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Impact of Treatment With Chelating Agents Depends on the Stability of Administered GBCAs

A Comparative Study in Rats

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Objective: This study investigated the potential effect of the chelating agent calcium trisodium pentetate (Ca-DTPA) on the urinary excretion of gadolinium and the subsequent elimination of gadolinium (Gd) in the brain after a single intravenous administration of either a linear (gadodiamide) or a macrocyclic (gadobutrol) Gd-based contrast agent in rats.

Materials and Methods: Rats received either a single injection of gadodiamide or gadobutrol (1.8 mmol/kg, each) or saline (n = 18 per group). Seven weeks after the injection, 6 animals of each group were killed before the treatment period. From the remaining 12 animals, 6 received either 3 intravenous injections of Ca-DTPA (180 μ mol/kg) or saline. Urine was collected daily for 3 days after each infusion. Gadolinium measurements by ICP-MS were performed in urine and tissue samples.

Results: In animals that initially received the linear gadodiamide, Ca-DTPA infusion increased the urinary excretion of Gd by a factor of 10 (cumulative amount of 114 ± 21 nmol Gd vs 10 ± 4 nmol Gd after saline infusion, $P \leq 0.0001$). In contrast, animals that received the macrocyclic gadobutrol exhibited a higher spontaneous urinary excretion of Gd (33 ± 12 nmol after saline infusion) and Ca-DTPA had no impact (30 ± 11 nmol Gd, $P = 0.68$).

The urinary excretion of Gd was associated with Gd brain content. Seven weeks after the initial Gd-based contrast agent administration, a total amount of 0.74 ± 0.053 nmol Gd was quantified in the brain after administration of gadodiamide. The Gd brain burden was partially reduced at the end of the treatment period in the animals that were repeatedly infused with Ca-DTPA (0.56 ± 0.13 nmol Gd, $P = 0.009$) but not with saline (0.66 ± 0.081 nmol, $P = 0.32$). In contrast, the total amount of macrocyclic gadobutrol measured in the brain was lower (0.11 ± 0.029 nmol Gd) and still spontaneously cleared during the 3-week saline infusion period (0.057 ± 0.019 nmol Gd ($P = 0.003$)). Gadolinium quantified in the brain after infusions with Ca-DTPA did not differ from saline-infused animals (0.049 ± 0.014 nmol Gd).

Conclusions: Administration of the chelating agent Ca-DTPA 7 weeks after injection of linear gadodiamide induced relevant urinary Gd excretion. In parallel, the Gd amount in the brain tissue decreased. This indicates a dechelated pool among the chemical Gd forms present in the rat brain after linear gadodiamide administration that can be mobilized by chelation with Ca-DTPA. In contrast, Ca-DTPA did not mobilize Gd in animals that received macrocyclic gadobutrol, indicating that the Gd measured is intact gadobutrol.

Key Words: gadolinium-based contrast agents, gadobutrol, gadodiamide, gadolinium retention, chelation treatment

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Gadolinium-based contrast agents (GBCAs) have been widely used in diagnostic magnetic resonance imaging (MRI) for almost 3 decades and are pivotal for the diagnosis and monitoring of diseases.¹ In recent years, hyperintensity on unenhanced MRI scans and gadolinium (Gd) presence in some brain areas has been observed in patients with normal renal function after multiple contrast-enhanced MRI procedures.^{2–4} There is an increasing body of evidence from retrospective clinical^{5–10} and preclinical animal studies^{11,12} that MRI hyperintensity in the brain is primarily associated with repeated injections of linear GBCAs. Thus the pharmacokinetic profiles and the physicochemical properties (in particular the stability) of the different GBCA types have been revisited in detail. Based on their molecular properties, specifically their thermodynamic and kinetic stabilities, linear GBCAs are more susceptible to dechelation and release of free Gd ions than macrocyclic GBCA.^{13–15} In accordance to the known stability differences, the European Commission decided in November 2017 to suspend the marketing authorization (MA) of multipurpose linear GBCAs (gadodiamide, gadoversetamide, gadopentetate dimeglumine, and gadobenate dimeglumine).¹⁶

Preclinical animal studies have confirmed the difference in stability by analyzing the form of Gd that is present in tissue samples. The studies demonstrated that linear agents release Gd in vivo yielding insoluble Gd species and soluble species bound to macromolecules in the brain, which were not found after administration of macrocyclic GBCA and may account for the observed hyperintensities on T1-weighted MRI scans.^{17,18} The exact molecular component present in the high molecular weight species as well as the precise intracellular or extracellular location of the Gd present in the brain remains unknown.

