Pathophysiology of gadolinium-associated systemic fibrosis

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PATIENTS WITH CHRONIC KIDNEY disease, including end-stage renal disease, tend to be burdened with cutaneous afflictions that are still not well understood (34). Among these, gadolinium-associated systemic fibrosis is an underrecognized disorder (93) and in a class separate from other described fibrosing disorders (30).

Gadolinium-associated systemic fibrosis was originally termed “nephrogenic fibrosing dermopathy” and then changed to “nephrogenic systemic fibrosis” after the suspicion that more than just the skin was involved. However, the term “nephrogenic” is a misnomer (54); renal insufficiency is requisite, but this alone does not cause the disease; it is caused by gadolinium-based magnetic resonance imaging contrast (42). Therefore, gadolinium-associated systemic fibrosis has been proposed as an alternative, and more accurate, term (54).

Obviously, gadolinium-associated systemic fibrosis is iatrogenic: it joins a number of cutaneous fibrosing disorders that have been drug or environmentally induced. Such agents include toxic/denatured rapeseed oil, tainted L-tryptophan supplements (the eosinophilia-myalgia syndrome), polyvinyl chloride, bleomycin, and carbidopa (10).

There is a registry for reporting cases, the International Center for Nephrogenic Systemic Fibrosis Research (http://www.icnfdr.org) (19). Since this was last updated on June 15, 2013 (at the time of this writing), no cases of gadolinium-associated fibrosis have been recorded.

Clearance of Gadolinium-Based Contrast

“The composition of the blood is determined not by what the mouth ingests but by what the kidneys keep” (91). One can estimate the plasma concentration (C) of a drug by using the equation,

\[ C = C_0 e^{-kt} \]

where \( C_0 \) is the initial plasma concentration (once distributed in the vascular compartment), \( k \) is a rate constant, and \( t \) is time. (The volumes of distribution for magnetic resonance imaging contrast agents are almost entirely the extracellular compartment; therefore, the distributive phase is negligible.) With substances that are eliminated by the kidney, renal insufficiency attenuates the rate constant \( k \) (Fig. 1A). Most contrast (whether it is iodinated or gadolinium-based) is cleared by the kidney. Gadolinium-associated systemic fibrosis is not found in patients with normal renal function; therefore, prolonged retention of the contrast seemed to be a reasonable hypothesis regarding the initiation of the disease.

Types of Gadolinium-Based Contrast

An odds ratio provides a quantification of an association between a risk factor and contracting a disease. For gadolinium-associated systemic fibrosis (GASF), this can be derived by examining the numbers of patients with and without the disease relative to whether they have been exposed to contrast or not,

\[
\text{odds ratio} = \frac{(\text{GASF}/\text{contrast exposure})(\text{disease} - \text{free}/\text{no exposure})}{(\text{disease} - \text{free}/\text{contrast exposure})(\text{GASF}/\text{no exposure})}
\] (1)
The odds ratio for patients with end-stage renal disease contracting systemic fibrosis after an exposure to gadolinium-based contrast ranges from 20.6 to 41.3 (Table 1) (12, 17, 25, 79).

Six million doses of magnetic resonance imaging contrast are administered annually (47). Gadolinium is a marker of anthropomorphic pollution, having been detected downstream of many cities harboring magnetic resonance imagers (8, 64). It is a transition metal of the lanthanide series with paramagnetic properties, making it ideal for magnetic resonance imaging (66). Gadolinium, such as that delivered in the form of GdCl₃, is toxic; therefore, gadolinium-based contrast is composed of a variety of organic ligands that avidly bind the metal, greatly reducing its harm. Before 2006, the safety of gadolinium-based contrast was unparalleled. Patients with kidney disease would routinely undergo contrast-enhanced magnetic resonance imaging or arteriography to avoid the (suspected) nephrotoxicity of iodine-based contrast. Now we know that those with renal insufficiency and an exposure to gadolinium-based contrast are exclusively at risk. Furthermore, a new disorder, “gadolinium deposition disease,” has been proposed within the last year: patients who are exposed to repeated contrast-enhanced magnetic resonance scans demonstrate brain deposition of the metal (74).
With gadolinium being so noxious, there are a variety of amine-containing organic ligands with different properties that have been manufactured to tightly couple with the metal, greatly reducing its toxicity (Fig. 1B). Some of these organic molecules are (roughly) linear, and others are (relatively) large rings, i.e., “macrocyclic.” When the number of acidic residues (typically as side chains) on a ligand is 3, i.e., equal to the valence of Gd$^{3+}$, the formulation is regarded as “nonionic”; otherwise it is “ionic.” For these ligands, the affinities for the ligand-bound form, $\text{Gd(H}_2\text{O)}_{\text{8}}^{3+} + \text{ligand} \rightleftharpoons \text{Gd(ligand)H}_2\text{O} + 7\text{H}_2\text{O}$, (2)

