Linear Gadolinium-Based Contrast Agents Are Associated With Brain Gadolinium Retention in Healthy Rats

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SG and SL report no conflict of interest.

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Objective: The aim of this study was to evaluate Gd retention in the deep cerebellar nuclei (DCN) of linear gadolinium-based contrast agents (GBCAs) compared with a macrocyclic contrast agent.

Materials and Methods: The brain tissue retention of Gd of 3 linear GBCAs (gadobenate dimeglumine, gadopentetate dimeglumine, and gadodiamide) and a macrocyclic GBCA (gadoterate meglumine) was compared in healthy rats (n = 8 per group) that received 20 intravenous injections of 0.6 mmol Gd/kg (4 injections per week for 5 weeks). An additional control group with saline was included. T1-weighted magnetic resonance imaging was performed before injection and once a week during the 5 weeks of injections and for another 4 additional weeks after contrast period. Total gadolinium concentration was measured with inductively coupled plasma mass spectrometry. Blinded qualitative and quantitative evaluations of the T1 signal intensity in DCN were performed, as well as a statistical analysis on quantitative data.

Results: At completion of the injection period, all the linear contrast agents (gadobenate dimeglumine, gadopentetate dimeglumine, and gadodiamide) induced a significant increase in signal intensity in DCN, unlike the macrocyclic GBCA (gadoterate meglumine) or saline. The T1 hypersignal enhancement kinetic was fast for gadodiamide. Total Gd concentrations for the 3 linear GBCAs groups at week 10 were significantly higher in the cerebellum (1.21 ± 0.48, 1.67 ± 0.17, and 3.75 ± 0.18 nmol/g for gadobenate dimeglumine, gadopentetate dimeglumine, and gadodiamide, respectively) than with the gadoterate meglumine (0.27 ± 0.16 nmol/g, P < 0.05) and saline (0.09 ± 0.12 nmol/g, P < 0.05). No significant difference was observed between the macrocyclic agent and saline.

Conclusions: Repeated administrations of the linear GBCAs gadobenate, gadodiamide, and gadopentetate dimeglumine to healthy rats were associated with progressive and significant T1 signal hyperintensity in the DCN, along with Gd deposition in the cerebellum. This is in contrast with the macrocyclic GBCA gadoterate meglumine for which no effect was observed.

Key Words: dentate nucleus, deep cerebellar nuclei, magnetic resonance, cumulative doses, gadolinium contrast agent, gadodiamide, gadolaurate dimeglumine, gadopentetate dimeglumine

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GBCA gadoterate meglumine (Dotarem®) on the T1 signal in DCN (including the dentate nucleus) of rats.

MATERIALS AND METHODS

The method applied was previously described by Robert et al., with additional quantitative R1 mapping. All animal experiments were conducted in accordance with French regulations and in compliance with the European Union Directive 2010/63/EU. The studies were performed in the Guerbet research laboratories by Guerbet employees except for the qualitative MRI scan analysis, which was performed by 2 certified neuroradiologists (S.L., S.G.). Statistical analysis was performed by Soladis (Lyon, France). All experiments (injections, MRI, image analysis, and dosage) were conducted blindly. Group allocation of animals was randomized.

Animal Model and Contrast Agents Injection Protocols

All injections were performed under general anesthesia (3%–3.5% isoflurane; IsoFlo, Axience, Pantin, France). Healthy female Sprague Dawley rats (SPF/OFA female rats from Charles River, L’Arbresle, France), weighing 227.7 ± 17.8 g at the beginning of the study, received 20 intravenous injections of 0.6 mmol Gd/kg (1.2 mL/kg) over a period of 5 weeks (4 daily and sequential injections per week). The daily dose of 0.6 mmol Gd/kg is equivalent to the usual human dose of GBCA (0.1 mmol Gd/kg) after adjusting for body surface area as recommended by the Food and Drug Administration. For the comparison of the contrast agents with the reference injection protocol (Fig. 1A),

FIGURE 1. Injection and MRI schemes. A, Comparative study with 5 groups of n = 8 rats and 4 injections of 0.6 mmol of Gd per kilogram per week over a period of 5 weeks. B and C, Simplified injection protocols (n = 4 gadodiamide-treated rats/group): 2 injections of 1.2 mmol of Gd per kilogram per week for 5 weeks (B) and 1 injection of 2.4 mmol of Gd per kilogram per week for 5 weeks (C).