Dechelation of GBCA may result in toxic effects caused by interference with biological processes. Unchelated Gd ions can compete with endogenous cations, for example, calcium, potentially blocking Ca-dependent channels and enzymes.¹⁹

Considerations from the clinical use of chelating agents to remove heavy metals from the body suggest that released Gd may be rechelated and eliminated in vivo.²⁰ The chelating agents calcium and zinc trisodium pentetate (Ca-DTPA or Zn-DTPA) are used as first-line treatment options after contamination of the human body by transuranic radionuclides.²¹ In addition, DTPA is the chelating ligand in the first marketed GBCA, gadopentetate.

The current study evaluated the potential effect of Ca-DTPA to mobilize and remove Gd from the rat brain and the impact thereof whether the rats received linear gadodiamide or macrocyclic gadobutrol. The study investigated the impact of Ca-DTPA treatment after a Gd-free period of 7 weeks, a time point where potentially dechelated Gd forms are present in the brain and intact chelates have been eliminated.²² This study will also provide an insight into the accessibility of retained Gd in the brain structures. The excretion and tissue content

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of the trace elements manganese (Mn) and zinc (Zn) were analyzed, because depletion of these elements is suspected.

MATERIALS AND METHODS

Contrast Agents and Chelating Agents

Commercially available GBCAs gadodiamide (Omniscan; GE Healthcare Buchler GmbH and Co KG, Braunschweig, Germany) and gadobutrol (Gadovist, Bayer Vital GmbH) and the chelating agent calcium trisodium pentetate (Ca-DTPA, Ditriventat-Heyl; Heyl GmbH and Co KG, Berlin, Germany) were used in this study.

Animal Study and Administration Protocol

Fifty-four Han-Wistar rats (CrI:WI; males; 275–325 g) were obtained from Charles River (Sulzfeld, Germany). The animals were kept under standard laboratory conditions, and the food and water were provided ad libitum. The study was approved by the Animal Welfare Administration of Berlin's State Office of Health and Social Affairs in accordance with the German Animal Protection Law, and the experiments were carried out in accordance with the approved guidelines.

Rats were randomly divided into a control (saline), a gadodiamide, and a gadobutrol group ($n = 18$ per group). The animals received one intravenous GBCA injection with a dose of 1.8 mmol Gd/kg body weight (equivalent to a triple standard dose in humans after body surface adaptation²³) or saline. Seven weeks after the administration of GBCA, the treatment regimen consisted of 3 infusions of Ca-DTPA or saline, once weekly (Fig. 1). A portion of the animals were killed before the treatment to determine the initial amount of Gd present in tissues (pre). The remaining animals were randomly divided into a Ca-DTPA subgroup ($n = 6$) and a saline control subgroup ($n = 6$) for comparison to the spontaneous rate of Gd excretion via the urine. Ca-DTPA (180 $\mu\text{mol/kg}$ in 0.9% NaCl solution) or 0.9% saline was administered to unanesthetized animals by slow infusion over a period of approximately 30 minutes through the tail vein using a pump at a flow rate of 2 mL/h. The urine was collected quantitatively for the subsequent 3 days after each single infusion by placing the animals in metabolism cages. The infusion and urine collection were performed 3 times in total, once weekly. Four days after the last infusion, animals were killed, and tissue samples were collected (post).

Tissue Processing and ICP-MS Measurements

Quantification of Gd, Zn, and Mn in urine, brain homogenates (brainstem, cerebellum, and cerebrum), bone (femur), and skin was performed by inductively coupled plasma mass spectrometry (ICP-MS Agilent 7900; Waldbronn, Germany). Samples (in triplicates, 10–20 mg) were mixed with 50 μL of 100 nM Tb-nitrate + 100 nM Co-nitrate as internal standards and dried for 2 hours at 95°C. Subsequently, 50 μL of concentrated nitric acid (65% HNO_3 , Suprapur; Merck KGaA, Darmstadt, Germany) and 20 μL of hydrogen peroxide (H_2O_2 , Emsure; Merck KGaA) were added, and samples were heated to dissolve the tissue for 2 hours at 95°C in a microwave oven (MDS 2000; CEM, Kamp-Lintfort, Germany). Quantification of Gd, Zn, and Mn was performed as nanomole per gram for all analyzed tissue. Values are reported with 2 significant digits but not more than 3 decimal places based on the precision and accuracy of the used ICP-MS method. To calculate the total amount of the element present in the brain, the brain parts (brainstem, cerebellum, and cerebrum) were weighed before homogenization. The total amount of the element present in bone and skin are extrapolated values. They were calculated based on the measured concentration (nanomole per gram) and the body weight factor of bone (estimated 15%) and skin (estimated 18%).²⁴ The body weight of the animal was determined on the day of killing.