The measurements of the ligand-gadolinium affinities are expressed in terms of “thermodynamic stabilities,” $K_{\text{therm}}$ (26):

$$K_{\text{therm}} = \frac{[\text{Gd(ligand)}]}{[\text{Gd}^{3+}][\text{ligand}]}$$  \hspace{1cm} (3)

These thermodynamic stabilities are among several in vitro measurements that describe the propensity of the ligands to retain gadolinium. At a physiological pH, though, the amines of the ligands will be partially protonated:

$$\text{Gd(H}_2\text{O)}_{\text{8}}^{3+} + \text{H}_2\text{O}_\text{ligand} \rightleftharpoons \text{Gd(ligand)H}_2\text{O} + n\text{H}^+ + 7\text{H}_2\text{O}$$  \hspace{1cm} (4)

The conditional stability constant, $K_{\text{eff}}$, accounts for this protonation,

$$K_{\text{eff}} = \frac{[\text{Gd(ligand)[H}^+][n]}{[\text{Gd}^{3+}][\text{H}_2\text{ligand}]}$$  \hspace{1cm} (5)

This implies that the degree of acidity in vivo will reduce the binding of the ligand to gadolinium (influencing the reaction in Eq. 3 to tend left). Therefore, the behaviors of the gadolinium-ligand complexes in vivo may not be predictable based on the simple, single values of their thermodynamic stabilities (88).

From 1997 to 2006, a commonly used gadolinium-based contrast agent was Omniscan, which is largely gadodiamide {gadolinium 5,8-bis(carboxymethyl)-11-[2-(methylamino)-2-oxoethyl]-3-oxo-2,5,8,11-tetraazatricyclo-13-0ic acid, Gd-(DTPA-BMA)}. [Note that there is an excess of calcium-bound ligand, caldiamide, in the brand Omniscan. The purpose of this additional chelate is to provide an excess amount of ligand (81); given the relatively low conditional stability constant of DTPA-BMA, excess nongadolinium-bound ligand favors the movement of the reactions depicted in Eqs. 2 and 4 to the right (88).] Gadodiamide has been tied to a large number of gadolinium-associated systemic fibrosis cases, and it has a relatively low conditional stability constant compared with the other contrast agents. This led to the speculation that the release of gadolinium from the chelate is an initial step in triggering fibrosis (85).

The displacement of gadolinium from a ligand by another metal, such as zinc, is termed “transmetallation” (83), i.e., the expulsion of the paramagnetic ion by an endogenous ion. In terms of pathogenesis, transmetallation is more likely to occur with linear ligands than with macrocyclic ones, where the gadolinium ion is “caged.” Body cations (calcium, zinc, or iron, for instance) may alter the thermodynamic equilibrium with gadolinium and a ligand, promoting the liberation of the lanthanide ion (47). Once liberated from the chelate, it has been speculated that Gd$^{3+}$ is prone to form low-solubility compounds, such as Gd(OH)$_3$ or Gd(PO$_4$)$_3$ with subsequent pathological effects (23, 70). In fact, both the European Medicines Agency and the American College of Radiology have categorized the various ligands into different safety classes (Table 2) despite being grounded in purely observational data. Despite this, a class effect of gadolinium-based contrast agents has been assumed by the United States Federal Drug Administration (2, 53).

