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Small Brain Lesion Enhancement and Gadolinium Deposition in the Rat Brain

Comparison Between Gadopiclenol and Gadobenate Dimeglumine

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Objectives: The aim of the set of studies was to compare gadopiclenol, a new high relaxivity gadolinium (Gd)–based contrast agent (GBCA) to gadobenate dimeglumine in terms of small brain lesion enhancement and Gd retention, including T1 enhancement in the cerebellum.

Materials and Methods: In a first study, T1 enhancement at 0.1 mmol/kg body weight (bw) of gadopiclenol or gadobenate dimeglumine was evaluated in a small brain lesions rat model at 2.35 T. The 2 GBCAs were injected in an alternated and cross-over manner separated by an interval of 4.4 ± 1.0 hours (minimum, 3.5 hours; maximum, 6.1 hours; n = 6). In a second study, the passage of the GBCAs into cerebrospinal fluid (CSF) was evaluated by measuring the fourth ventricle T1 enhancement in healthy rats at 4.7 T over 23 minutes after a single intravenous (IV) injection of 1.2 mmol/kg bw of gadopiclenol or gadobenate dimeglumine (n = 6/group). In a third study, Gd retention at 1 month was evaluated in healthy rats who had received 20 IV injections of 1 of the 2 GBCAs (0.6 mmol/kg bw) or a similar volume of saline (n = 10/group) over 5 weeks. T1 enhancement of the deep cerebellar nuclei (DCN) was assessed by T1-weighted magnetic resonance imaging at 2.35 T, performed before the injection and thereafter once a week up to 1 month after the last injection. Elemental Gd levels in central nervous system structures, in muscle and in plasma were determined by inductively coupled plasma mass spectrometry (ICP-MS) 1 month after the last injection.

Results: The first study in a small brain lesion rat model showed a ≈2-fold higher number of enhanced voxels in lesions with gadopiclenol compared with gadobenate dimeglumine. T1 enhancement of the fourth ventricle was observed in the first minutes after a single IV injection of gadopiclenol or gadobenate dimeglumine (study 2), resulting, in the case of gadopiclenol, in transient enhancement during the injection period of the repeated administrations study (study 3). In terms of Gd retention, T1 enhancement of the DCN was noted in the gadobenate dimeglumine group during the month after the injection period. No such enhancement of the DCN was observed in the gadopiclenol group. Gadolinium concentrations 1 month after the injection period in the gadopiclenol group were slightly increased in plasma and lower by a factor of 2 to 3 in the CNS structures and muscles, compared with gadobenate dimeglumine.

Conclusions: In the small brain lesion rat model, gadopiclenol provides significantly higher enhancement of brain lesions compared with gadobentate dimeglumine at the same dose. After repeated IV injections, as expected for a macrocyclic GBCA, Gd retention is minimalized in the case of gadopiclenol compared with gadobenate dimeglumine, resulting in no T1 hypersignal in the DCN.

Key Words: gadopiclenol, gadobenate dimeglumine, magnetic resonance imaging, gadolinium-based contrast agents, brain lesion, cerebrospinal fluid, rats, deep cerebellar nuclei

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Contrast-enhanced (CE) magnetic resonance imaging (MRI) is crucial for detecting brain lesions, including small brain metastases with disrupted blood-brain barrier (BBB). Compared with noncontrast procedures, it substantially improves the detection of additional lesions and makes it possible to distinguish nonneoplastic white matter disease from metastases.[1] One approach to improve MR sensitivity for the detection of brain metastases is to increase the dose of gadolinium (Gd)–based contrast agents (GBCAs).[2] However, given current issues raised by Health Authorities regarding the safety of GBCAs related to deposition of Gd in the body, especially linear GBCAs, another approach consists in achieving equivalent diagnostic efficacy with the use of a lower dose of macrocyclic GBCA with higher r_1 relaxivity.[3]

Gadopiclenol is a macrocyclic GBCA with 2- to 3-fold higher relaxivity than currently approved GBCAs (r_1 = 12.8 $mM^{-1}{\cdot}s^{-1}$ in human serum at 37°C and a field of 1.5 T). Interestingly, the r_1 relaxivity value does not markedly change with an increase in field strength (r_1 = 11.6 $mM^{-1}{\cdot}s^{-1}$ in human serum at 37°C and a field of 3 T).[4] Phase 1/2a and phase 2b clinical studies concluded that the product's safety and efficacy profiles were good.[5,6] Phase 3 clinical trials have just been completed.

The initial report in 2014 of hypersignals in the dentate nucleus and globus pallidus on unenhanced T1-weighted (T1w) MRI scans of patients having received multiple administrations of GBCAs[7] was confirmed by a large number of clinical and nonclinical studies.[8] Except for a few disputed studies,[9,10] the majority of the literature indicates that the effect is associated with prior administration of linear-based GBCAs,[11] but not macrocyclic agents.[8,12] These studies also challenged the old dogma that GBCAs could not cross into brain tissues when the BBB was intact.[13]

All nonclinical studies in different animal models including mice,[14,15] rats,[16–20] rabbits,[21] sheep,[22] and swine[23] concluded that Gd retention in brain was substantially higher with repeated administration of linear GBCAs compared with macrocyclic GBCAs. Comparative long-term rat studies[24,25] support the claim that the normal pathway of intact GBCA through brain tissue should not be confused with potential deposition of permanently dissociated Gd, a phenomenon demonstrated with linear GBCAs.[26]

Among all the brain compartments, numerous studies have described the passage of GBCAs into cerebrospinal fluid (CSF). In a rat study, Gd was observed in the CSF 4.5 hours after a single administration of GBCA.[27] In both nonclinical[18,27] and clinical[26,28,29] studies, GBCA-related hypersignals were found in CSF using heavily T2-weighted fluid-attenuated inversion recovery sequences. Intra-CSF distribution was found for all GBCAs with similar kinetics, and Gd was almost completely cleared from the CSF 24 hours after single administration to rats.[27] An increasingly popular hypothesis regarding GBCA brain distribution and clearance is that it occurs via the waste clearance system of the CSF, through the perivascular and interstitial spaces (the so-called glymphatic pathway).[30] It should however be noted that certain aspects of the glymphatic system hypothesis are being challenged.[31]

So far, no toxicity specifically associated with Gd retention in the central nervous system (CNS) has been reported.[8] However, the subject has been investigated by Health Authorities throughout the world. In

November 2017, the European Commission endorsed the European Medicines Agency (EMA)–recommended suspension of the marketing authorizations of most linear GBCAs in the European Union. Nevertheless, 3 linear GBCAs can still be used for specific indications (gadobenate dimeglumine and gadotexate disodium, only for MRI of the liver, and gadopentetic acid for MR arthrography after direct intra-articular administration for joint scans). The favorable benefit-risk balance of macrocyclic GBCAs has been confirmed in all their indications.[32] The US Food and Drug Administration (FDA) did not suspend any GBCA but stated that radiologists should consider the Gd retention characteristics of each agent when selecting a GBCA for patients who may be at higher risk (patients requiring multiple lifetime administrations, children, and patients with inflammatory conditions). The FDA also requested that radiologists minimize repeated CE procedures whenever possible and that market authorization holders carry out nonclinical and prospective postmarketing clinical studies.[33]

In the context of the clinical development of gadopiclenol, the aims of the present studies in rats were (*a*) to evaluate the ability of gadopiclenol to detect small brain lesions compared with gadobenate dimeglumine (study 1), (*b*) to evaluate the early kinetics of the fourth ventricle enhancement in the rat brain (study 2), and (*c*) to investigate the putative occurrence of T1 hypersignals in the deep cerebellar nuclei (DCN) and Gd retention in the brain tissues of healthy rats repeatedly treated with gadopiclenol or gadobenate dimeglumine (study 3).

