Type of MRI contrast, tissue gadolinium, and fibrosis

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Do C, Barnes JL, Tan C, Wagner B. Type of MRI contrast, tissue gadolinium, and fibrosis. Am J Physiol Renal Physiol 307: F844–F855, 2014. First published August 6, 2014; doi:10.1152/ajprenal.00379.2014.—It has been presupposed that the thermodynamic stability constant ($K_{\text{therm}}$) of gadolinium-based MRI chelates relate to the risk of precipitating nephrogenic systemic fibrosis. The present study compared low-$K_{\text{therm}}$ gadodiamide with high-$K_{\text{therm}}$ gadoteridol in cultured fibroblasts and rats with uninephrectomies. Gadolinium content was assessed using scanning electron microscopy equipped with energy-dispersive X-ray spectroscopy in paraffin-embedded tissues. In vitro, fibroblasts demonstrated dose-dependent fibronectin generation, transforming growth factor-$\beta$ production, and expression of activated myofibroblast stress fiber protein $\alpha$-smooth muscle actin. There were negligible differences with respect to toxicity or proliferation between the two contrast agents. In the rodent model, gadodiamide treatment led to greater skin fibrosis and dermal cellularity than gadoteridol. In the kidney, both contrast agents led to proximal tubule atrophy and tubular epithelial cells accumulation. Experimental MRI contrast-induced fibrosis model. Curiously, skin findings is that when chelate is deposited in the involved tissue, gadolinium becomes liberated and then triggers the disease. This led to the hypothesis that low-$K_{\text{therm}}$ chelates have a greater propensity to elicit nephrogenic systemic fibrosis and demonstrate that certain tissues are resistant to these effects.

Nephrogenic systemic fibrosis (NSF) is a systemic disease that is associated with exposure to gadolinium-based MRI contrast in patients with compromised renal function. For those afflicted, this disorder has caused indescribable suffering, permanent disability, and increased mortality.

Gadolinium is a unique element in terms of its paramagnetic properties and, particularly advantageous for clinical use, a spin-lattice relaxation rate that is ideal for T1-weighted MRI. Because free ionized gadolinium ($\text{Gd}^{3+}$) is highly toxic, a number of proprietary chelates have been formulated for clinical use as MRI contrast. The American College of Radiology has three categories for contrast agents approved for use in the United States (Table 1). These agents each have different chemical structures as well as a range of affinities to bind gadolinium. The later is characterized as the thermodynamic equilibrium for the binding between the metal and ligand (15), as follows:

$$[\text{Gd}^{3+}] + [\text{chelate}^{3-}] \leftrightarrow [\text{gadolinium chelate}]$$

The affinities of each proprietary chelate for gadolinium are expressed in terms of a thermodynamic stability constant ($K_{\text{therm}}$):

$$K_{\text{therm}} = \frac{[\text{gadolinium chelate}]}{[\text{Gd}^{3+}][\text{chelate}^{3-}]}$$

The lower the $K_{\text{therm}}$, the more the propensity to liberate $\text{Gd}^{3+}$. Multiple gadolinium-based contrast agents are available, and the numbers of NSF cases that have been associated with different brands has led to the assumption that the lower the $K_{\text{therm}}$ of the chelate, the more risk than others. Among the various commercially available contrast agents for MRI, gadodiamide (Omniscan) and gadoteridol (ProHance) are at opposite ends of the $K_{\text{therm}}$ spectrum (Table 2) (4, 28).

The half-life of the contrast agent is prolonged in any subject with compromised renal function (15). This led to the hypothesis that prolonged elimination times of the contrast provide an environment for dechelation and subsequent tissue deposition of gadolinium (31).

Supporting this concept, gadolinium has been detected in many organs in patients with NSF, including the spleen (on autopsy). Although rodent models have been established, to date no laboratory has assayed tissue gadolinium content in an experimental MRI contrast-induced fibrosis model. Curiously, it has been known that after administration of gadolinium-based MRI contrast, the metal accumulates in organs other than skin, such as the kidney and liver (with barely any retention in the spleen) (13, 35).