Statistical Evaluation

Statistical comparisons were performed within GBCA groups. For the treatment factor on urinary excretion, comparisons were done with an unpaired t test. Statistical comparisons of Gd tissue concentrations between 2 time points (the pretreatment group and 2 post-treatment groups) were performed with analysis of variance followed by Dunnett post hoc test for multiple comparison. The calculations were performed with GraphPad Prism (GraphPad Software, La Jolla, CA) using a significance level of 5%.

RESULTS

The experimental design of the present study aimed to investigate the urinary excretion and organ concentration of Gd in rats after infusion of the clinically used chelating agent Ca-DTPA after a single dose of either linear gadodiamide or macrocyclic gadobutrol (Fig. 1).

The amount of Gd in the urine is the sum of the spontaneous excreted Gd and the Gd amount that is additionally excreted as a consequence of Ca-DTPA infusion. To distinguish between spontaneous

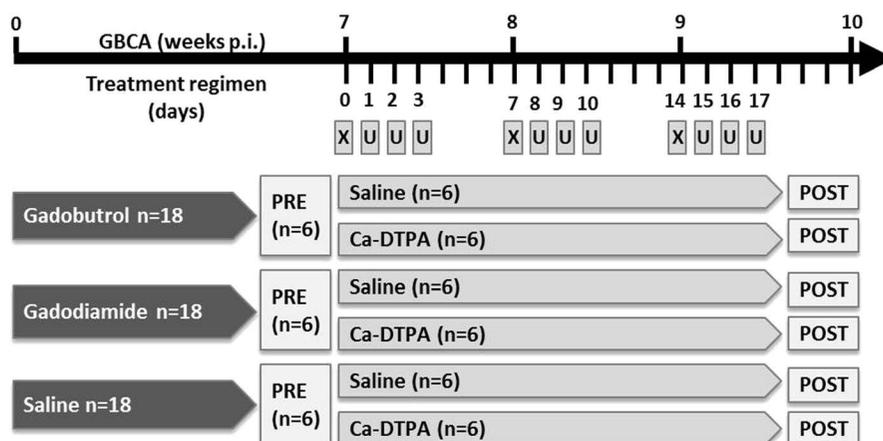


FIGURE 1. Schematic overview of the study design. Male rats were randomly allocated into 3 groups ($n = 18$ per group) and received either single injection of saline (control group) or gadodiamide or gadobutrol at a dose of 1.8 mmol/kg followed by a 7-week recovery period. One part of each group ($n = 6$) was killed before the treatment (pre), the remaining animals received either Ca-DTPA ($n = 6$) or a saline vehicle control ($n = 6$) and were killed 18 days later at the end of the treatment period (post). Ca-DTPA (180 $\mu\text{mol/kg}$) or saline were administered by slow infusion 3 times, once per week with urinary excretion monitored daily on the 3 following days. X indicates day of infusion; U, day with urine collection.

and Ca-DTPA–induced Gd excretion, we subtracted the amount of Gd determined in the saline-infusion animals from the amount determined in the Ca-DTPA–infused animals and defined the remaining amount as mobilized Gd.

The ability of Ca-DTPA to mobilize Gd was dependent on the GBCA administered whether it was linear gadodiamide or macrocyclic gadobutrol. Figure 2A shows the urinary excretion of Gd

mobilized by Ca-DTPA. The infusion of Ca-DTPA did not mobilize Gd after administration of macrocyclic gadobutrol (Fig. 2A). The amount of Gd determined in urine samples upon Ca-DTPA infusion was equal to the spontaneous Gd clearance after saline infusion at all time points analyzed (mean Gd difference of -0.14 ± 0.6 nmol Gd to saline-infused animals). In contrast, in animals that received gadodiamide, Ca-DTPA infusion induced