MATERIALS AND METHODS

All experimental procedures and animal care were carried out in full compliance with Directive 2010/63/EU of the European Parliament and of the Council for the Protection of Animals used for scientific purposes. All experiments (GBCA administrations, MRIs, image analyses, and elemental Gd measurements) were blinded. The rats were housed at an ambiant temperature of 22 ± 2°C, with a relative humidity of 45% ± 10%, in a room with 12:12 light/dark cycles. The rats had ad libitum access to water and food.

Animal Model and Administration Protocols

The 3 studies flowcharts are shown in Figure 1.

Study 1: Detection of Small Brain Lesions

Brain lesions were induced in 6 female Sprague-Dawley rats (SPF/OFA rats; Charles River, L'Arbresle, France) by C6 glioma tumor cell implantation. The C6 glioma cells were extracted from *Rattus norvegicus* tissue (ATCC, Manassas, VA)[34] and implanted stereotaxically in anesthetized animals (mixture of 3.8 mL of ketamine, 2 mL of xylazine, and 2.2 mL of NaCl 0.9%) in the right caudate nucleus, 3.5 mm to the right of the bregma by referring to a rat brain atlas.[35,36] All the rats received the same number of cells (4×10^4 cells/μL; volume, 5 μL). To evaluate the very first brain damage after induction, the animals were imaged 2 days after C6 cell transplantation. Each rat received 2 intravenous (IV) injections of 0.1 mmol/kg bw of gadopiclenol (Guerbet, Roissy Charles de Gaulle, France; 0.5 M) and gadobenate dimeglumine (Multihance; Bracco, Milan, Italy; 0.5 M) in randomized order on the same day separated by an interval of 4.4 ± 1.0 hours (minimum, 3.5 hours; maximum, 6.1 hours). The blood elimination half-life of GBCAs being around 20 minutes in rats (for comparison, it is

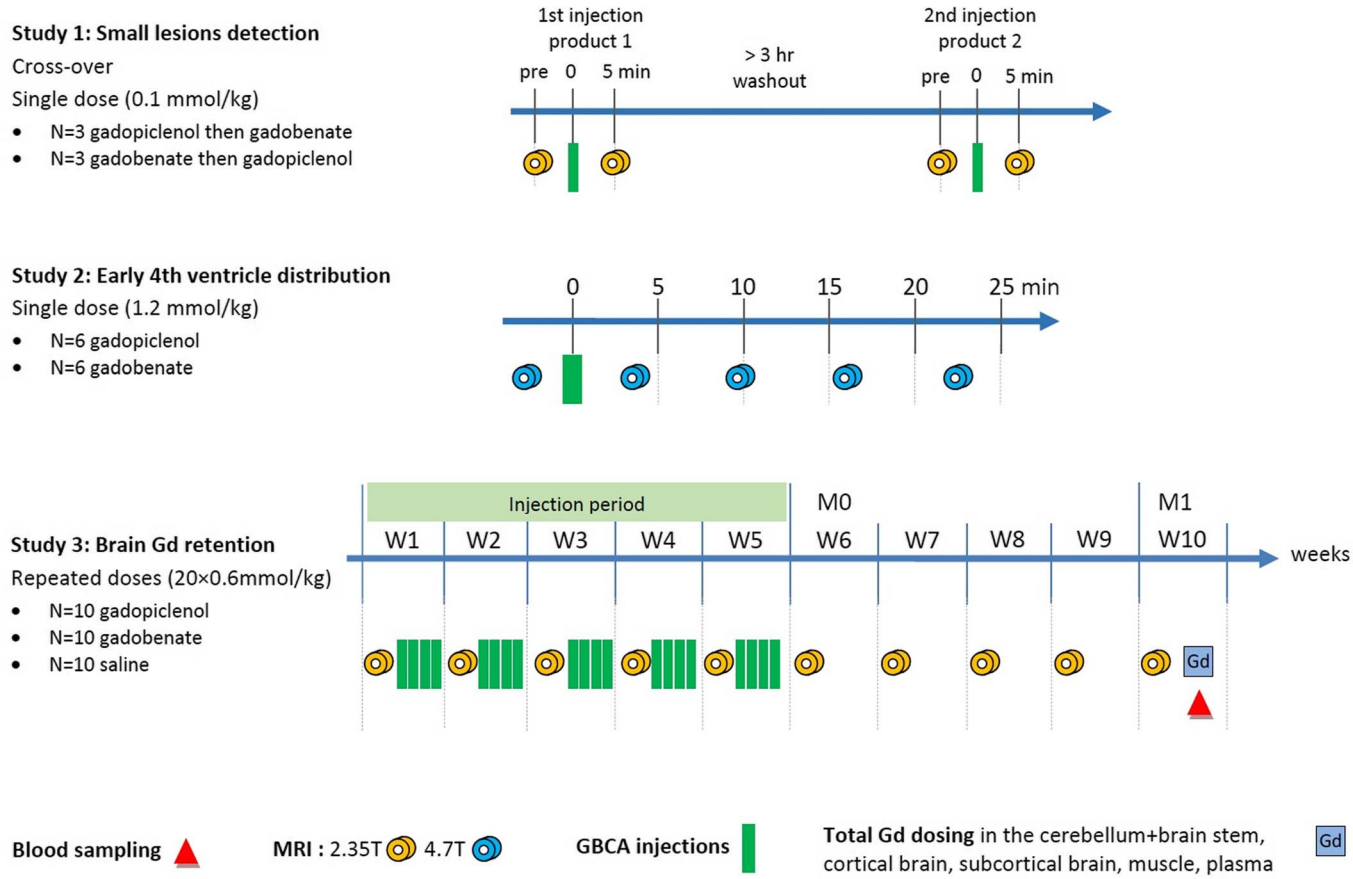

FIGURE 1. Studies flowcharts. The 2 GBCAs were compared over 3 different studies. The 2 first studies are on a short-term after a single administration, whereas the last one lasted 1 month after a period of repeated injections.

