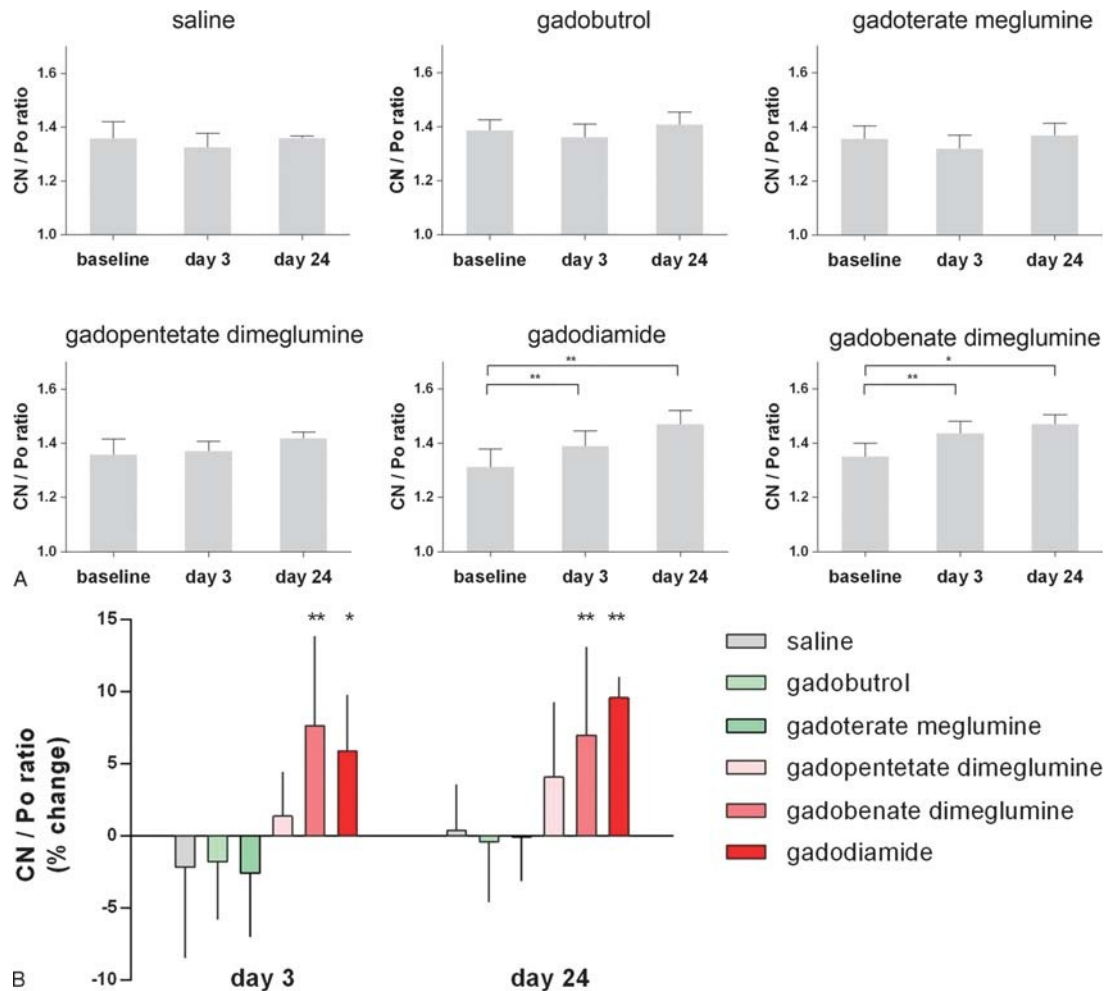


FIGURE 3. A, Change over time for signal intensity ratios between deep CN and pons (CN/Po) compared with baseline after injection of saline, gadobutrol, gadoterate meglumine, gadopentetate dimeglumine, gadobenate dimeglumine, and gadodiamide. * $P < 0.05$ and ** $P < 0.01$ indicate statistical significance of CN/Po compared with baseline. B, Percent change of CN/Po for day 3 and day 24 p.i. compared with baseline. * $P < 0.05$ and ** $P < 0.01$ indicate statistical significance of GBCA group compared with saline.