Whether transmetallation occurs in vivo and is an initial step in triggering the disease is controversial (88, 98). In vivo, the candidate metals, such as zinc or copper, are an order less in concentration than the peak dose of gadolinium-bound ligand, and these physiological elements are often protein-bound, thereby reducing the free, ionized forms that could participate

### Table 2. Categorization of magnetic resonance imaging contrast agents according to the American College of Radiology and the European Medicines Agency

<table>
<thead>
<tr>
<th>European Medicines Agency</th>
<th>American College of Radiology Group</th>
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<tr>
<td>High</td>
<td>Gadodiamide</td>
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<tr>
<td>High</td>
<td>Gadopentate</td>
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<tr>
<td>High</td>
<td>Gadoversetamide</td>
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<tr>
<td>Medium</td>
<td>Gadoxenate dimethylglucamine</td>
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<tr>
<td>Medium</td>
<td>Gadofoveset</td>
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<tr>
<td>Low</td>
<td>Gadobutrol</td>
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<tr>
<td>Low</td>
<td>Gadoteric acid</td>
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<tr>
<td>Low</td>
<td>Gadoteridol</td>
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(F3) AJP-Renal Physiol • doi:10.1152/ajprenal.00166.2016 • www.ajprenal.org
in such a reaction (96). Nonetheless, regardless of the conditional stability constants, all gadolinium-based contrast agents lead to residual metal deposition in several different organs (97). (Radiolabeled gadolinium in different formulations has been studied in both mice and rats. The formulations tested included gadodiamide, gadopentetate, gadoterate meglumine, and gadoteridol. Fourteen days after administration, the isotope was detectable in several organs, regardless of the ligand structure.) A meta-analysis has supported the United States Food and Drug Administration’s original supposition of a class effect, and all gadolinium-based contrast agents are assumed to carry some risk of precipitating the disease (2). The pathological effects of gadolinium, such a commonly used substance in contemporary clinical medicine, have been largely unexplored.

Contrast Dose

The United States Food and Drug Administration-approved dose of most gadolinium-based contrast, such as gadodiamide, for an adult undergoing an imaging procedure is 0.1 mmol/kg. Commonly used doses range from 0.2 up to 0.9 mmol/kg, with larger amounts often employed in magnetic resonance angiography (95). Among 116 patients with creatinine ranging from 1.6 to 9.5 mg/dl, all of them with creatinine clearances ≤15 ml/min, who underwent abdominal magnetic resonance angiographies with single doses of gadolinium-based contrast, none developed systemic fibrosis within a year (78). That being said, the original papers linking contrast to systemic fibrosis did not indicate dose dependence (70). In an analysis from four different centers, the cumulative lifetime exposures in afflicted patients ranged from the label dose of 0.1 mmol/kg up to 0.95 mmol/kg (103). Therefore, the evidence that the risk of systemic fibrosis relates to the dose of gadolinium-based contrast is weak (1). The caveat to pinning dose dependence, like many other purported risk factors, to gadolinium-associated systemic fibrosis, is that it is a very rare condition: outside of gadolinium exposure and renal insufficiency, other hazards will be difficult to accurately assess and quantify. The odds ratios for end-stage renal disease patients to develop systemic fibrosis after single, label doses of gadodiamide may range from 22 (12) to 32.5 (70). Curiously, the onset to the disease after exposure varies immensely, ranging from 24 h to several years (43, 70, 71, 73).

Since a variety of contrast formulations have been linked to this ailment, gadolinium is theorized to be a causative factor. As mentioned, the type of ligand may be a factor (15, 25, 62, 70), but there are little prospective or experimental data to support this contention. More cases have been reported with “linear” and “nonionic” agents (Omniscan: gadodiamide; Op-tiMark: gadoversetamide) (52) than for cyclic and “ionic” formulations (ProHance: gadoteridol; Gadovist: gadobutrol) (85). However, mountains of narrative reviews have gilded the “high thermodynamic stability” (often cyclic) ligands.