90 minutes in humans),[37] this interval of more than 7 half-lives guaranteed no contrast interference between both imaging studies due to significant remaining Gd in the blood. Brain lesion enhancement with gadopiclenol was compared with that with gadobenate dimeglumine 5 minutes after the injections.

Study 2: Investigation of Fourth Ventricle Enhancement Immediately After Injection

Twelve healthy female Sprague Dawley rats (6 per group) under anesthesia (isoflurane gas) received a single IV dose of 1.2 mmol/kg bw of gadopiclenol or gadobenate dimeglumine in the tail vein. The dose of 1.2 mmol/kg was chosen to maximize detection levels based on previous studies.[18,27] T1 enhancement of the fourth ventricle was assessed by 4.7-T MRI short-term follow-up at 3.5, 10, 16.5, and 23 minutes post-administration, and compared with images taken before injection.

Study 3: Assessment of DCN Enhancement and Gd Retention in Brain After Repeated Administrations

Thirty healthy female Sprague Dawley rats weighing 235 ± 12 g at the beginning of the study were randomized into 3 groups of 10 rats. They received 20 IV injections of 0.6 mmol/kg bw (1.2 mL/kg bw) gadopiclenol or gadobenate dimeglumine. The control group received 0.9% saline solution (CDM Lavoisier, Paris, France) (1.2 mL/kg bw). The 0.6 mmol/kg dose corresponds to the clinical dose (0.1 mmol/kg) adjusted to the body surface area of the rat according to FDA guidelines.[38] The products were administered intravenously in the tail vein once a day for 4 consecutive days a week for 5 weeks under isoflurane anesthesia (IsoFlo; Axience, Pantin, France), as described earlier.[16] After the last MRI examination, all the animals were euthanized by exsanguination under anesthesia and the cerebellum/brain stem, cerebral cortex, subcortical brain, tracheal muscle, and plasma were harvested to measure total Gd concentrations.[24]

Measurement of Elemental Gadolinium Tissue Concentrations

Elemental Gd concentrations were measured in study 3, only in biological samples, by inductively coupled plasma mass spectrometry (ICP-MS, 7700x; Agilent Technologies, Santa Clara, CA) after sample mineralization in 65% nitric acid for 8 hours at a temperature of 80°C. The lower limit of quantification (LLOQ) was 0.32 nmol/L in the nitric acid matrix, that is, 0.02 nmol/g for tissues and 0.02 nmol/mL for plasma after taking into account sample preparation. The acceptance limits (total error) were set at ±14%. Results are expressed in nmol Gd/g of wet tissue (tissue samples) or µmol Gd/L of plasma. Values inferior to the limit of detection (LD) were arbitrarily replaced by 0. Values between the LD and LLOQ were arbitrarily replaced by LLOQ/2 for calculation of the means, standard deviations (SDs), and statistical analyses.

Magnetic Resonance Imaging Protocols

The animals were anesthetized with isoflurane. Breathing and body temperature were continuously monitored during all the experiments.

Study 1: Small Brain Lesion Detection

Magnetic resonance imaging was performed at 2.35 T (BioSpec24/40; Bruker, Ettlingen, Germany). T1 enhancement of brain lesions was assessed before and 5 minutes after injection with a 2-dimensional spin echo sequence (repetition time/echo time, 500/10 milliseconds; field of view, 40 × 40 mm^2; matrix, 192 × 192; 2 averages; acquisition time, 3 minutes 12 seconds).

Study 2: Early Fourth Ventricle Distribution

T1 enhancement of the fourth ventricle was assessed by MRI short-term follow-up at 3.5, 10, 16.5, and 23 minutes post-administration and compared with images taken before the injections. Magnetic resonance imaging was performed using a dedicated phased-array quadrature head coil on a 4.7-T preclinical magnet (BioSpec 47/40; Bruker, Ettlingen, Germany) using a T1w gradient echo sequence (repetition time/echo time, 50/1.78 milliseconds; flip angle, 60 degrees; 48 averages; in-plane resolution, 164 × 164 µm^2; slice thickness, 700 µm; acquisition time, 6 minutes 36 seconds). The scan range of the MRI sequence covered only the cerebellum (11 slices).

Study 3: Gadolinium Retention in the Brain

T1-weighted MRI was performed before the first GBCA administration and once a week during the treatment period (ie, after the 4th, 8th, 12th, 16th, and 20th injections) and for an additional 4-week treatment-free period (Fig. 1). During the administration period, the MRI examination was performed 3 days after the last administration, corresponding to a minimal 72 hours treatment-free period. Magnetic resonance imaging was performed at 2.35 T using a T1w spin echo sequence (repetition time/echo time, 525/10 milliseconds; 36 averages; in-plane resolution, 156 × 156 µm^2; slice thickness, 800 µm; acquisition time, 15 minutes 8 seconds). The scan range of the MRI sequence covered only the cerebellum (10 slices).

Magnetic Resonance Imaging Scans Postprocessing

All image analyses were performed under blinded (for groups and time points) and randomized conditions with a dedicated in-house software (Guerbet Oriented Analysis, GOA V1.6).

Qualitative Analysis (Study 3 Only)

Qualitative assessment of DCN enhancement was carried out by 3 different readers who were blinded for the rats, the groups, and time points. The readers comprised 2 researchers experienced in animal imaging and 1 neuroradiologist. A 3-point scoring scale for the DCN relative to adjacent areas was applied as described before.[16] A score of 0 was given for no DCN enhancement, 1 for doubtful enhancement,

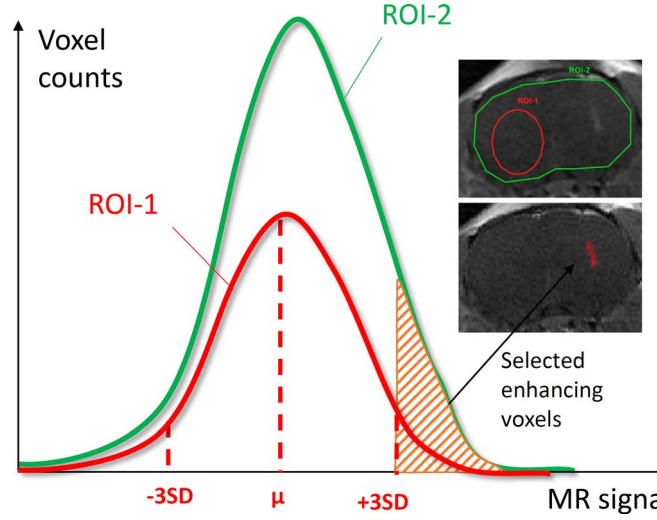

FIGURE 2. Study 1: thresholding of the enhancing voxels. The first region of interest (ROI-1) (in red) corresponds to the contralateral healthy brain and determines the mean (µ) and standard deviation (SD) of the signal in healthy tissue. Enhancing voxels (corresponding to the brain lesions) of the whole brain slice (ROI-2, in green) are selected as the voxels with an intensity superior to the mean + 3.SD.