FIGURE 2. Region of interest positioning.
FIGURE 3. A–E, Representative T1-weighted MRI follow-up. A, Representative T1-weighted MRI follow-up in the gadodiamide group (rat 18) revealed significant T1 signal hyperintensity in the DCN from week 4. Arrows on positive DCN are shown only in the cases where consensus on scoring 2 was reached between the 2 readers. B, Representative T1-weighted MRI follow-up in the gadobenate dimeglumine group (rat 39) revealed significant T1 signal hyperintensity in the DCN from week 4. Arrows on positive DCN are shown only in the cases where consensus on scoring 2 was reached between the 2 readers. C, Representative T1-weighted MRI follow-up in the gadopentetate dimeglumine group (rat 25) revealed significant T1 signal hyperintensity in the DCN from week 7. Arrows on positive DCN are shown only in the cases where consensus on scoring 2 was reached between the 2 readers. D, Representative T1-weighted MRI follow-up in the gadoterate meglumine group (rat 5) did not reveal any T1 signal increase in DCN. E, Representative T1-weighted MRI follow-up in the control group (saline, rat 15) did not reveal any T1 signal increase in DCN.
5 groups (8 rats per group) were used: gadodiamide (linear and non-ionic GBCA, Omniscan, 500 mM; GE Healthcare, Chalfont St Giles, United Kingdom, batch 12538837), gadopentetate dimeglumine (linear and ionic GBCA, Magnevist, 500 mM; Bayer Healthcare (linear and ionic GBCA, Magnesit, 500 mM; Bayer Healthcare, Berlin, Germany, batches 33150F and 32553C), gadobenate dimeglumine (linear and ionic GBCA, MultiHance, 500 mM; Bracco Imaging, Milan, Italy, batch S4P267B), gadoterate meglumine (macrocyclic and ionic GBCA, Dotarem, 500 mM; Guerbet, Roissy CDG, France, batch 14G05D7A), and saline as a control group (300 mOsm/kg H2O). A behavioral examination was performed daily. In addition, 2 simplified gadodiamide injection protocols were evaluated with n = 4 rats per group: 2 injections of 1.2 mmol of Gd per kilogram per week were performed for 5 weeks (Fig. 1B) and 1 injection of 2.4 mmol of Gd per kilogram of bodyweight per week was performed for 5 weeks (Fig. 1C).

**MRI Protocol**

T1-weighted MRI was performed under general anesthesia (3%–3.5% isoflurane) before the first GBCA administration and once a week during the treatment period (ie, after the fourth, eighth, 12th, 16th, and 20th injections) and for an additional 4-week treatment-free period (Fig. 1). During the administration period, MRI examination was performed 3 days after the last administration, corresponding to a minimal 72-hour clearance period. Magnetic resonance imaging was performed with a dedicated quadrature brain coil (RAPID Biomedical GmbH, Rimpar, Germany) on a 2.35-T preclinical magnet (BioSpec 24/40; Bruker, Ettlingen, Germany) using a T1-weighted multi spin echo RARE sequence: TR/TE = 525 ms/10 ms; 36 averages; in-plane resolution, 156 × 156 μm²; slice thickness, 800 μm; acquisition time, 15 minutes 8 seconds. The scan range of the MRI sequence covered the cerebellum only (10 slices).

R1 mapping on the slice containing the DCN was added during the final MRI examination. Spin echo imaging TR/TE = 2000 ms/57.2 ms; TI = 0, 200, 400, 700, 1000, 2000 ms; 3 averages; in-plane resolution, 153 × 195 μm²; slice thickness, 1000 μm; multi spin echo RARE factor 8.

After the final MRI examination, all animals were killed by exsanguination under anesthesia, and the brains were harvested to determine total gadolinium.

**Image Analysis**

All image analysis was performed under blinded and randomized conditions. Qualitative and quantitative evaluations of DCN T1 signal intensity were performed.