A great number of cases of NSF are attributed to gadodiamide, a low-$K_{\text{therm}}$ chelate. Fewer cases have been reported with gadoteridol, a cyclic chelate with high $K_{\text{therm}}$. When it was recognized that MRI contrast exposure was an important risk factor for the development of NSF, many editorials and narrative reviews implied that the different proprietary chelate structures with resultant variable affinities for gadolinium may have figured into some brands having more propensity to elicit the disease than others. Competition for $\text{Gd}^{3+}$ by anions (PO$_4^{3-}$, CO$_3^{2-}$, or OH$^-$) and for the ligand by cations (e.g., Fe$^{2+}$, Ca$^{2+}$, or physiological trace metals) has been proposed to be a mechanism by which gadolinium is more prone to become liberated and trigger systemic fibrosis (23). This mechanism has been invoked to explain why certain formulations of MRI contrast (gadodiamide/caldiamide > gadobenate dimeglumine > gadoteridol) have been tied to more cases of NSF than others. Moreover, a retrospective examination failed to pin any cases of NSF on gadoteridol exposure in chronic hemodialysis patients (27).

Over 40% of dermal cellularity in a NSF model is bone marrow derived (39). A popular model that attempts to explain the skin findings is that when chelate is deposited in the involved tissue, gadolinium becomes liberated and then triggers the disease. However, this is at odds with the tissue distributions of gadolinium, particularly because we have yet
to see liver involvement, an organ that accumulates gadolinium to a high degree and one with a great mass of phagocytic cells; this should promote the liberation of gadolinium from chelates at this site, and, therefore, this organ should be a principle organ affected in NSF. Because there was no liver pathology, it was decided to quantify gadolinium deposition in the skin, muscle, spleen, kidney, and liver in MRI contrast-induced systemic fibrosis and correlate the organ accumulation of gadolinium with tissue histology. Furthermore, the same conditions that were used to induce skin fibrosis were compared between low- and high-$K_{\text{therm}}$ contrast agents (gadodiamide and gadoteridol, respectively).

Gadolinium has been detected in the tissues of subjects exposed to MRI contrast, with high levels in afflicted patients (7, 14, 29). Because scanning electron microscopes equipped with energy-dispersive X-ray spectrographic (EDS) detectors have been used to analyze human tissue specimens for gadolinium (32), this technique was used in comparing tissues from animals treated with low- or high-$K_{\text{therm}}$ MRI contrast agents to correlate with the extent of disease.

**MATERIALS AND METHODS**

**Cell Culture, DNA Synthesis, and Toxicity Assay**

Human foreskin fibroblasts (HFFs) were kindly provided by Hanna E. Abboud (Division of Nephrology, University of Texas Health Science Center at San Antonio). Gadodiamide (Ommiscan) was purchased from General Electric Healthcare (Little Chalfont, UK), and gadoteridol (ProHance) was purchased from Bracco Pharma (Milan, Italy). Twenty-four-well dishes were seeded with 50,000 cells/well and incubated for 48 h (12). Monolayers were washed and incubated for another 48 h in serum-deprived medium. Cells were treated with the indicated doses of MRI contrast and pulsed with $[^3H]$thymidine for 24 h. DNA synthesis was measured as $[^3H]$thymidine incorporation in the tricarboxylic acid precipitate. For toxicity assays, cells were seeded in 96-well dishes, serum deprived for 48 h, and treated with the indicated substances using 800 $\mu$M H$_2$O$_2$ as a positive control. The addition of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was followed by a 4-h incubation per the manufacture’s protocol (Sigma, St. Louis, MO).

**Animals**

Female Fischer 344 rats underwent 5/6 nephrectomy at 8 wk of age followed by 2 wk of acclimation. Experimental animals received MRI contrast (gadodiamide, n = 6; gadoteridol, n = 3) at 2.5 mmol/kg ip (1 dose daily to a total of 20 doses over the 4-wk period). Experimental procedures and protocols were in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee.