Some have proposed that gadolinium deposition in the involved organ, such as the skin, is the trigger for the disease, and this contention is supported by the finding of gadolinium in fibrotic lesions (28). It is postulated that pinocytosis of the contrast leads to a low-pH lysosomal environment, greater protonation of the ligand, and this promotes liberation of the gadolinium cation as an initial step in triggering fibrosis. This is how a ligand’s “thermodynamic stability” may relate to the propensity to induce systemic fibrosis. Of note, under semiphysiological conditions (human serum, 37°C, in vitro), the percentage of gadolinium released from ligands varied from nearly 0% for gadopentetate to 20–35% for gadodiamide (31). [Frenzel and colleagues (31) measured the liberation of gadolinium ion from a number of different ligands in vitro, incubating the substances in human serum at 37°C. Despite demonstrating that gadoversetamide having the greatest propensity to release gadolinium, ranging from 30 to 45% after 15 days of incubation, and Magnevist among the agents with the least release (2% over the same time period), Magnevist has been associated with several cases of systemic fibrosis.] Despite these differences, a mechanism to explain why certain formulations seem to carry less risk than others, gadopentetate is still the second most common formulation tied to systemic fibrosis (80, 85). This suggests that very minute quantities of gadolinium meet the threshold to trigger the disorder, or the mechanism and degree of gadolinium release in the body are different than the in vitro setup.

Gadolinium from contrast-treated animals accumulates primarily in the liver. Gadodiamide-treated mice (with intact renal function) demonstrated residual 153Gd 14 days after exposure (97). Contrary to all in vivo publications using gadolinium-based contrast and our own experience with gadodiamide, GdCl3 has a profound effect on liver histology (92). Furthermore, in mice with renal insufficiency, the biodistribution of gadolinium deposition is altered in a manner where the liver is by far the largest reservoir organ, especially with respect to the skin (99). John Haylor et al. (44) (Academic Nephrology Unit, Medical School, Sheffield, UK) found that when rats with an 80% nephrectomy were given a single dose of gadodiamide (2.5 mmol/kg), the accumulation of gadolinium in the skin was remarkably low. Of particular interest is the relative accumulation of gadolinium in the liver with respect to the skin (44). Tissues obtained from autopsy analyzed with energy-dispersive X-ray spectroscopy had detectable gadolinium in the skin, liver, lungs, intestinal wall, kidney, and skeletal muscle. The liver was noted to have chronic venous stasis, but not fibrosis (87).

Perhaps it is not the dose of gadolinium-based contrast, but the cumulative lifetime exposure that increases the risk or severity of systemic fibrosis (17). After all, one retrospective study found the odds ratio for systemic fibrosis in hemodialysis patients was 6.7, but this increased to 44.5 after having multiple exposures (79).

Gadolinium exposure and renal insufficiency cannot be the sole and rigid effectors of systemic fibrosis; many patients with end-stage renal disease on chronic dialysis do not acquire the disease, despite several exposures to gadolinium-based contrast (72).

It should be mentioned that there are a few cases that resemble gadolinium-associated systemic fibrosis, but no history of contrast exposure could be found. A 19-yr-old underwent a noncontrast magnetic resonance imaging scan before a heart transplant, yet he developed symptoms 3 mo later. In another, a 22-yr-old woman received a cadaveric renal transplant after 5 yr of chronic hemodialysis. Within 6 wk, she had signs of systemic fibrosis, which was congruent with her skin biopsy. There was no evidence of exposure to either magnetic resonance imaging or gadolinium exposure (102). Another woman, 34 yr of age, underwent a transplant in her mid-20s. After this allograft failed, she began hemodialysis. Despite not
having a history of magnetic resonance imaging contrast exposure, she developed fibrotic symptoms with a biopsy consistent with the disease (94). A man in his 50s underwent a kidney transplant in 1999 for end-stage renal disease. Shortly after the transplant, he developed spontaneous hyperpigmented plaques and firm nodules on his trunk and extremities. He did not have a history of gadolinium exposure, and the only magnetic resonance imaging scan was 5–6 yr after the transplant and onset of symptoms. Skin biopsy showed wall-to-wall fibroblast-like cells with disorganized collagen (11). Whether the donors of these organs had undergone gadolinium-enhanced radiological procedures was not reported. (Therefore, we entertain the possibility that systemic fibrosis can be transferred by a transplanted solid organ.)