Therefore, the change of the signal intensities after GBCA administration in fluid attenuated sequences was investigated. In Figure 5 (left row), MRC images represent the CSF cavities in 1 slice of the brain, which shows the dorsal and lateral arachnoid space, the aqueduct, and the internal auditory canal. Figure 5 (middle row, precontrast) illustrates the identical slice after the CSF signal has been fully attenuated by the inversion pulse. In Figure 5 (right row, postcontrast), qualitative evaluation of the CSF cavities shows signal enhancement after administration of all GBCAs in the CSF. All 3 animals per GBCA group demonstrated signal enhancement of the CSF. In contrast, after saline injection, no signal enhancement was measured in the CSF in the rat brains.

DISCUSSION

In this preclinical study, the signal enhancement on T1w images in the CN and GP was investigated in rats. For this purpose, the signal intensity ratios CN/Po and GP/Th were evaluated after repeated administration of extended doses of GBCAs (2.5 mmol Gd/kg b.w.) with different structural and physicochemical properties up to 24 days p.i. Two macrocyclic and 3 linear agents were systematically compared in this study. The groups that were administered with macrocyclic GBCAs (gadobutrol, gadoterate meglumine) and saline exhibited no elevated signal intensities in either the CN or GP. In contrast, the application of

the linear GBCAs gadodiamide, gadobenate dimeglumine, and, to a lesser extent, gadopentetate dimeglumine resulted in increased and persistent CN/Po signal intensity ratios. Thus, the previously described differences between linear and macrocyclic GBCAs regarding signal enhancement in dentate nucleus were reproduced by comparing for the first time several commonly used GBCAs from each class.

Smaller differences between the linear GBCAs seem to exist as gadodiamide showed the most intense increase in CN/Po signal intensity ratio followed by gadobenate dimeglumine. Gadopentetate dimeglumine exhibited only a moderate increase in the CN/Po signal intensity ratio, which was statistically not significant. This is in contrast to clinical observations where patients showed significant hypersignal intensities of the dentate nucleus after serial applications of gadopentetate dimeglumine.^{7,11,12} The deviations of significantly increased signal intensity ratios in the clinical studies, and the nonsignificant increase in the present rat study may have several explanations. First of all, this study was conducted in rats. An animal model can approach, but is not able to replicate the clinical conditions in patients in all details. The rat model represented healthy animals at the same age and weight, and all animals have a similar genetic background. Conversely, in the clinical situation, the medical background of patients is diverse, and the disease process and treatment are highly variable. The second was the experimental setup, especially the high GBCA dose per injection (~4 times based

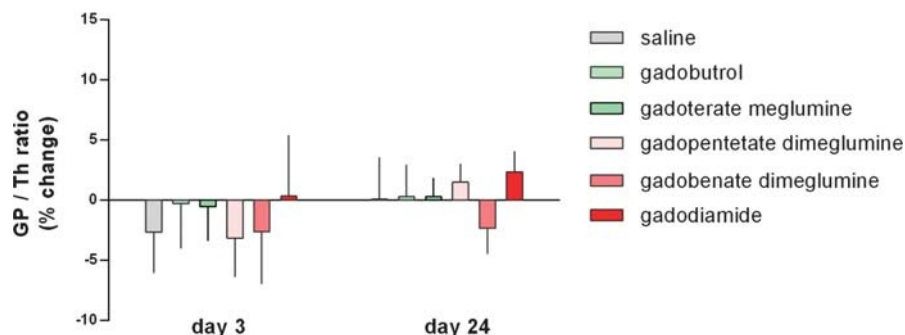


FIGURE 4. Percent change of GP/thalamus signal intensity ratio (GP/Th) for day 3 and day 24 p.i. compared with baseline.

upon body surface area, but physically 25-fold of a single human standard dose). The influence of the osmolality of the administered GBCAs might become an issue with such a dosing regime because hyperosmolar solutions were sometimes used to open the blood-brain barrier in intra-arterial injections.²³ However, in this study, a slow, intravenous injection (0.8 mL/min) was used, which should lead to sufficient dilution before the bolus reaches the brain. In this respect, it is remarkable that gadodiamide has the lowest osmolality (780 mOsm/kg, package insert) but the strongest increase in signal intensity, while gadobutrol (1603 mOsm/kg, package insert) and gadoterate meglumine (1350 mOsm/kg, package insert) have a much higher osmolality but did not evoke an increased signal intensity. In addition, the interval between the 10 injections and the time between the last injection and the MRI significantly differ from clinical studies. A possible explanation for the observed difference between gadopentetate dimeglumine and gadobenate dimeglumine might be the higher relaxivity of the latter and its weak binding to proteins resulting in stronger signal enhancement. A further important distinction between gadobenate dimeglumine and gadopentetate dimeglumine is their different pharmacokinetic profile and excretion pathway. Gadobenate dimeglumine is taken up by hepatocytes to a different extent in rats (~50%) and humans (3%–5%),²⁴ while gadopentetate dimeglumine is only excreted via the kidneys. Despite a more pronounced dual excretion pathway for gadobenate dimeglumine in rats, in this study, surprisingly higher signal intensities in the neuronal brain regions were observed for gadobenate dimeglumine than for gadopentetate dimeglumine. However, this discrepancy can only be resolved once the chemical species of the deposited gadolinium has been discovered.