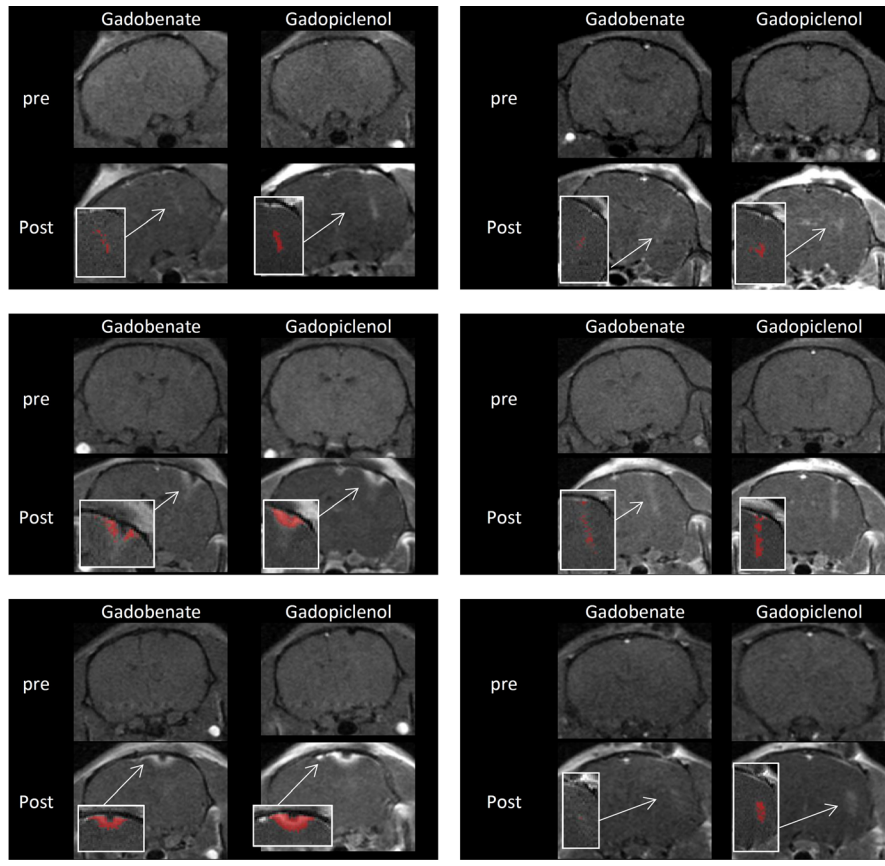

FIGURE 3. Study 1: precontrast and 5 minutes postcontrast images of the 6 studied animals, 2 days after intracerebral injection of C6 glioma tumor cells. Comparison of enhanced voxels in red (threshold of 3 × SD of contralateral nonlesion brain signal). The arrows aim at the lesion areas, based on the detection of enhanced voxels. The delay between the 2 injections (and associated images) was superior to 3 hours.

and 2 for definite enhancement. The mean score was subsequently plotted for each reader.

Quantitative Analysis

For study 1, evaluation of T1 contrast enhancement was performed by "3.SD" thresholding. Briefly, a region of interest (ROI) was positioned on the contralateral healthy brain to extract the mean and SD of the "normal brain." From these 2 data, animal per animal, voxels with a signal intensity (SI) superior to the mean + 3-fold the SD of the contralateral region were selected as "enhancing voxels," see Figure 2.

For study 2, enhancement was followed by positioning ROIs on the fourth ventricle and the background noise and recording the corresponding signal intensities. The enhancement was reported as a percent of SNR (signal-to-noise ratio) increase, compared with the baseline SNR (post-injection/pre-injection SNR).

For study 3, blinded quantitative analysis of the signals on randomized images was performed by positioning ROIs in different cerebellar structures: the cerebellar parenchyma, brain stem, left and right DCN, and the fourth ventricle. Signal intensity was calculated as a ratio of the most visible of the 2 DCN zones to the brain stem signal (DCN/BS ratio). Signal intensity ratio of the fourth ventricle to the cerebellar parenchyma signal (fourth ventricle/cerebellar parenchyma ratio) was followed by positioning ROIs on these both regions.

Statistical Analyses

The statistical analysis was performed using Soladis on SAS v9.3 software for study 2, and using Graphpad (GraphPad Prism,

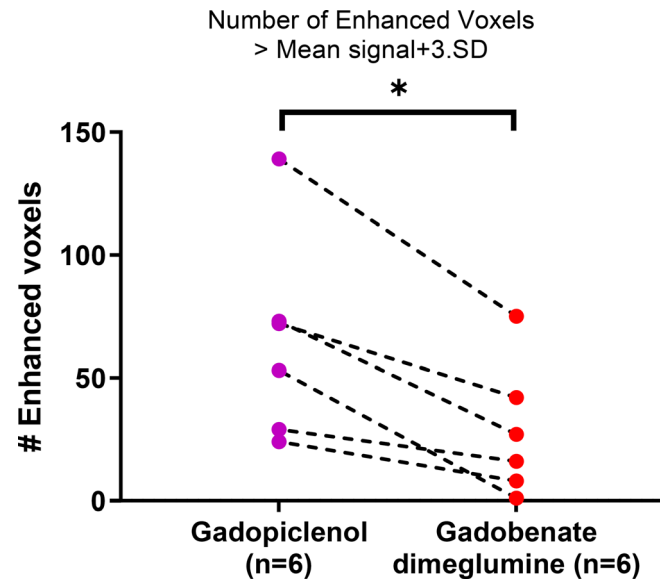

FIGURE 4. Study 1: comparison of the number of enhanced voxels in small brain lesions after gadopiclenol or gadobenate dimeglumine at 0.1 mmol/kg bw. The points of a same lesion are connected by a discontinued line. Statistics: asterisk corresponds to $P < 0.05$ (Wilcoxon test result: $P = 0.0313$). Mean voxels: gadopiclenol 65 ± 42 vs gadobenate dimeglumine, 28 ± 27 voxels.