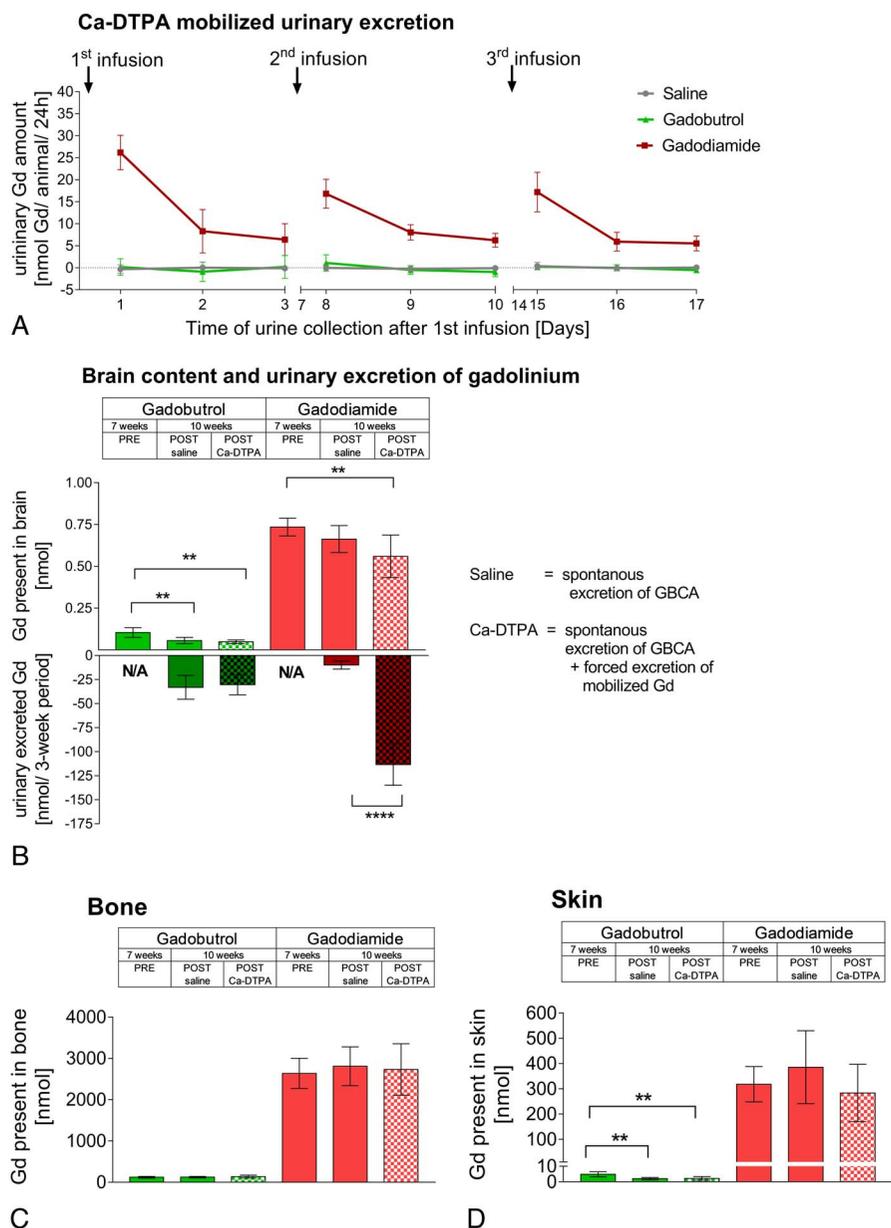


FIGURE 2. A, Time course of urinary excretion of Gd over the treatment period. Data are excreted Gd after Ca-DTPA infusion subtracted by the spontaneous excreted Gd after saline infusion. Mean amount of Gd \pm SD quantified in urine samples collected for 24 hours, $n = 6$ per group. B, Upper panel: Total Gd amount in nanomole present in the brain at the start (7 weeks post injection) and at the end of the treatment period (10 weeks post injection), $**P \leq 0.01$. Lower panel: Cumulative amount of Gd eliminated from the body by urinary excretion during the treatment period. Nine individual samples/animal, mean \pm SD, $n = 6$ per group and time point, $****P = 0.0001$. N/A indicates not applicable as urine was not collected before the start of the treatment period. C, Gd amount present in the bone at the start (7 weeks post injection) and at the end of the treatment period (10 weeks post injection). Data are total bone burden calculated on the estimation of bone as 15% of body weight. D, Gd amount present in the skin at the start (7 weeks post injection) and at the end of the treatment period (10 weeks post injection). Data are total skin burden in nanomole calculated on the estimation of skin as 18% of body weight. The amounts of Gd present in urine and tissues in animals that received saline instead of GBCA were close to or below the limit of quantification and were not included in panels B to D for simplification.

urinary Gd excretion that exceeded the spontaneous Gd urine excretion (Fig. 2A). Urine Gd excretion was increased for at least 3 consecutive days after each infusion of Ca-DTPA. The highest amount of Ca-DTPA-mobilized Gd in the urine was achieved within 24 hours after the first dose of Ca-DTPA (26 ± 4.3 nmol). The Gd-mobilization process declined after this rapid elimination, but was not completed within the observation period of 3 days as the excreted Gd amount remained elevated (6.4 ± 3.6 nmol). The second and third Ca-DTPA infusion resulted in further Gd mobilization; however, both were less efficient than the initial infusion (17 ± 3.3 and 17 ± 4.5 nmol Gd in the first 24 hours).