**Quantitative Analysis**

Randomized qualitative evaluation of DCN enhancement was performed by 2 independent and board-certified neuroradiologists (S.L. and S.G.) with no conflict of interest. Readers were blinded to both the group and the time points. Variable window and level settings were used when reviewing MRI scans. A 3-point scoring scale of DCN relative to adjacent cerebellar cortex high signal intensity was applied: score = 0 for no detectable signal enhancement, score = 1 for doubtful enhancement, and score = 2 for definite enhancement. The mean score per group was then plotted for each reader.

**Quantitative Analysis**

Randomized quantitative evaluation of the signal was performed by positioning a region of interest over the more visible of the 2 DCN zones and over a reference zone in the adjacent cerebellar cortex (Fig. 2). Region of interest positioning was performed blindly for the groups and time points. The signal intensity ratio was calculated as the ratio of the max DCN signal to the cerebellar cortex signal: DCN/cerebellar cortex ratio.

**R1 Mapping**

R1 mapping was calculated on a pixel-by-pixel basis with a homemade software coded in MATLAB (The Mathworks Inc. Natick, MA, USA) with the following formula: Mz(TI) = Mz,eq (1 − 2 ·e−TI/τ1).

**Determination of Brain and Plasma Total Gadolinium Concentrations**

Rats were euthanized under isoflurane anesthesia by exsanguination at the end of the treatment-free period. All the rat brains were removed and dissected to sample cerebellum and blood. Total Gd concentrations were measured in biological samples with inductively coupled plasma mass spectrometry (7700x, Agilent Technologies, Santa Clara, CA) after mineralization in 65% nitric acid at 80°C for 8 hours followed by dilution in water. A standard curve of inorganic Gd (0.05–100 μg/L) in 6.5% HNO3 was used by monitoring the response of the 157Gd isotope. The lower limit of quantification with our inductively coupled plasma mass spectrometry instrument is 0.32 nmol/L in HNO3 matrix, that is, 0.02 nmol/g for the cerebellum and 0.02 nmol/mL for plasma after taking into account the sample preparation. The acceptance limits (total error) were set at ±14%. Results are expressed in nanomole Gd per gram of wet tissue weight (tissue samples) or micromole Gd per liter of plasma.

**FIGURE 4.** T1-weighted MRI at week 10 (completion of the treatment-free period, all rats).
**Statistical Analysis**

Differences between the 5 contrast agents were tested at each time point using an analysis of variance model with 2 factors for the DCN/cerebellar cortex ratio. The time course of this ratio was evaluated by analyzing the potential progressive increase of the signal ratio during the injection phase and the all follow-up period by means of a regression test and a lack-of-fit test. Lastly, differences in tissue Gd content and R1 values were tested with an analysis of variance or Kruskal-Wallis test after checking the normal distribution. All analyses were performed with SAS v9.2 (SAS Institute Inc, Cary, NC). A $P$ value below 0.05 was considered to indicate significant difference.

A box plot graph was used to identify unusual data, and then a Dixon test was performed to detect outlier values.

**RESULTS**

Thirty-eight rats completed the protocol: 7 in the control and gadoterate meglumine groups and 8 in the gadopentetate dimeglumine, gadobenate dimeglumine, and gadodiamide groups. The death of the 2 remaining rats was ascribed to anesthetic complication.

**Qualitative Analysis**

Reference Injection Protocol, Comparison of the 5 Contrast Agents

Figure 3, A to E shows typical examples of brain MRIs during both the injection and the treatment-free periods for all 5 tested compounds; only the linear GBCAs induced detectable T1 hypersignal intensity in the DCN. Arrows on positive DCN are shown only in the cases where consensus on scoring 2 was reached between the 2 readers.

Figure 4 displays DCN T1-weighted MRIs for all animals upon completion of the 10-week study period. Deep cerebellar nuclei signal enhancement was obvious for all the 3 linear GBCAs, but not for the macrocyclic gadoterate meglumine and saline. Qualitatively, after a randomized reading of the 380 images (38 rats × 10 weeks) by the 2 blinded neuroradiologists, an increase in signal intensity was detected only with linear GBCAs by both readers (Fig. 5). For both readers, T1 signal hyperintensity in the gadodiamide group increased very much more than the other groups.