**Immunofluorescence**

Quiescent HFFs grown on Permanox-chambered slides were treated with gadodiamide (0.2 mM) for 48 h, fixed with ice-cold methanol, and washed. Transforming growth factor (TGF)-$\beta$ (2 ng/ml) was used as a positive control. Primary antibody was Cy3-conjugated anti-$\alpha$-smooth muscle actin ($\alpha$-SMA; 1:100 dilution), and coverslips were adhered with SlowFade Gold antifade reagent with 4',6-diamidino-2-phenylindole (Molecular Probes); the exposure time (Cy3) was 200 ms. For in vivo experiments, frozen sections were stained with the indicated primary antibodies. This was followed by Cy3-conjugated secondary antibody (red). Coverslips were fixed using SlowFade Gold with 4',6-diamidino-2-phenylindole (Molecular Probes) to stain the nuclei (blue), and photographs were taken at $\times10$. Antibodies against $\alpha$-SMA were from Sigma, CD34 and procollagen type I were from Santa Cruz Biotechnology, and TGF-$\beta$ and factor XIIIa were from Abcam (Cambridge, MA).

**Histology**

Midline, posterior, dorsal skin was obtained and processed as previously described (39). The kidney, liver, gastrocnemius skeletal muscle, and spleen were sliced into equal sections. One section each was flash frozen for immunohistochemistry, flash frozen for immunoblot, and fixed in 10% neutral-buffered formalin (Richard-Allan Scientific, Kalamazoo, MI) overnight. These formalin-fixed organ sections were desiccated in 70% ethanol the following day. Skin was mounted edge on in cassettes. Paraffin-embedded tissues were stained

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**Table 1. Categorization of MRI contrast agents according to the American College of Radiology and European Medicines Agency**

<table>
<thead>
<tr>
<th>European Medicines Agency</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
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<tr>
<td>High</td>
<td>Gadodiamide, gadopentetate, and gadoversetamide</td>
<td>Gadobenate and dimeglumine</td>
<td>Gadofosveset and gadoxetic acid</td>
</tr>
<tr>
<td>Medium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td></td>
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Group I agents are associated with the greatest number of nephrogenic systemic fibrosis cases. Group II agents are associated with few (if any) unconfounded cases. Group III includes agents that have recently appeared on the market. Data are from Refs. 5 and 10a.

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**Table 2. Characteristics of the MRI contrasts used in this study**

<table>
<thead>
<tr>
<th>Contrast</th>
<th>$V_d$, ml/kg</th>
<th>Renal $K_t$</th>
<th>Plasma $K_t$</th>
<th>$\log(K_{\text{therm}})$</th>
</tr>
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<tbody>
<tr>
<td>Gadodiamide</td>
<td>200 ± 61</td>
<td>1.7</td>
<td>1.8</td>
<td>16.9</td>
</tr>
<tr>
<td>Gadoteridol</td>
<td>204 ± 58</td>
<td>1.41 ± 0.33</td>
<td>1.5 ± 0.35</td>
<td>23.8</td>
</tr>
</tbody>
</table>

Data are from Refs. 4 and 28. $V_d$, volume of distribution; DTPA, diethylenetriaminepentaacetic acid; DOT A, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid; $K_{\text{therm}}$, thermodynamic equilibrium constant.
with trichrome and hematoxylin and eosin (Department of Pathology, University of Texas Health Science Center at San Antonio).

Immunohistochemistry

Five-micrometer-thick sections of frozen skin were air dried for 1 h, fixed in cold acetone for 10 min, air dried, rehydrated in Tris-buffered saline (TBS) for 15 min, peroxidase blocked (Peroxidized 1, Biocare Medical, Concord, CA) for 6 min, rinsed three times with TBS, blocked (Background Sniper, Biocare Medical) for 20 min, and incubated with rabbit fibronectin antibody (1:800, Sigma-Aldrich) overnight. Tissue was rinsed with TBS, stained with horseradish peroxidase-conjugated secondary antibody (Rabbit-on-Rodent HRP-Polymer, Biocare) for 20 min, washed with TBS, and stained with chromagen (BetaZoid DAB, Biocare) for 1–2.5 min. The reaction stopped with TBS, and the tissue was washed, counterstained with hematoxylin for 1–2 min, rinsed, and then mounted. Photographs were taken using a light microscope (Imager.A1, Carl Zeiss, Oberkochen, Germany).