The image acquisition and evaluation were performed in an analogous manner to the published clinical retrospective studies. A clinical 1.5 T MRI system was used, the whole brain was imaged with a standard T1w sequence, and the quantitative image evaluation was done using signal intensity ratios in a similar manner as described previously.^{7–11,14} A recently published study in rats by Robert et al¹⁵ focused on the cerebellum only and performed MRI on a dedicated preclinical system. In this approach, the whole deep CN (including the lateral cerebellar nucleus, which is equivalent to the dentate nucleus in humans) was used for the ROI-based analysis. In contrast to the present study, the cerebellum and not the pons was used as the reference region, which, however, resulted in an analogous difference between linear and macrocyclic agents. Interestingly, Robert et al observed a slight decrease in this signal intensity ratio for gadoterate meglumine and hyperosmolar saline, similar to the present study with slightly reduced CN/Po signal intensity ratios for gadoterate meglumine, gadobutrol, and saline on day 3 p.i. The reason for this decrease is unclear, but might be correlated to the repeated treatment under anesthesia because, in the present study, the signal intensity ratios returned to their baseline values in these groups on day

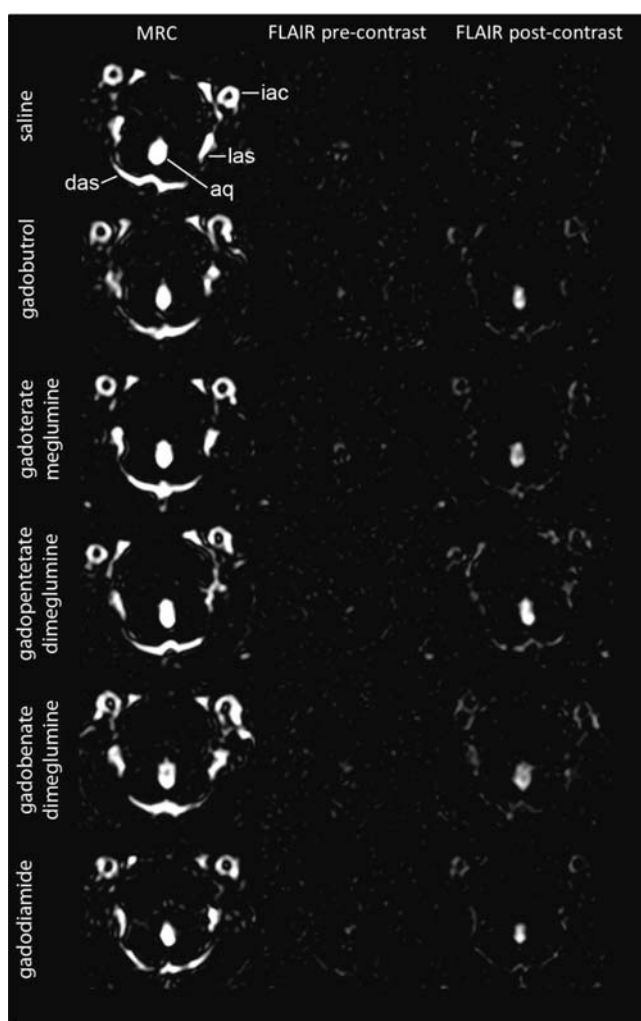


FIGURE 5. Representative transversally acquired MRCs (left column) of the rat brains at a level showing the dorsal (das) and lateral arachnoid space (las) as well as the aqueduct (aq) and inner auditory canal (iac). The middle column illustrates the respective slices acquired with FLAIR before GBCA administration. The right column represents FLAIR images acquired 1 minute p.i. of saline, gadobutrol, gadoterate meglumine, gadopentetate dimeglumine, gadobenate dimeglumine, and gadodiamide.