The difference in Gd tissue presence between linear gadodiamide and macrocyclic gadobutrol relies, in part, on the urinary excretion of Gd. In animals that received gadobutrol, a clearance of Gd from tissue/brain was still evident 7 weeks after GBCA injection, which was independent of the Ca-DTPA infusion. In saline-infused animals, the Gd brain content was reduced from 0.11 ± 0.029 nmol Gd (mean \pm SD) at 7 weeks to 0.057 ± 0.019 nmol ($P = 0.003$) at 10 weeks after the GBCA administration (Fig. 2B). Spontaneous urinary excretion of Gd was still evident in animals that received gadobutrol (cumulative amount of 33 ± 12 nmol Gd) with no impact of Ca-DTPA (30 ± 11 nmol, $P = 0.68$). In accordance, the infusion of Ca-DTPA did not induce any additional decrease of Gd brain content (0.049 ± 0.011 nmol Gd).

The Gd brain burden in gadodiamide-injected animals before the first infusion was 7-fold higher (0.74 ± 0.052 nmol Gd) compared with gadobutrol-injected animals (Fig. 2B). Moreover, the total amount of Gd measured in the brain was not significantly reduced at the end of the experiment in the gadodiamide group that received the saline infusions (0.66 ± 0.081 nmol Gd, $P = 0.32$; Fig. 2B, upper panel). This is in line with the almost complete lack of spontaneous urinary excretion of gadodiamide (Fig. 2B, lower panel). The cumulative amount of Gd recovered in the urine was 10 ± 4 nmol Gd, which is close to the background of Gd present in urine of animals that were not exposed to GBCA (5 ± 5 nmol).

Ca-DTPA infusion partially decreased the Gd amount in the brain of animals that received gadodiamide, which is in line with the Ca-DTPA-enhanced urinary Gd excretion. After 3 infusions of Ca-DTPA, the brain Gd amount was reduced to 0.56 ± 0.13 nmol ($P = 0.009$) compared with the Gd amount determined in the brain before treatment.

The total amount of Gd found in the urine (114 ± 21 nmol Gd) exceeded by far the total amount eliminated from the whole brain (~ 0.2 nmol Gd), indicating that Gd is additionally eliminated from other organs after Ca-DTPA infusion in gadodiamide animals. As bone (estimated 15% of the body weight) and skin (estimated 18% of the body weight) have a greater organ-to-body weight ratio than the brain, these organs have the potential to store larger amounts of Gd. In animals that received gadodiamide, a total amount of 2637 ± 365 nmol Gd (extrapolated value) was present in the bone at the start of the treatment period (Fig. 2C) and Ca-DTPA infusion did not significantly mobilize Gd from bone (2732 ± 625 nmol Gd, $P = 0.923$). In the skin, a total amount of 318 ± 70 nmol Gd (extrapolated value) was found at the time before treatment and 283 ± 113 nmol Gd ($P = 0.82$) after the Ca-DTPA infusion.

The Gd burden in animals that received gadobutrol was approximately 20-fold lower in the bone (120 ± 22 nmol Gd, extrapolated value) and more than 60-fold lower in the skin (4.8 ± 1.6 nmol Gd, extrapolated value) compared with gadodiamide-injected animals 7 weeks after the GBCA administration. In line with the data obtained from brain, Ca-DTPA infusion did not mobilize Gd from these organs (Fig. 2C).

The amount of Gd measured in animals that received saline instead of a GBCA were close to or below the limit of quantification for all analyzed organs and all treatment groups (data not shown).

Ca-DTPA is not specific for Gd and additionally mobilized endogenous metals. Ca-DTPA infusion mobilized 2.5 ± 0.2 μ mol Zn after each infusion (Fig. 3A). Unlike the Gd excretion, the mobilization process of Zn was terminated within 24 hours, as the urinary Zn excretion returned to a physiological level after 24 hours (mean difference of 0.06 ± 0.04 μ mol Zn after 48 hours). The efficacy of Ca-DTPA on Zn excretion was equivalent for all 3 injections, and no difference was observed between animals injected with gadodiamide, gadobutrol, or with saline. A cumulative amount of 10.0 ± 0.9 , 10.4 ± 0.8 , and 10.2 ± 0.8 μ mol Zn was excreted after 3 Ca-DTPA infusions in the saline, gadobutrol, and gadodiamide groups, which corresponds to a nearly 5-fold increase compared with the amount excreted after saline infusion.