EDS

Formalin-fixed and embedded tissues (e.g., the skin, spleen, and liver) were analyzed with scanning electron microscopy equipped with an EDS silicone detector (Genesis, EDAX, Mahwah, NJ). Spectra were obtained under low vacuum (30 Pa) at ×600 with a 10-mm working distance and 20-kV accelerating voltage. The estimated gadolinium content was compared.

Immunoblot analysis

Fibronectin antibody was from Sigma-Aldrich. GAPDH antibody was from Santa Cruz Biotechnology. Collagen type IV and TGF-β antibodies were from Abcam. Pixel densitometry was measured using the gel analysis tool with ImageJ (1.45s, NIH).

Statistical Analysis

Results are expressed as means ± SE. Experiments were performed at least three times and compared using one-way ANOVA with a Tukey post hoc test.

RESULTS

In Vitro Experiments

**Gadodiamide induces fibronectin expression in cultured fibroblasts.** Differentiated myofibroblasts express α-SMA-rich stress fibers (6, 11, 34). Therefore, the effect of gadodiamide on fibronectin and α-SMA expression in cultured HFFs was investigated (Fig. 1). Quiescent HFFs were treated with increasing doses of gadodiamide for 24 h, and fibronectin accumulation was assessed by immunoblot (Fig. 1A). Peak expression was with a clinically relevant dose of 0.2 mM. This was the concentration used for all in vitro experiments reported here unless otherwise noted. Fibronectin accumulation was notable after 24 and 48 h of treatment (Fig. 1B). Fibronectin and the formation of α-SMA-rich stress fibers was assessed by immunofluorescent microscopy. Gadodiamide was a potent stimulant for fibronectin accumulation and α-SMA-rich stress fiber expression in vitro (Fig. 1C).

**Other than fibronectin expression, gadodiamide and gadoteridol have negligible biological effects on cultured fibroblasts.** Some have assumed that the biological effect of high-\(k_{\text{therm}}\) chelates is minimal. Therefore, the effects of gadoteridol (high \(k_{\text{therm}}\)) was compared with those of gadodiamide (low \(k_{\text{therm}}\), Fig. 2A). In quiescent cells, gadodiamide induced DNA synthesis at only a low dose, whereas gadoteridol was without effect (Fig. 2B).

Because mild toxicity of a compound can stimulate mitogenesis by cellular lysis and the subsequent release of cytokines and growth factors, the toxicities of these compounds were compared with a MTT incorporation assay (Fig. 2C). Only gadoteridol demonstrated dose-dependent cytotoxicity. With 24 h of treatment, gadoteridol induced a milder accumulation of collagen and fibronectin (Fig. 2D). These data indicate that a low-\(k_{\text{therm}}\) MRI contrast agent has comparable effects to a high-\(k_{\text{therm}}\) MRI contrast agent (gadoteridol) with respect to DNA synthesis and toxicity. That gadodiamide promoted the accumulation of fibronectin and collagen to a greater degree than gadoteridol, however, supports the free gadolinium hypothesis, i.e., that Gd\(^{3+}\) liberated from the chelate is one pathogenic mechanism of MRI contrast-induced fibrosis.

**Confirmation of gadolinium detection using EDS.** Elements have specific profiles that can be detected and quantified using EDS (Fig. 3A). The presence of gadolinium was examined in organs of contrast-treated animals. To verify that gadolinium could be detected using EDS, increasing quantities (0–10%)...
of gadodiamide in agarose were examined (Fig. 3B). Peaks in the M and L regions correlated with contrast concentra-
tion (Fig. 3C).

In Vivo Experiments

A comparison of gadodiamide- and gadoteridol-treated Fisher 344 rats with 5/6 nephrectomy was conducted (Fig. 4). The doses were the same as used in the original rodent model of NSF (31) and that used in vitro (39). Weights did not differ from control paired animals throughout the 4 wk of treatment (Fig. 4A). In contrast to gadodiamide-treated animals, gadoteridol-treated animals did not demonstrate chromodacryorrhea. After 4 wk, animals were killed and tissues were collected. Skin fold thicknesses were increased in gadodiamide-treated animals, whereas gadoteridol had no effect (Fig. 4B). The thickness of the epidermal layer was only increased in gadodo-

iame-treated animals (Figs. 4C and 5A). Unlike gadodi-
amiame, gadoteridol did not induce dermal hypercellularity (Figs. 4C and 5B).