**FIGURE 5.** Temporal changes in qualitative blinded evaluation of the T1-weighted signal hyperintensity between the DCN area and cerebellar cortex (mean ± SD). Each box plot represents 1 week of follow-up (10 box plots per product).
rapidly, reaching its maximum value after 12 injections and remaining stable during the treatment-free period. No difference was detected between gadoterate meglumine and saline regardless of the time point. The gadopentetate dimeglumine and gadobenate dimeglumine groups had different profiles. The number of positive rats progressively increased during the treatment period and continued to grow after completion of the injection period. At week 10, nearly all the rats in the gadopentetate dimeglumine and gadobenate dimeglumine groups displayed a DCN T1 hypersignal, as did the gadodiamide group, at completion of the follow-up period.

**Simplified Injection Protocol With Gadodiamide**

Figure 6 shows the images of all rats after the 2 injections a week (A) and 1 injection a week injection protocols (B) at week 1 (preinjection), week 6 (end of the injection period), and week 10 (after the treatment-free period). All the rats were classified as positive at both weeks 6 and 10 (arrows). No quantitative analysis was performed on these images.

**Quantitative Analysis**

**DCN/Cerebellum Signal Ratio**

Rapid T1 hypersignal between DCN and the surrounding cerebellum was observed after gadodiamide administration. Enhancement after gadobenate dimeglumine or gadopentetate dimeglumine appeared more progressively during the 10 weeks of imaging as compared with gadodiamide (Fig. 7). No such enhancement was observed with gadoterate meglumine or saline, which remained at baseline levels. At week 10, the DCN/cerebellum ratio was significantly different for gadodiamide ($P = 0.003$) and gadopentetate dimeglumine ($P = 0.007$) compared with saline. For gadobenate dimeglumine, the difference versus saline was not significant ($P = 0.06$), and no difference was observed for gadoterate meglumine ($P = 0.78$) versus saline.

The progressive increase of ratio was studied by measuring the gradients during the injection phase (week 1 to 6) and during the entire follow-up period (week 1 to week 10) (Fig. 8). Significant positive slopes were observed during the follow-up period for all the linear contrast agents, as opposed to the macrocyclic gadoterate meglumine and

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**FIGURE 6.** Modified injection protocols with (A) $2 \times 1.2$ mmol/kg and (B) $1 \times 2.4$ mmol/kg gadodiamide injections per week for 5 weeks.
Brain Gd Retention After Linear GBCA Injections

The results after repeated injections of gadodiamide are consistent with previously published data and are consistent with a simplified injection protocol. No T1 hyperintensity was observed in rats treated with the macrocyclic gadoterate meglumine or saline. Half-lives of GBCAs in rat are approximately 20 minutes and 1.2 to 2 hours in healthy humans. If we consider the ratio of half-lives between humans and rats, our protocol involving 4 injections per week would correspond to 4 injections to patients in a period of 1 to 6 months. For comparison, Radbruch et al have included patients who underwent 5 to 15 MRI scans in a period of 11 to 17 weeks.

The T1 signal intensity measured after administration of gadobenate dimeglumine and gadopentetate dimeglumine progressively increased during the 10 weeks of follow-up, not only during the injection period (W1–W5) but also during the 5 subsequent treatment-free weeks (W6–W10). Two hypotheses might explain this prolonged increase of T1 contrast even after stopping the injection of linear GBCAs even though no more Gd was detected in the plasma at week 10. The first hypothesis could be that the increase in signal intensity is due to a progressive change of the Gd form, from a low-relaxivity (eg, Gd under a chelated form) to a high-relaxivity molecule (eg, dissociated Gd bound to a macromolecule). A second hypothesis is that some organs, such as bones, can be a reservoir for gadolinium and may slowly release enough metal back into extracellular space to gradually reach the brain, as suggested by Hirano and Suzuki.

At week 10, the cerebellum retention of gadolinium was significantly higher for gadodiamide as compared with that of gadoterate meglumine (P<0.05). The DCN R1 value was significantly higher for gadodiamide as compared with those of saline (P<0.01) and gadoterate meglumine (P<0.001).