A rapid Ca-DTPA-induced increase independent of prior GBCA administration was also evident for the urinary excretion of Mn (mean urinary amount of 0.19 ± 0.06 μ mol Mn within 24 hours for all groups; Fig. 3B). Similar to Zn, the Ca-DTPA-induced Mn mobilization was terminated within 24 hours with no difference to the spontaneous urinary excretion after 48 hours and 72 hours (mean difference, 0.02 ± 0.04 μ mol Mn and 0.01 ± 0.03 μ mol Mn). No lasting effects on the endogenous Zn and Mn levels in the brain were observed 4 days post Ca-DTPA infusion (Figs. 3C, D). This suggests either no effect or a rapid replenishment of these minerals as both Zn and Mn are minerals present in the standard diet of rats.

DISCUSSION

Based on the clinical use of Ca/Zn-trisodium pentetate (Ca/Zn-DTPA) for the treatment of heavy metal contamination,²⁵ Ca/Zn-DTPA has been recently evaluated for the treatment of patients who reported persistent symptoms not attributable to other causes and which they attributed with gadolinium presence after GBCA administration.^{26–28} To date, however, there has been no confirmation that these persistent symptoms are causally related to gadolinium presence in patients with normal renal function.

In the study by Semelka et al, the chelating agent Ca/Zn-DTPA was applied on a weekly or monthly dosing regimen and the authors reported an increased (13-fold and 30-fold) amount of Gd in urine demonstrating the presence of Gd in these patients. In our study, Ca-DTPA induced a 10-fold increase of urinary excreted Gd in rats administered linear gadodiamide but not after macrocyclic gadobutrol. Compared with our study in rats, Semelka et al reported in their study an increased Gd excretion for both GBCA classes (linears and macrocyclics) with less Gd urine content observed after macrocyclic agents than linear agents. However, the number of patients that received macrocyclic GBCA only was low (5 patients according to the text, 4 according to a table) and the authors could not exclude that these patients might have received unrecorded linear GBCA injections previously. Moreover, patients without GBCA administration were not included. Previous autopsy studies have shown that control groups with no GBCA history contain small amounts of Gd in the body,^{29,30} indicating a GBCA-unrelated background of Gd in humans. This raises the possibility that the Gd found in the urine of these patients might not have originated from macrocyclic GBCAs.

With regard to a potential effect of Ca-DTPA for rechelation of any released Gd in tissue, Ca-DTPA, like all GBCAs, is distributed mainly in extracellular fluids and has only limited capability to enter cells. As a consequence, the main compartments accessible to the Ca-DTPA chelate are the blood plasma and the extracellular space. This has become evident from a rat study that aimed to investigate chelating agent 3,4,3-LI(1,2-HOPO) and DTPA as a treatment for Gd accumulation.³¹ In this study, the body burden of Gd originated from Gd salts (Gd citrate) and not from the administration of chelated GBCA. The distribution of Gd citrate completely differs from that of highly stable chelates,³² and it was suggested that Gd was taken up by the