In fibrosis, TGF-β1 is pivotal (18). TGF-β has also been postulated as a trigger for fibrocyte differentiation (1) and as a mediator of NSF (16). Gadodiamide administration has been associated with an increase of TGF-β mRNA (25). It has been reported that there is a massive increase in TGF-β1 transcript (16) and protein expression (21) in affected skin. Therefore, the expression of fibronectin and TGF-β1 was examined (Figs. 5 and 6, respectively). After 4 wk of treatment, both gadodi-
amiame and gadoteridol induced fibronectin accumulation in the dermis with respect to control (Fig. 5C). The accumulation of fibronectin and basement membrane collagen type IV was assessed in homogenized skin with immunoblot analysis. Fi-
bronectin and collagen type IV expression tended to be higher in the skin of MRI contrast-treated animals (Fig. 5D). TGF-β1 expression was increased in the MRI contrast-treated animal dermis, as detected by immunofluorescence (Fig. 6A). Of note, skin from the gadoteridol-treated group demonstrated greater TGF-β protein than the control group at the 4-wk time point (Fig. 6B). In total, these data demonstrate that although the histological differences between gadoteridol-treated and control animals are subtle, there are early markers of fibrosis even with this high-Ktherm contrast agent.

Fibrocyte markers are induced by MRI contrast in skin. Because of the symmetric nature of the disease, the rapid development of lesions, the absence of mitotic figures among the numerous spindle-shaped cells (resembling wound heal-
ing), and the cell markers CD34 and procollagen type I, it has been hypothesized that the fibrosis is mediated by circulating fibrocytes (8). Therefore, the expression of these and α-SMA-rich activated myofibroblasts in the dermis in MRI contrast-
treated rats was examined (Fig. 7). Regardless of the MRI contrast, there was an increase in α-SMA-expressing myofi-
broblasts. Furthermore, both procollagen type I and CD34 were increased with respect to control (Fig. 7, A and B). The histology of NSF-afflicted skin was also remarkable for increased cellularity, with factor XIIIa+ dendritic cells (9, 19). In experimental animals, factor XIIIa was increased in the dermis

Fig. 2. Differential effects of low- and high-thermodynamic stability constant (Ktherm) MRI contrast on cultured fibroblasts. A: comparison of gadodiamide and gadoteridol with respect to thermodynamic stability. Gadodiamide and gadoteridol differ with respect to their affinities to associate with cations, and these physical properties can be measured by Ktherm = log(Ktherm) values for Gd2+, Ca2+, Cu2+, and Zn2+ for gadodiamide and gadoteridol are shown with each radar plot. Each axis ranges from 0 to 25 in 5-unit increments [log(Ktherm)] of the ligand. Data from Ref. 26. These agents are at the opposite ends of the spectrum with respect to Gd2+ affinity. B: DNA synthesis was assessed by [3H]thymidine incorporation in quiescent human foreskin fibroblasts (HFFs). Cells were treated for 24 h and pulsed at the time of treat-
m-ment as previously described (38). C: gadodi-
amiame demonstrated no cellular toxicity in vitro. Cells were 
treated for 4 h, and toxicity was assessed by MTT incorporation. H2O2 (800 μM) was used as a positive control. ***p < 0.001 with respect to control by one-way ANOVA and Tukey post hoc analysis. D: MRI contrast led to increased collagen type IV and fibronectin ac-
cumulation in vitro. Quiescent HFFs were treated for 48 h with the indicated contrast (0.2 mM), and immunoblot analysis was performed. Bar plots show the pixel densities of each group relative to the loading control in arbitrary units. *p < 0.05 compared with control by one-way ANOVA and Tukey post hoc test.
Fig. 3. Energy-dispersive X-ray spectroscopy (EDS) of physiological elements with respect to gadolinium. A: gadolinium has a unique spectral signature compared with all physiological elements by EDS. Shells are successively filled by electrons for each element, with the innermost being termed K, L, and M. Excitation of these electrons by EDS results in measurable spectra. Shown are the energies of the highest spectral peaks (a) of all elements found in the human and gadolinium (which is not normally found in tissue). Note that gadolinium has several specific emission energies that are distinct from normal biological elements found in vertebrates. These histograms demonstrate signature K, L, and M emissions. The high L emission for gadolinium with respect to other elements that can be found in human are suitable for detection in flash-frozen or fixed and paraffin-embedded tissues. For K, the lower atomic number elements (6C through 34Se) are not labeled, and these comprise the energies ranging from 0 to 10 KeV. For L, the high peak represents elements 23V through 33As. B: detection of gadolinium in MRI contrast-saturated material by EDS. Increasing amounts of gadodiamide (Omniscan) were mixed in agarose and analyzed in low vacuum (30 Pa) with EDS at $(\ldots)$ (spot size: 70). Spectra were obtained for 50 live seconds with a silicon drift detector [Apollo X, AMETEK (EDAX), Berwyn, PA]. The accelerating voltage was 25 kV, and the take-off angle was 36.02° (50 live seconds). C: M and L data were quantified by Genesis software using the Z (atomic number), A (absorption), and F (fluorescence) method. ZAF corrections allow calculation of the composition of the sample. Box and whisker plots of the peak-to-background ratios (P/B) for gadolinium based on M (left) and L (right) peaks are shown.