Total Gadolinium Concentrations in the Cerebellum

The total gadolinium concentration in the cerebellum was 4 to 14 times higher after linear chelate injections compared with gadoterate meglumine, and 13 to 42 times higher compared with the control group (Fig. 10A). In the cerebellum, the highest concentration and percentage of injected dose (%ID) were observed with gadodiamide (3.75 ± 0.18 mmol/g, n = 7 corresponding to 0.128 ± 0.008 × 10−3 %ID/g) then the concentrations of gadopentetate dimeglumine (1.67 ± 0.17 mmol/g, n = 8 corresponding to 0.056 ± 0.005 × 10−3 %ID/g) and gadobenate dimeglumine (1.21 ± 0.48 mmol/g, n = 7 corresponding to 0.040 ± 0.015 × 10−3 %ID/g) and finally the lowest concentration for gadoterate meglumine (0.27 ± 0.16 mmol/g, n = 7 corresponding to 0.010 ± 0.005 × 10−3 %ID/g) and saline (0.09 ± 0.12 mmol/g, n = 6). The cerebellar total Gd concentration of all linear contrast agents was significantly greater than that measured in the gadoterate meglumine and saline groups. No significant difference was observed between the macrocyclic agent and saline.

Total plasma concentrations were very low and inferior to the limit of quantification for all GBCAs except for gadodiamide with a concentration of 0.04 ± 0.02 nmol/mL (Fig. 10B). Only the total plasma concentration of Gd after gadodiamide was significantly different from saline (P<0.05).

DISCUSSION

The present study reveals the presence of a T1 hypersignal in DCN of healthy rats after injection of 3 linear GBCAs: gadodiamide, gadobenate dimeglumine, and gadopentetate dimeglumine. The results after repeated injections of gadodiamide are consistent with previously published data and are consistent with a simplified injection protocol. No T1 hyperintensity was observed in rats treated with the macrocyclic gadoterate meglumine or saline. Half-lives of GBCAs in rat are approximately 20 minutes and 1.2 to 2 hours in healthy humans. If we consider the ratio of half-lives between humans and rats, our protocol involving 4 injections per week would correspond to 4 injections to patients in a period of 1 to 6 months. For comparison, Radbruch et al have included patients who underwent 5 to 15 MRI scans in a period of 11 to 17 weeks.

The T1 signal intensity measured after administration of gadobenate dimeglumine and gadopentetate dimeglumine progressively increased during the 10 weeks of follow-up, not only during the injection period (W1–W5) but also during the 5 subsequent treatment-free weeks (W6–W10). Two hypotheses might explain this prolonged increase of T1 contrast even after stopping the injection of linear GBCAs even though no more Gd was detected in the plasma at week 10. The first hypothesis could be that the increase in signal intensity is due to a progressive change of the Gd form, from a low-relaxivity (eg, Gd under a chelated form) to a high-relaxivity molecule (eg, dissociated Gd bound to a macromolecule). A second hypothesis is that some organs, such as bones, can be a reservoir for gadolinium and may slowly release enough metal back into extracellular space to gradually reach the brain, as suggested by Hirano and Suzuki.

At week 10, the cerebellum retention of gadolinium was confirmed for the linear GBCA groups, with a significantly higher gadolinium concentration (factor of 4 to 14) than that of the macrocyclic GBCA or control groups. Nevertheless, a very low but not significant (0.010 ± 0.005 × 10−3 %ID/g) quantifiable Gd concentration was measured.
after gadoterate meglumine in cerebellum compared with saline (0.27 ± 0.16 nmol/g vs 0.09 ± 0.12 nmol/g, respectively, not significant, \( P = 0.16 \)).

Previous studies performed in mice treated with a much lower dose (single intravenous administration of 0.48 mmol/kg of \(^{153}\)Gd-labeled GBCAs) have shown a rapid disappearance of Gd in the brain Gd (<limit of detection at 1 day after administration), even with macrocyclic GBCAs.\(^{20}\) The injection protocol we applied resulted in a high cumulated dose at completion of the 5 treatment weeks (ie, 12 mmol/kg). We therefore hypothesize that when a very high dose is applied to rats, remaining macrocyclic product content in tissues can be evidenced even with the high-stability GBCA gadoterate meglumine. Moreover, as recently shown by Birka et al\(^{32}\) in the case of the macrocyclic chelate gadoteridol, the GBCA can be stored during a long period while remaining chelated.