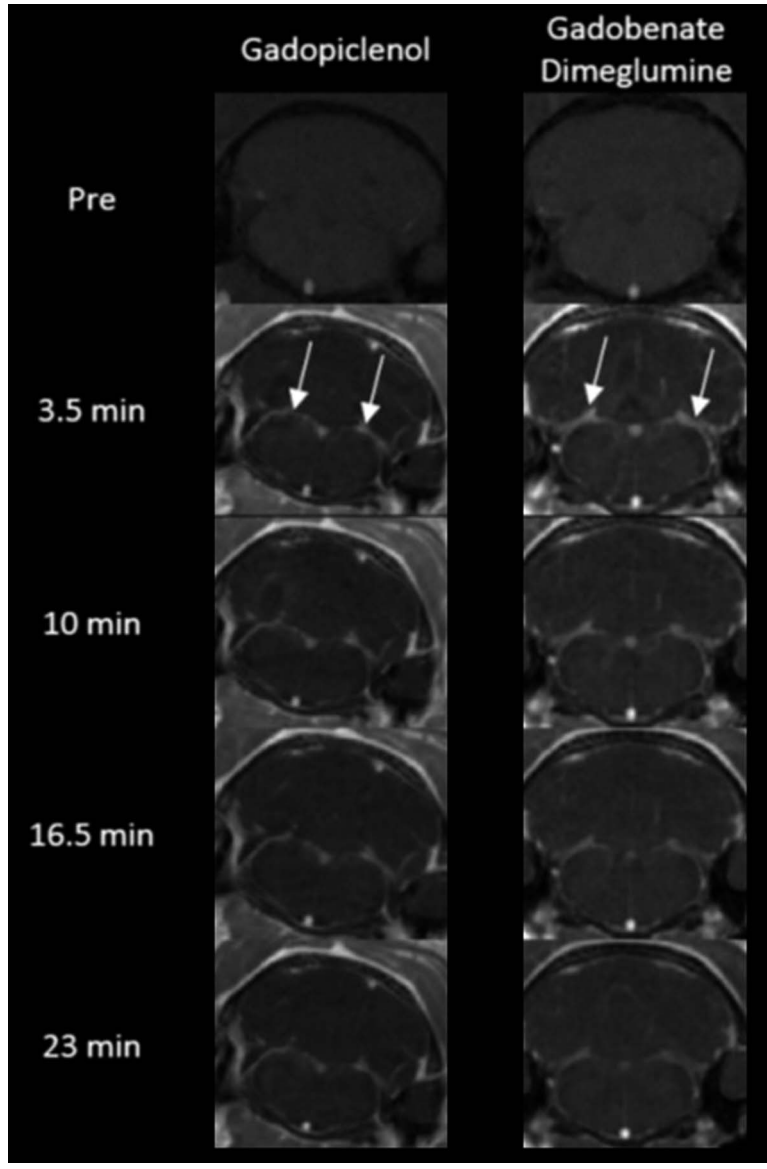

FIGURE 5. Study 2: example of early fourth ventricle enhancement (arrows) after injection of gadopiclenol or gadobenate dimeglumine at 1.2 mmol/kg bw.

v8.0.2; GraphPad Software Inc, San Diego, CA) for studies 1 and 3. Only the significant differences between gadopiclenol and the other test compounds are cited in the graphs. An arbitrary significance level of 5% was adopted.

The Shapiro-Wilk ($n < 50$) or Kolmogorov-Smirnov ($n > 50$) tests were used to examine normality, and homogeneity of variance was tested using Bartlett's test. If homogeneity of variance was not met across the groups, heterogeneity was taken into account in the model.

Magnetic Resonance Imaging Follow-up

For study 1, small brain lesion enhancement was compared using a nonparameteric test (Wilcoxson test). For study 3, a mixed model was built with 2 factors (contrast product and week) and the interaction between these 2 factors (contrast product × week). In case of nonnormality of model residuals, rank transformation of data were performed. If the interaction (contrast product × week) had no significant effect, it was removed from the model, and if a significant contrast product effect was found, pairwise comparisons were performed between groups for all weeks confused, with Tukey adjustment on the generated *P* values. In the case of a significant interaction (contrast product × week), all product pairwise comparisons were performed by week with Tukey adjustment on the generated *P* values.

Gadolinium Concentrations

Outliers were highlighted with Dixon's test and an α level of 5%, and the decision to exclude or keep them was made based on experience. In the case of assumption of normality acceptance, an analysis of variance with 1 factor (contrast product) was performed. If significant, multiple pairwise comparisons were performed with Tukey adjustment on the generated *P* values. In the case of normality rejection, a Kruskal-Wallis test was performed to compare products. In case of significant contrast product effect, pairwise comparisons were performed

with nonparametric Wilcoxon signed rank tests on all contrast products with no adjustment of the calculated *P* values.

RESULTS

Small Brain Lesion Detection (Study 1)

In study 1, all rats completed the whole protocol. Enhancing lesions in the right hemisphere were observed in all animals (Fig. 3). Some lesions were located more peripherally at the level of the brain cortex and some deaper in the parenchyma. After image postprocessing with 3 × SD thresholding, significantly more enhancing voxels (≈2-fold) were detected with gadopiclenol than with gadobenate dimeglumine (65 ± 42 vs 28 ± 27 for gadopiclenol and gadobenate dimeglumine, respectively; $P = 0.0313$, Fig. 4).

Evaluation of Early Fourth Ventricle Enhancement (Study 2)

One rat in the gadobenate dimeglumine group was excluded from analysis due to 5-fold less enhancement than others. A Dixon test on muscle enhancement concluded in an outlier, maybe due to a badly performed injection. Fourth ventricle enhancement was observed with both GBCAs and was strongest at the first time point, 3 minutes after injection, decreasing slowly thereafter (Figs. 5, 6). Baseline values had still not been reached at 23 minutes, the last studied time point. Irrespective of the time point, enhancement in the gadopiclenol group was stronger (nearly doubled) than in the gadobenate dimeglumine group ($P < 0.01$ at 3.5–10 and 16.5 minutes, and $P < 0.05$ at 23 minute postinjection).

Assessment of T1 Enhancement in the Cerebellum (Study 3)

Two rats in the gadopiclenol group and 3 in the gadobenate group died due to anesthesia issues during the injections or MRI examinations. No deaths were attributed to the administration of the GBCAs. Example of images are given in Figure 7. Progressive T1 enhancement of the signal in the fourth ventricle was observed in the gadopiclenol group, starting with the MRI performed after 8 injections (week 3).

The enhancement increased with the number of injections during the administration period, reaching a peak at week 6. This increased signal in the fourth ventricle was significantly different from the observations in the saline and gadobenate dimeglumine groups between weeks 3 and 7 (Fig. 8C, $P < 0.001$). During the washout period (from week 7), enhancement started to slowly decrease, while remaining significantly higher than the saline group only in weeks 8 and 9. In week 10 (1 month after the administration period), the signal values in the gadopiclenol group had reached the baseline values in the gadobenate dimeglumine and control groups.

Increased SI was observed in the DCN after the administration period only in the gadobenate dimeglumine group, and not in the gadopiclenol or in saline groups, as shown qualitatively (Fig. 8A). Quantitatively, the DCN/BS signal ratio was significantly higher in the gadobenate dimeglumine group compared with the saline group at weeks 7, 9, and 10, and significantly higher compared with the gadopiclenol group at weeks 6 and 8 to 10 (Fig. 8B).