Fig. 4. Comparison of the effects of low- and high-$K_t$ MRI contrast in vivo. Fisher 344 rats with 5/6 nephrectomy were treated with gadodiamide ($n = 6$) or gadoteridol ($n = 3$) at 2.5 mmol/kg ip for 5 days/wk over a 4-wk period. A: there were no significant differences in the weights of treated animals and their matched controls ($n = 5$ and 2, respectively). B: skin fold thicknesses were measured in triplicate for each animals using digital calipers. C: effects of MRI contrast on skin histology. Dorsal skin from the lumbar area was fixed overnight in 10% neutral-buffered formalin (Richard-Allan Scientific, Kalamazoo, MI) followed by 70% ethanol. Tissues were paraffin embedded for light microscopic sections. Trichrome-stained skin demonstrated denser collagen in gadodiamide-treated animals. Skin from gadodiamide-treated animals demonstrated dermal hypercellularity, epidermal thickening, and enlargement of sebocytes. Bars = 0.1 mm. TC, trichrome.
regardless of the contrast agent (Fig. 7, C and D). In total, these findings demonstrate that both a low- or high-\(K_{\text{therm}}\) contrast agent will lead to evidence of dermal fibrosis, active myofibroblasts, and increased CD34/factor XIIIa expression. These data are consistent with fibrocyte recruitment.

**Effect of MRI contrast on other organs.** NSF is believed to be a disorder with promiscuous involvement of numerous organs. It has been demonstrated in rodents that MRI contrast treatment leads to gadolinium deposition in all tissues, particularly the liver. The degree of gadolinium deposition may correlate with the chemical structure of the chelate. Gadodiamide-treated mice demonstrate more liver gadolinium compared with the gadoteridol-treated group (35). In mice with renal insufficiency, the biodistribution of gadolinium deposition is altered where the liver is a substantially larger reservoir organ, especially with respect to the skin (37). GdCl\(_3\) has a profound effect on liver histology (33). Therefore, given the systemic nature of the disease, several organs were examined histologically (Fig. 8). Other than the kidney, no differences were detected at this 4-wk time point. Renal proximal tubules from either MRI contrast showed vacuolization. Fibronectin expression was increased in the kidney of contrast-treated animals (Fig. 9). There was a trend toward higher fibronectin accumulation in the liver from gadodiamide-treated animals. These data suggest that the skin, although not among the major reservoirs of gadolinium accumulation, has a propensity for fibrocyte homing with respect to the other organs examined.