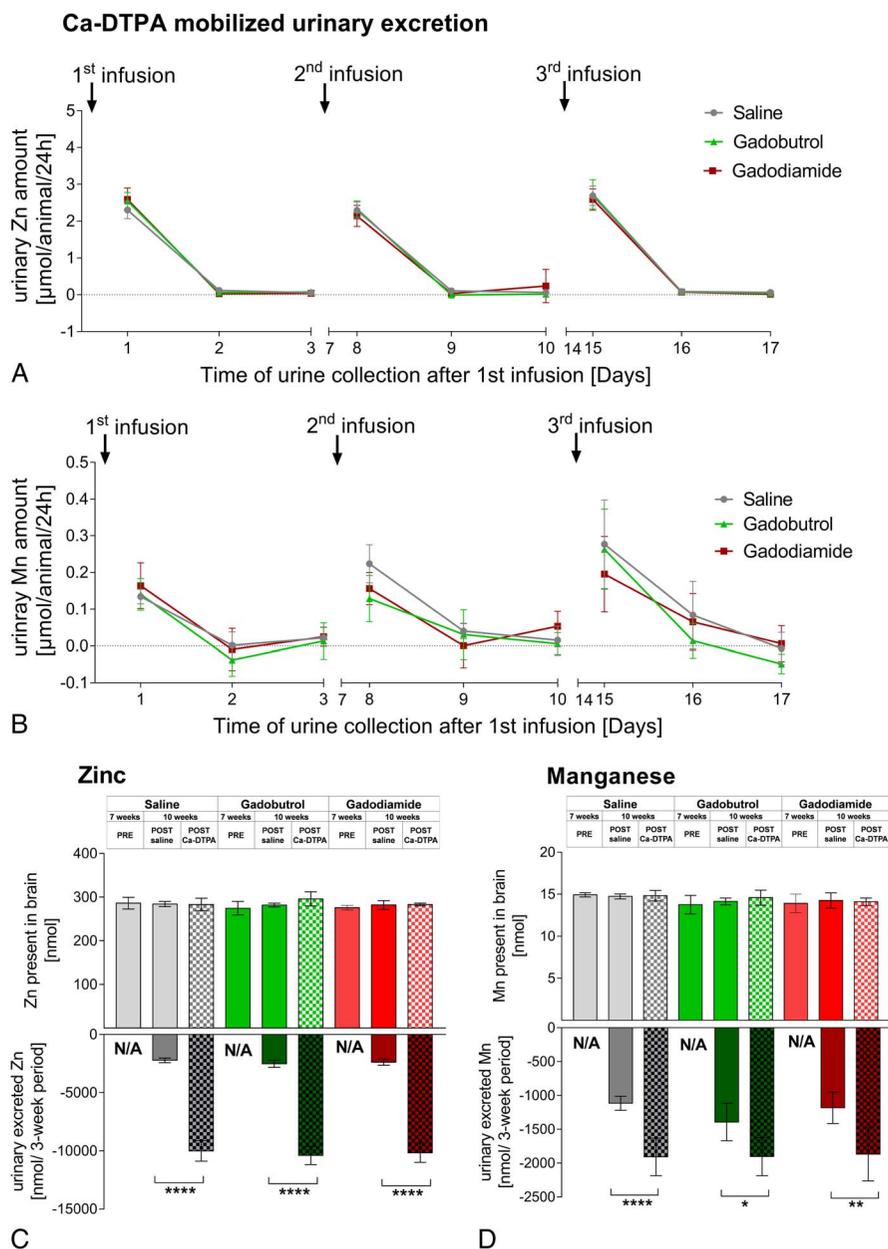


FIGURE 3. A and B, Time course of urinary excretion of Zn and Mn over the treatment period. Data are excreted Zn (Mn) after Ca-DTPA infusion subtracted by the spontaneous excreted Zn (Mn) after saline infusion. Mean amount of Zn ± SD quantified in urine samples collected for 24 hours, n = 6 per group. C and D, Total amount of Zn and Mn in nanomole present in the brain at the start (7 weeks post injection) and at the end of the treatment period (10 weeks post injection) and the cumulative amount of Zn and Mn eliminated from the body by urinary excretion during the treatment period.

mononuclear phagocyte system and deposited in tissue.^{32,33} This might explain why prophylactic infusion with Ca-DTPA, which is a much stronger ligand to Gd than citrate, mitigated Gd burden by immediate chelation of the administered Gd citrate, whereas post hoc treatment showed only moderate efficacy. A reduction in brain Gd content was not achieved in the preclinical study.³¹

Our study showed that Ca-DTPA reduced the Gd amount in the brain of rats to a small extent in animals injected with gadodiamide. Although the current study did not provide information on the Gd species mobilized by Ca-DTPA, released Gd bound to macromolecules is among the different Gd species most likely sensitive to Ca-DTPA chelation. A rat study by Frenzel et al¹⁷ showed that 3 weeks after repeated injection of gadodiamide, 18% of soluble Gd species

in the brain were associated with or bound to macromolecular structures. Further studies are needed to determine the Gd species from which Gd is mobilized by Ca-DTPA and to evaluate whether the Gd elimination is accompanied by a decrease of the MRI signal in the dentate nucleus from hyperintensity toward baseline level.

Data obtained from the administration of chelating agents after contamination by heavy metals suggest that the efficacy of chelation diminishes with time after exposure due to long-term storage in organs that are largely inaccessible to Ca-DTPA such as mineralized bone.³⁴ Our current study investigated delayed infusion with Ca-DTPA after administration of a linear or a macrocyclic GBCAs with no major reduction in the bone Gd concentration. However, the relatively low amount of eliminated Gd (114 nmol/animal) compared with the total

Gd burden in bone (~2700 nmol/animal) and skin (~500 nmol/animal) might not have been sufficient to detect significant changes in the Gd concentration in these organs. The Gd skin values showed a nonsignificant but slight decrease, but additionally showed the highest degree of standard deviation, which possibly masks small reductions of Gd in this organ.