24 p.i. Potentially, this effect is also present in the linear GBCA groups but concealed by potential Gd deposition. For the GP, no elevated signal intensities were observed for any GBCA. The respective fluctuations of the GP/Th signal intensity ratios most likely reflect variations due to the experimental conditions. This is in contrast to clinical studies that reported a T1w signal increase for both regions (the dentate nucleus and the GP) after administration of gadodiamide and gadopentetate dimeglumine^{7,9,11} and indicates a possible limitation of the current animal model.

As the signal enhancement in the neuronal structures of the brain is prominent for linear but not for macrocyclic agents, these findings suggest that the differences could be attributed to differences in complex stabilities. It is tempting to draw parallels to the differences seen between linear and macrocyclic GBCA with respect to complex stability demonstrated in vitro and by long-term retention experiments in rats.^{6,25} However, the present study did not investigate the chemical species of Gd³⁺ in the brain, which would be necessary to learn more about the underlying mechanism. T1-weighted signal enhancement can result either by accumulation of intact GBCA or by accumulation of other chemical species, which incorporated the Gd³⁺ ion by transmetalation from the GBCA. If these not yet identified species do not precipitate in physiological environment, such as, Gd-phosphate, they may exhibit a significant r1-relaxivity and may thus also contribute to the observed signal intensity.

Although on T1w images no enhancement of CSF cavities was observed, a heavily T2-weighted FLAIR imaging sequence provided a clear enhancement of CSF cavities after the administration of all GBCAs, independently of their chemical structure. This FLAIR sequence is highly sensitive to concentrations of GBCA as low as 15.6 μ M as shown in phantoms.²⁶ Hence, the results demonstrate the presence of GBCA in the CSF at very low concentrations. An enhancement of the CSF in the internal auditory canal has been described before in patients with Ménière diseases undergoing MRI for the visualization of endolymphatic hydrops.²⁷ However, very little knowledge exists about the impact of the physicochemical properties and the structure of GBCA on the distribution and on the intensity of enhancement of various CSF cavities. Most of the imaging studies of the endolymphatic hydrops were performed after intravenous injection of gadodiamide, but some also after gadoteridol, with both GBCAs giving similar findings.²⁷ The time course of contrast enhancement in the CSF internal auditory canal was investigated in a small cohort of healthy volunteers with a maximum at 1.5 hours after administration of gadoteridol.²⁸ Hence, the observed CSF signal enhancement in the present rat study may not represent the time point of maximal enhancement. In addition, the primary location of blood CSF infiltration still remains to be elucidated. The flow of the CSF is directed from its origin of formation, the choroid plexus, to the arachnoid granules where it is returned into the venous blood system. The observed widespread distribution of the GBCAs in the CSF cavity system may be explained by this CSF flow, induced by ependymal cilia movement, by choroid plexus pulsation, and by contraction and expansion of the ventricles.²⁹ Cerebrospinal fluid is one of several compartments in the brain, which are usually well separated by highly specific barriers. Several publications reviewed by Brinker et al²⁹ address exchange mechanisms of fluids and substances between these brain compartments. However, the infiltration of GBCAs into the CSF and the connection to the observed gadolinium deposition in the brain still remain obscure.

In summary, similar to recent retrospective clinical studies, an increased and persistent T1w signal intensity increase in the CN (which contains the dentate nucleus) quantified by the CN/Po signal intensity ratio was observed after multiple extended doses of linear GBCAs in rats. However, the reported signal enhancement in T1w images in the GP quantified by the GP/Th signal intensity ratio could not be reproduced in this animal model. No elevated signal intensities in either the CN or the GP were found after administration of macrocyclic agents.

Thus, the previously described differences between linear and macrocyclic GBCAs regarding signal enhancement in the dentate nucleus were reproduced qualitatively by comparing for the first time several commonly used GBCAs from each class. A CSF signal enhancement on FLAIR images was observed for all GBCAs independent of their chemical structure. Thus, the GBCA passage from the blood into CSF might represent an initial pathway of GBCA infiltration into the brain. However, as of yet, no connection can be made between the CSF enhancement and the signal increase in the dentate nucleus and GP. Neither the underlying mechanism of GBCA exchange between different brain fluids nor the accumulation of Gd in the dentate nucleus and GP is known yet. A logical next step is the establishment of advanced analytical methods for gadolinium speciation in the dissected brain tissues. This would lead to a more detailed understanding of the observed signal enhancement and the role of the different GBCAs.

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