**Detection of gadolinium in paraffin-embedded tissues.** Gadolinium has been detected in biopsy specimens from NSF-affected patients by EDS (14). Although the preferential organ of gadolinium deposition is the liver in rodents (35), there is no evidence of hepatic disease in animals with renal insufficiency.
Dermal disease has been repeatedly demonstrated in MRI contrast-treated rats (31, 39). If the liberation of Gd\(^{3+}\) initiates the disease, then the metal should be found in fibrotic sites. EDS was conducted (Figs. 10 and 11). In every tissue examined other than the heart and spleen, gadolinium was detected in the gadodiamide-treated group. Gadolinium content was less in the kidney and skin from gadoteridol-treated animals. These data demonstrate that there are quantifiably higher amounts of gadolinium in the organs of gadodiamide-treated animals with respect to gadoteridol. Furthermore, in these experiments, muscle, skin, and the liver appear to be large reservoirs for gadolinium.

DISCUSSION

Gadolinium-based contrast agents are widely used. Other than causing NSF or rare cases of nephrotoxicity, MRI contrast is considered to be safe (24). Hemodialysis and renal transplant patients have a high risk of contracting NSF when exposed to MRI contrast (20). The largest risk factors for NSF are 1) renal insufficiency and 2) exposure to MRI contrast. No one has yet proved that the type of contrast determines whether NSF can be induced.

It is difficult to single out particular brands of contrast for precipitating the disease because the relative market shares of each are unknown (22). Given the paucity and quality of strictly observational data, a class effect of gadolinium-based MRI contrast agents has been assumed (2, 17). A meta-analysis has supported the Federal Drug Administration’s original supposition of a class effect, and all gadolinium-based contrast are assumed to carry some risk of precipitating the disease (2).
Gadodiamide and gadoteridol differ with respect to the propensity to release gadolinium (26). Whether this metal is free or complexed with the original chelate in skin lesions has yet to be determined. Therefore, the transmetallation hypothesis has been noted to be an attractive yet controversial hypothesis (36). Further complicating the free gadolinium theory of NSF, GdCl₃ does not stimulate fibroblast proliferation (10). In this regard, it has been noted that “a model solely relying on thermodynamic stability constants and stressing the thermodynamic selectivity of a ligand for Gd³⁺ over Zn²⁺ is not supported by the body of animal data and, increasingly, NSF data relying on a single physical measurement to predict in vivo behavior can be naïve…” (30).

Gadodiamide does leave 10 times more residual gadolinium than gadoteridol when rodents with normal renal function are administered a single dose (35). Gadodiamide (5 mM) does not differ from any other MRI contrast agent tested with respect to cytokine/chemokine gene expression or the generation of pro-inflammatory or profibrotic cytokines or chemokines (40). Conditioned media from MRI contrast-treated monocytes stimulated collagen and fibronectin production in addition to α-SMA stress fiber expression regardless of the chelate. Therefore, the transmetallation hypothesis has not been supported by prior in vitro experiments (40). Until our study, there have been no prospective and experimental data to support the contention that one formulation had less propensity to elicit NSF than another.

The theory that some MRI contrast chelates are less prone to elicit NSF than others (gadoteridol < gadodiamide) is based on the affinities each has for gadolinium (27). In a comparison of wild-type mice with chronic renal insufficiency treated with three different contrast agents (gadoterate meglumine, gadopentetate, and gadodiamide), there was a large accumulation of labeled gadolinium in every organ tested, regardless of the chelate (37). The organs serving as the largest reservoirs were the kidney, liver, and spleen. There was a tendency for the accumulation of gadolinium in the bone of gadodiamide-treated mice.

The data in the present study demonstrate that studying the in vitro actions of MRI contrast does not reveal the complex multiorgan effects witnessed in NSF. Gadodiamide and gadoteridol had negligible differences in DNA synthesis, toxicity, and apoptosis. Interestingly, gadodiamide did induce more fibronectin and collagen type IV accumulation in cultured fibroblasts, properties that correlate directly with the effect each MRI contrast agent had in the skin in vivo, with gadodiamide leading to greater fibrosis than gadoteridol when histology, fibronectin accumulation, and cellularity were compared. Most importantly, this project was the first to use EDS in a systematic fashion to compare low- and high-\(K_{\text{therm}}\) gadolinium chelates. As predicted by the high thermodynamic stability hypothesis, gadoteridol led to less gadolinium tissue deposition and less evidence of histological disease than gadodiamide.