Ca-DTPA chelation is not specific for exogenous heavy metals and also resulted in increased elimination of the essential minerals such as Zn and Mn. Manganese and especially Zn were rapidly excreted via the urine within 24 hours, but their excretion returned to baseline level 48 hours after Ca-DTPA administration. The enhancement of urinary excretion of Gd was observed for longer times (at least 72 hours), indicating additional mobilization of Gd from a compartment with slower pharmacokinetics³⁵ or depletion of extractable Zn and Mn. Increased urinary excretion of Zn after GBCA administration has also been reported for gadodiamide and gadopentetate in patients³⁶ and for gadodiamide and gadobenate in rats.³⁷ Gadodiamide is supplied with excess free ligand caldiumide (5 mol%), which has the ability to chelate endogenous metals similar to Ca-DTPA. A lack of minerals as a consequence of the increased excretion of essential trace elements is a potential cause of adverse effects associated with the clinical use of Ca-DTPA and after initial treatment with Ca-DTPA maintenance treatment with Zn-DTPA is recommended. In the clinical study by Semelka et al,²⁶ Ca-DTPA treatment resulted in a higher rate of elimination of Gd in the urine compared with Zn-DTPA.

Ca-DTPA infusion had no impact on Gd content and elimination in rats administered with macrocyclic gadobutrol, indicating that the Gd measured is the intact gadobutrol. Macrocyclic GBCAs are kinetically inert and the high kinetic stability of macrocyclic agents prevents spontaneous dissociation of Gd, transmetallation of Gd by endogenous metals,¹⁵ and therefore deposition of Gd in tissue.

Importantly, the present study also demonstrates—by both brain tissue and urine data—that between 7 and 10 weeks after a single injection of gadobutrol, Gd is still physiologically excreted from the body, thus adding to the growing body of evidence for a continuous elimination of macrocyclic GBCAs only. Recently, an ongoing elimination of Gd from brain tissue after repeated administration of macrocyclic GBCA was shown “short-term” (between 3 and 24 days) for gadobutrol and gadoterate¹⁷ and “long-term” between 5 and 52 weeks in rats for gadobutrol,³⁸ gadoteridol,³⁸ and gadoterate.^{22,38} No elimination of Gd was observed for linear GBCAs during the latter observation period.

The current study has certain limitations. The study aimed to evaluate the potential of retained Gd after administration of GBCAs to be mobilized by Ca-DTPA. Infusion of Ca-DTPA was initiated 7 weeks after a single high dose of GBCAs. At such late time point, the fraction of gadodiamide in the total eliminated gadolinium is presumably very low. This had been demonstrated in a previous rat study that evaluated Gd brain species after multiple administration of gadodiamide,²² suggesting that at that time tissue Gd was most likely present in another Gd species but not as intact gadodiamide. The effect of Ca-DTPA to reduce the Gd burden as a preventive or acute measure after GBCA administration was not evaluated. The single GBCA administration used in this study is not sufficient to induce hyperintensities in T1-weighted MRI scans, therefore MRI was omitted. As a consequence, the association between MRI hyperintensity and Gd removal remains to be investigated. The applied single dose of Ca-DTPA was based on the human dose with body surface adaption for the rat (180 μmol/kg body weight), but remains a factor 10 below the initial GBCA dose (1.8 mmol Gd/kg body weight). The concentration of Ca-DTPA reaching the brain might not be sufficient to rechelate Gd in the interstitial space of the brain. Further studies are needed to evaluate if additional infusions of Ca-DTPA may increase the amount of Gd removable after linear GBCAs from the brain or if a static point will be reached indicative for a Gd species inaccessible for Ca-DTPA or an intracellular location of Gd. Furthermore, we did not collect and analyze other organs. Thus the net balance between

excreted Gd and reduction of Gd content in tissue was incomplete, and Gd eliminated from additional organs such as the kidney or liver might contribute to the Gd found in the urine after infusion of Ca-DTPA in rats that received gadodiamide. Lastly, the current study did not evaluate all marketed GBCAs.

In conclusion, Ca-DTPA infusion forced the urinary excretion of Gd and partially reduced Gd content in the brain after linear gadodiamide, indicating mobilization of Gd from tissue. For the macrocyclic agent gadobutrol, Ca-DTPA infusion had no impact and no relevance, because the trace amounts of Gd detectable in the brain and body are continuously and spontaneously excreted via its physiological route through the kidney, most likely as intact Gd-chelate.

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