T1 signal hyperintensity was observed exclusively in the case of linear GBCAs. Both the thermodynamic stability constant (\( \log K_{\text{cond}} \) at physiological pH) and the kinetic dissociation rate must be taken into account to describe their behavior.\(^{22,28}\) It has been shown that at a pH of 1.0 and 25°C, the half-life of chelate dissociation is lower than 5 seconds for all linear GBCAs.\(^{28}\) In human serum at pH 7.4 and 37°C, the Gd release from the chelate was found to be in the descending order: gadodiamide > gadopentetate dimeglumine > gadobenate dimeglumine. For macrocyclic GBCAs, no Gd dissociation was evidenced for 15 days.\(^{22}\) The \( \log K_{\text{cond}} \) values (pH 7.4) are higher with gadopentetate dimeglumine (17.7) and gadobenate dimeglumine (18.4) than with gadodiamide (14.9).\(^{28}\) Interestingly, in our study, the rate of the T1 signal DCN/cerebellar cortex ratios increases were higher for gadodiamide than for ionic and linear GBCAs. No significant T1 hyperintensity was observed for gadoterate meglumine. Gadoterate meglumine is characterized by both high thermodynamic and kinetic stabilities.\(^{28,33}\) In the case of macrocyclic chelates, the relevance of the thermodynamic stability remains debated while that of the kinetic stability is well agreed upon.\(^{28,34}\) Our data support the assumption that the gradual T1 hyperintensity across time is associated with the gradual dechelation of the linear chelates characterized by a low kinetic stability and for which the thermodynamic component also plays a substantial role, as demonstrated by the difference between gadopentetate dimeglumine and gadobenate dimeglumine.

In this work, we performed quantitative R1 mapping to investigate the pure T1 effect of gadolinium accumulation on DCN. At week 10, R1 of DCN is clearly increased with linear contrast agents as compared with gadoterate meglumine or saline. The concentrations found in the cerebellum are in the micromolar range (eg, for gadodiamide: 3.75 ± 0.18 nmol/g ~ 3.8 \( \mu \)M assuming a tissue density of 1 g/L). If we hypothesize that the Gd dosed is mainly located in the DCN, representing only a few percentage of the total volume of cerebellum, this would correspond to a concentration higher than 10 \( \mu \)M, which is in the level of MRI sensitivity for contrast detection. In addition, with such R1 maps, if we were able to know with precision the DCN Gd concentration by any other technic, we could estimate the local relaxivities of the Gd, and therefore investigate the form in which the Gd is in the brain tissue.
The thermodynamic value of Fe-DTPA-BMA is 21.9, while it in the capillary endothelium and interstitium of A1NR Am J excretion Brain Gd Retention After Linear GBCA Injections

In the DCN of one gadodiamide-which needs highly sensitive nonclinical models to it is worth noting that Gd accumula-

tion in rats (55%) is far greater than in humans (3%–5%). Since our rats had normal renal and liver functions and since $T_1$ half-lives are similar in healthy rats for all GBCAs including gadobenate dimeglumine (~20 minutes), it is worth noting that Gd deposition still occurred with gadobenate dimeglumine despite that its substantial hepatobiliary excretion in this species.

In our study, no obvious behavioral abnormalities were detected in rats, regardless of the GBCA administered. It is likely that if Gd accumulation in the DCN has toxicological or functional consequences, these will be subtle and may occur at a later time point. Lesions of dentate nucleus (including from iatrogenic origin) are typically associated with ataxia, which needs highly sensitive nonclinical models to be detected. They may also lead to dysarthria, which, of course, cannot be explored in this experimental model. Dedicated experimental models are therefore needed for in-depth analysis of the potential deleterious consequences of Gd accumulation in the dentate nucleus.

So far, there are no available data on our animal model with respect to 5 of the marketed GBCAs: the nonionic macrocyclic GBCAs gadobutrol (Gadovist/Gadavist, Bayer) and gadotodrol (ProHance, Bracco), the nonionic linear GBCA gadoversetamide (OptiMARK, Mallinkrodt), and the ionic and linear gadoxetic acid (Eovist/Primovist, Bayer) and gadofosveset trisodium (Ablavar, Lantheus).

In conclusion, cerebellar retention of gadolinium was observed for all 3 linear contrast agents tested gadobenate dimeglumine, gadopentetate dimeglumine, and gadodiamide. The T1 hypersignal in the DCN was highly consistent and observed regardless of the injection protocol. This animal model opens new perspectives for potentially comparing all the GBCAs and to investigate the mechanism underlying the accumulation of Gd in the brain.

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