Skin TGF-\(\beta\) was detected in the gadoteridol-treated group at the 4-wk end point. Furthermore, fibronectin and collagen type IV levels were elevated in these same animals. Therefore, gadoteridol, when used at the same doses as gadodiamide in this model, still cannot be considered NSF inert. The dose-dependent relationship to NSF has been overemphasized. The threshold of gadolinium exposure is still unknown. Our experiments demonstrate that 1) gadodiamide induces more skin

**Fig. 8.** Effects of MRI contrast on tissue histology. After 4 wk of treatment, there did not appear to be any differences among control and MRI contrast-treated animals with respect to the heart, skeletal muscle, liver, or spleen. Renal histology was notable for proximal tubule vacuolization only in MRI contrast-treated groups. Uniformly, the vacuoles were larger in the gadodiamide-treated group (\(n = 3\)) compared with the gadoteridol-treated group (\(n = 3\)). Bars = 0.1 mm. H&E, hematoxylin and eosin; PAS, periodic acid-Schiff.
fibrosis than gadoteridol at the same doses and 2) gadodiamide leads to a greater accumulation of gadolinium in every organ tested, as measured by EDS. This supports the hypothesis that the liberation of gadolinium from its chelate is the initial step in precipitating systemic fibrosis. Despite the histological differences between gadoteridol- and gadodiamide-treated animals, note that there was more fibronectin accumulation in organs from gadoteridol-treated animals than in control ani-

Fig. 9. Fibronectin expression in nondermal tissues from MRI contrast-treated animals. Only livers from gadodiamide-treated animals demonstrated a variable increase in fibronectin accumulation. Comparisons were performed using one-way ANOVA and Tukey post hoc analysis. **P < 0.01 with respect to the control group; #P < 0.05 with respect to the gadodiamide-treated group.
mals. This suggests that there is low dose threshold for gadolinium to initiate fibrosis. (Gadolinium was still found in every organ examined from gadoteridol-treated animals.)

Because there is controversy about the use of one chelate over another, yet no strong (particularly experimental) data to support this recommendation, the in vitro and in vivo effects of both low- and high-$K_{d}$ MRI contrast agents were compared in our study. Neither agent demonstrated impressive proliferatory effects on fibroblasts (particularly within the therapeutic ranges), whereas gadoteridol did have dose-dependent toxicity, as demonstrated by the MTT incorporation assay. Gadodiamide appears to have more propensity to stimulate both

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Fig. 10. Effects of MRI contrast on skin gadolinium content as detected by EDS. A: the blocks of paraffin-embedded tissue used for light microscopy (Fig. 4) were freshly faced (with the control tissues always cut before any tissues from MRI contrast-treated animals) and analyzed using scanning electron microscopy equipped with EDS. Spectra were obtained for 50 live seconds with a silicone drift detector (accelerating voltage: 20 kV, magnification: ×600, and working distance: 10 mm). Overall magnification: ×80–85. Bar = 200 μm. B: values are for the weight percentage, GdL$_{2}$O$_{3}$ spectra, above control. Comparisons were performed using one-way ANOVA and Tukey post hoc analysis. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ with respect to the control group; ##$P < 0.01$ and ###$P < 0.001$ with respect to the gadodiamide-treated group.
apoptosis and necrosis in these cells. Despite using the same doses over the same time period, rats treated with gadoteridol demonstrated far less dermal cellularity and extracellular matrix accumulation (fibronectin and collagen type IV) than those treated with gadodiamide. That TGFB- and fibronectin were greater in several organs from the gadoteridol-treated group with respect to the control group does not exonerate gadoteridol from higher levels from the gadoteridol-treated group. Although the average value was relatively high in the heart from the gadoteridol-treated group, there was no statistical significance (by one-way ANOVA and Tukey post hoc testing) for any of the outcomes (including the atomic percentage for GdL or GdM, weight/atomic percentages).

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DISCLOSURES
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AUTHOR CONTRIBUTIONS
Author contributions: C.D., C.T., and B.W. performed experiments; C.D., J.L.B., C.T., and B.W. approved final version of manuscript; J.L.B. and B.W. analyzed data; B.W. conception and design of research; B.W. interpreted results of experiments; B.W. prepared figures; B.W. drafted manuscript; B.W. edited and revised manuscript.

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