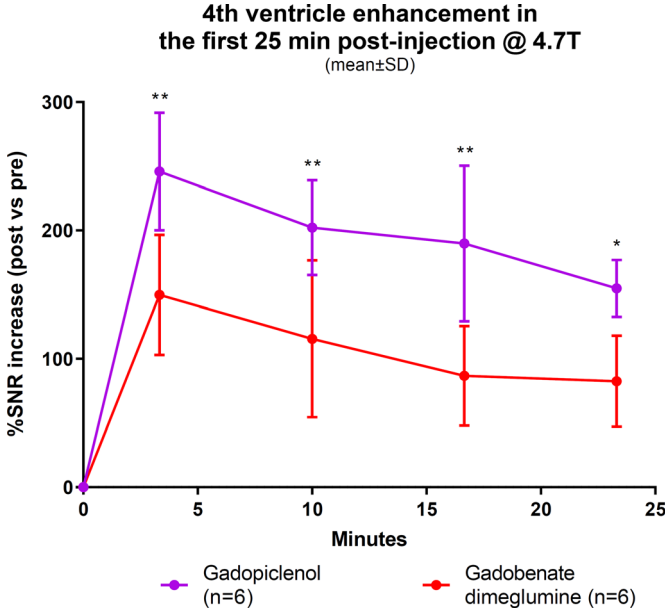

FIGURE 6. Study 2: mean (±SD) of the percentage of the fourth ventricle SNR (signal-to-noise ratio) increase (post-pre)/pre × 100 during the first 25 minutes after injection of 1.2 mmol/kg bw of gadopiclenol or gadobenate dimeglumine (n = 6 rats/GBCA).

Determination of Gd Concentrations at 1 Month Post Repeated Injections (Study 3)

In the saline group, a small amount of Gd was detected in the cerebellum (0.026 ± 0.014 nmol/g), the cortical brain (0.049 ± 0.053 nmol/g), subcortical brain (0.042 ± 0.044 nmol/g), in muscle (0.066 ± 0.071 nmol/g), and in plasma (0.008 ± 0.007 nmol/mL), demonstrating minor Gd contamination of the samples (Fig. 9). Among the 2 GBCA groups, significantly higher Gd concentrations were observed with gadobenate dimeglumine compared with gadopiclenol in the tissues of the cerebellum (1.11 ± 0.15 nmol/g and 0.61 ± 0.61 nmol/g, respectively, $P = 0.0177$), the cortical brain (1.11 ± 0.10 nmol/g and 0.32 ± 0.17 nmol/g, respectively, $P = 0.0015$), the subcortical brain (1.18 ± 0.15 nmol/g and 0.36 ± 0.14 nmol/g, respectively, $P = 0.0015$), and in muscle (2.18 ± 0.53 nmol/g and 1.29 ± 0.57 nmol/g, respectively, $P < 0.0001$). In plasma, Gd concentrations in the gadopiclenol group were significantly higher than those of the contaminated saline group (gadopiclenol vs saline: 0.025 ± 0.015 nmol/mL and 0.008 ± 0.007 nmol/mL, respectively, $P = 0.0126$); no significant difference was observed between the 2 GBCA groups (gadobenate dimeglumine: 0.015 ± 0.006 nmol/mL, $P = 0.0812$).

DISCUSSION

Gadopiclenol is a new high relaxivity macrocyclic GBCA. Preclinical studies have shown that this new macrocyclic chelate injected at the same dose as currently approved GBCAs improves the contrast-to-noise ratio in tumors by a factor of 2 to 3, as demonstrated in rat models of liver tumors[39] or brain gliomas.[40] Phase 3 clinical trials have just been completed. In these trials, gadopiclenol injected at half dose (0.05 mmol/kg) was compared with gadobutrol at 0.1 mmol/kg for the visualization and detection of lesions in the CNS and in other body areas (results not yet published).

Gadobenate dimeglumine is currently the approved GBCA with the highest relaxivity in biological medium.[4] For this reason, gadobenate dimeglumine was the chosen comparator in the phase 2 clinical trial of gadopiclenol,[6] as well as in the present studies. Blood half-life of elimination is comparable between gadopiclenol and gadobenate dimeglumine (around 20 minutes in rat), despite the bimodal excretion profile (renal and hepatobiliary) of the latter.[41]

In the study 1, the model used was induced by transcranial injection of tumoral cells. The C6-glioma model is known to produce large brain tumors in 12 to 13 days after cell implantation.[40] In this study, we performed the MRI study very shortly after cell implantation (day 2) to simulate the detection of small brain lesions. The comparison of the number of voxels with a signal superior to the mean signal+3.SD was

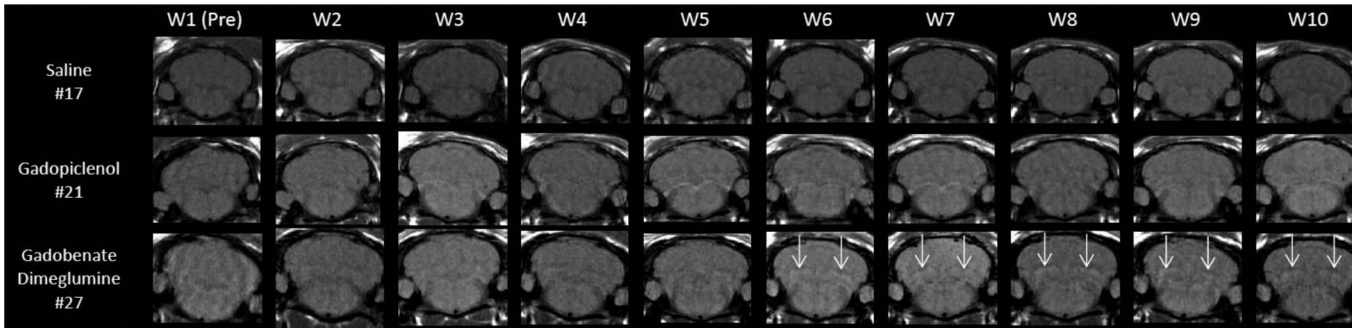

FIGURE 7. Study 3: MRI follow-up at 2.35 T of 3 animals, each one being representative of its group (identification number #17 for saline, #21 for gadopiclenol, #27 for gadobenate dimeglumine). W indicates week, W1 being the preinjection image. The arrows are pointing at the appearing T1 enhancement of the DCN, in the rat of the gadobenate dimeglumine group, from week 6.

chosen to identify the most enhancing voxels (beyond the 99.7% of the normal distribution). With this approach, we observed that gadopiclenol improved the depiction of small brain lesions, with approximately 2-fold more enhancing voxels compared with gadobenate dimeglumine. As no enhancement is expected in absence of BBB disruption (healthy brain), the enhanced voxels observed in this study should be attributed to abnormal vascular permeability. Clinically, this may be translated to a better brain lesions detectability. This has been confirmed in a C6 glioma rat-bearing model, where the higher enhancement of lesions after an injection of gadopiclenol at 0.1 mmol/kg resulted to a diagnostic preference (regarding border delineation and tumor morphology) of the 2 radiologist readers, compared with gadobenate injected at the same dose.[40] Phase 2b clinical trials also reported an overall preferred diagnostic preference for gadopiclenol compared with gadobenate at a same dose of 0.1 mmol/kg.[6] Further studies on small lesions (eg, small brain metastasis) would be however needed to confirm this.

The purpose of the study 2 was to document and compare the increase in T1 SI in the fourth ventricle shortly after single administration of gadopiclenol or of an ionic and linear GBCA, gadobenate dimeglumine. A dose of 1.2 mmol/kg was chosen to maximize the detection level based on previous studies.[18,27] A significant signal increase was observed with both GBCAs from the first time point (3.5 minutes after administration), which slowly decreased thereafter but which was still enhanced compared with baseline values 23 minutes after injection. Our data are consistent with the short-lasting filling of the ventricles reported by others.[18,27] It was observed with the 2 GBCAs tested, which is also consistent with the literature. However, enhancement in the fourth ventricle was almost doubled in the case of gadopiclenol compared with gadobenate dimeglumine. We hypothesize that the Gd responsible for the enhancement in the fourth ventricle corresponds to circulating Gd in blood passing by the choroid plexus. We assume that gadobenate dimeglumine bimodal excretion profile in rat should not have any significant impact on the access and elimination of Gd into the plexus choroid and CSF, because the blood half-lives between the 2 GBCAs are similar. However, its binding to proteins might indeed reduce its access to CSF or extend its stay in the plexus, as in humans. The proportion of gadobenate dimeglumine bound to blood protein is described to be low (7%–18% in rat[42]), so this cannot be the principal explanation to the observed difference, which is by a factor of 2. This double enhancement in the first minutes can be explained by the more than doubled relaxivity of the gadopiclenol molecule,[4] even if the signal to Gd concentration relationship in MRI is known not to be linear.[4,43]

In study 3, assessment of signal enhancement in the fourth ventricle showed a significant increase from week 3 (after 8 injections) in the gadopiclenol group, compared with the saline and gadobenate dimeglumine groups. This signal enhancement increased until the end of the injections and started decreasing during the washout period, reaching the baseline at week 10. The fourth ventricle comprises the choroid plexus and CSF. As mentioned earlier, GBCAs pass into the CSF after injection and are generally cleared from it in the following hours as suggested by the signal decrease in study 2. Interpreting the 2 studies together, it seems that, after injection of gadopiclenol, enhancement of the fourth ventricle does not return to the baseline signal value before the second injection 24 hours later. The follow-up should have been longer than 23 minutes to confirm this. The mechanism of passage of GBCAs into the CSF is unknown as small hydrophilic molecules are not supposed to cross the choroid plexus. However, it cannot be excluded that the tridimensional conformation of the gadopiclenol molecule may lead to specific interactions at the level of choroid plexus

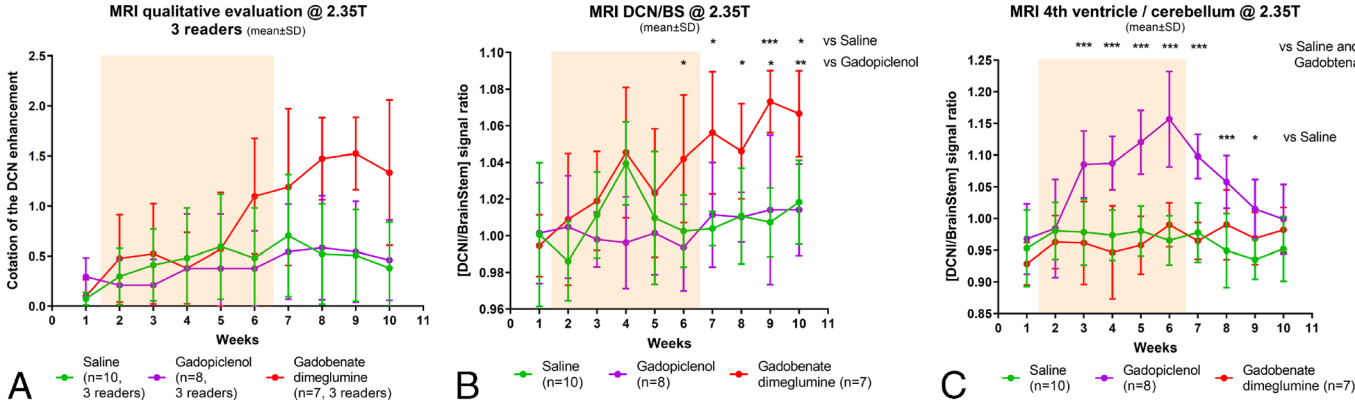

FIGURE 8. Study 3: MRI qualitative (A) and quantitative (B) analysis of the DCN hypersignal, and quantitative analysis of the fourth ventricle enhancement (C), over the injection period and the following month (statistics: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$).

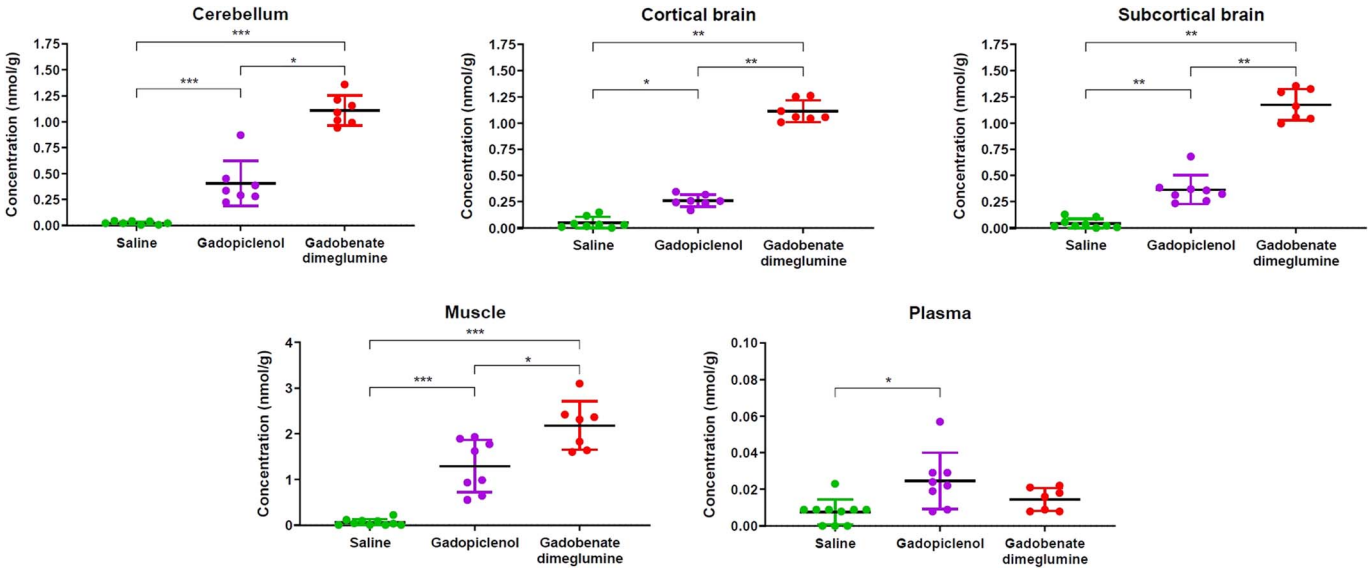

FIGURE 9. Study 2: total gadolinium concentration measured 1 month after the last injection in the cerebellum, cortical and subcortical brain, muscle, and plasma. Outliers corresponding to one systematically contaminated saline rat were withdrawn for all matrices (statistics: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$).

due to pyridine cycle planarity and hydrogen bonding of the alcohol groups. In this way, with gadopiclenol, the new baseline signal increased with each new injection during the week, thereby increasing enhancement throughout the injection period. Furthermore, the 3 days' washout period between 2 weeks of administration after the 4 cumulated injections of each week was not enough time for the signal to return to the baseline value. However, the fourth ventricle enhancement, probably due to GBCA trapping in the choroid plexus, is a reversible phenomenon. Once the injections stopped, enhancement slowly decreased, disappearing after a month. Gadolinium clearance from the choroid plexus could explain the higher Gd concentrations found in the plasma compared with the gadobenate dimeglumine group 1 month after the injection period. It can also be hypothesized that the transient increased enhancement of the fourth ventricle may have been potentialized by the repeated injection administration protocol.

With an administration protocol widely used in the literature,[16,17,19,44] study 3 results showed no T1 signal enhancement of the DCN after repeated administration of gadopiclenol (12 mmol/kg bw cumulated) regardless of the measurement approach (semiquantitative, blinded scoring, or quantitative DCN/BS ratio follow-up). Conversely, gadobenate dimeglumine, a linear GBCA, was associated with gradual enhancement of the T1 signal of the DCN. It should be underlined, however, that signal enhancement in the DCN in the gadobenate dimeglumine group appeared (quantitatively and qualitatively) only after the 20 injections. In another study[17] performed under similar conditions with gadodiamide, DCN enhancement appeared from 8 injections. Obviously, the amount of accumulated Gd might principally be responsible for that difference. However, it has also been proposed[17] that the effect may partly be related to a progressive change of the Gd species, from a low-relaxivity species (the GBCA itself) to a high-relaxivity molecule (eg, Gd bound to a soluble macromolecule, as has already been reported[24,45,46]). In fact, the phenomenon is likely similar for both linear GBCAs (gadodiamide and gadobenate dimeglumine), but because of its better dissociation kinetics in a biological medium compared with the nonionic GBCA gadodiamide,[47] it may be possible that gadobenate dimeglumine dissociates more slowly in the DCN. Conversely, no such effect was observed after repeated administrations of the macrocyclic GBCA gadopiclenol. This might be due to its excellent kinetic inertness.[4] Because all rat studies published so far[16–20] are consistent with available clinical data, it may reasonably be considered that the probability of a T1 hypersignal in the dentate nucleus of patients after repeated treatment with gadopiclenol is very low, despite its high intrinsic relaxivity. High kinetic inertness of marketed macrocyclic GBCAs is related to the high conformational rigidity of their ligands.[48] In the case of gadopiclenol, the presence of substituted arms in the ligand structure is likely to further enhance this rigidity.[4]

In study 3, total (elemental) Gd concentrations in various tissues, including plasma, were measured at study completion, that is, after 4 weeks of washout. The Gd concentrations measured in the cerebellum + brainstem, the cortical and subcortical telecephalon, and the muscle samples were significantly lower in the gadopiclenol group than those measured in the gadobenate dimeglumine group. Elemental Gd concentrations were, however, quantifiable with gadopiclenol (0.61 ± 0.60 nmol/g in the cerebellum + brain stem). It should be noted that circulating Gd was still measurable in the 2 GBCAs groups at week 10 (as well as, albeit to a lesser extent, in the saline group, indicating minor contamination). This can be explained by the ongoing washout process that takes weeks to months to be completed.[24] It should be stressed that inductively coupled plasma mass spectrometry provides no information on the chemical form of the element.[49] Therefore, no conclusion can be reached with regards to the Gd species measured in the various tissues studied. In vivo gradual dissociation of linear but not macrocyclic GBCAs has been reported in numerous nonclinical studies.[24,45–47,50–52] Lastly, GBCAs are distributed in the brain and cleared via the so-called glymphatic pathway or intramural periarterial drainage pathways to the lymph nodes,[30,53] regardless their molecular structure. This distribution/clearance is natural and must be distinguished from Gd deposition in tissues.[26]

Our studies present several limitations. Gadobenate dimeglumine is known to have a high hepatobiliary elimination in rat (fecal elimination approximately 30%) as compared with human (fecal elimination approximately 2%–4%). However, considering that the blood elimination kinetics is comparable between the 2 compounds, we hypothesize that this feature does not have a significant impact on the study. In study 1, no histopathological analysis was performed to characterize the nature and the morphometry of the brain lesions. It is very likely that the enhancing region was due to BBB disruption caused by the surgery (inflammation,

etc) and the beginning of the tumoral development process. For the basic objectives of this study, given that we were looking for BBB disruption, we considered that it was not critical to determine the nature of the lesions. In study 2, the follow-up of the fourth ventricle after a single injection should have been longer to describe the time required by both GBCAs for the signal to return to baseline. In study 3, the choroid plexus should have been dissected and the CSF sampled, in order to determine Gd concentrations in these compartments. Moreover, the brain stem (reference tissue for the assessment of T1 enhancement in the DCN) and the cerebellum should have been sampled separately.

In conclusion, our studies indicate that the new, high relaxivity, macrocyclic GBCA gadopiclenol provides better contrast enhancement of small brain lesions compared with gadobenate dimeglumine when used at the same dose. Gadopiclenol, like other GBCAs, passes into the CSF shortly after IV administration according to what seems to be a normal distribution pathway in the brain. Part of the gadopiclenol injected seemed to be present at the level of the choroid plexus for a longer period because of its different molecular structure, responsible for persisting but reversible enhancement of the fourth ventricle in our model of repeated administrations in a short timeframe. In terms of Gd retention in the CNS, as expected for a macrocyclic GBCA, no enhancement of the DCN signal was observed after repeated administrations of gadopiclenol. Gadopiclenol is a promising new GBCA that offers improved sensitivity for brain lesion detection while exhibiting low brain Gd retention behavior.

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