**Incidence and Prevalence**

Ten percent of patients with gadolinium-associated systemic fibrosis have never been dialyzed (86). In a retrospective study of patients referred to an academic nephrology group, 18% had been diagnosed with the disorder (69). Men and women appear to be equally susceptible, with an age range from 8 to 87 yr old (98).

**Diagnosis**

Some of the differential diagnoses for gadolinium-associated systemic fibrosis are provided in Table 3. The typical scenario occurs in patients with impaired renal function (acute or chronic) after exposure to gadolinium contrast. [However, gadolinium exposure is not requisite for the diagnosis; the full criteria are listed in Girardi et al. (35).] Gadolinium-associated systemic fibrosis resembles scleroderma, yet specific anatomic locations are involved (16). Patients present with lower extremity pain, paresthesias, or pruritus. Findings include edema, plaques and firm nodules on his trunk and extremities. He did not have a history of gadolinium exposure, and the only magnetic resonance imaging scan was 5–6 yr after the transplant and onset of symptoms. Skin biopsy showed wall-to-wall fibroblast-like cells with disorganized collagen (11). Whether the donors of these organs had undergone gadolinium-enhanced radiological procedures was not reported. (Therefore, we entertain the possibility that systemic fibrosis can be transferred by a transplanted solid organ.)

**Table 3. The differential diagnoses of gadolinium-associated systemic fibrosis (24)**

<table>
<thead>
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<th>Diagnosis</th>
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<tr>
<td>Amyloidosis</td>
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<td>α2-Microglobulin amyloidosis</td>
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<td>Borréliose</td>
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<td>Calciphylaxis</td>
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<td>Carcinoid syndrome</td>
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<td>Chronic graft vs.host disease</td>
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<td>Dermatomyositis</td>
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<tr>
<td>Drug-induced fibrosis [silica, polyvinyl chloride, toxic (“Spanish”) oil] (33)</td>
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<tr>
<td>Fibroblastic rheumatism (76)</td>
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<tr>
<td>Lipodermatosclerosis</td>
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<tr>
<td>Melorheostosis</td>
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<tr>
<td>Early cellulitis</td>
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<tr>
<td>Early panniculitis</td>
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<tr>
<td>Eosinophilic fasciitis</td>
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<tr>
<td>Eosinophilia-myalgia syndrome</td>
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<tr>
<td>Phenyleketonuria</td>
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<tr>
<td>Porphyria cutanea tarda</td>
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<tr>
<td>Pretibial myxedema</td>
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<tr>
<td>Progeria</td>
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<td>Scleredema of Buschke</td>
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<tr>
<td>Scleroderma</td>
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<td>Scleromyxedema (77)</td>
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<td>Systemic sclerosis/morphea</td>
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have contractures with a concomitant compromise in the range of motion. The fibrosis of skeletal muscle and a number of visceral organs have been attributed to gadolinium. The face, neck, and upper trunk are typically spared (other than occasional yellow plaques in the ocular sclerae) (35).

**Treatment**

There are no consistently effective treatments for gadolinium-associated systemic fibrosis (67).

**Histology and Immunohistology**

The index cases of gadolinium-associated systemic fibrosis were detailed by Shawn Cowper and colleagues in 2000 (21). Skin biopsies demonstrate dermal fibrosis with a significant increase in cellularity, but no inflammation (59). Often the cells are described as being spindle shaped (61, 77), fibroblasts (6), fibroblast like (56), fibrohistiocytic (68, 75, 82), or “polygonal epithelioid fibroblasts” (6). With the use of light microscopy, the dermal collagen is notably increased (27), with bundles haphazardly arranged (56) or “disorganized” (48). Mucin may be present (29, 55, 61, 105), but is not required for the diagnosis (59). The abundant, thin, and spindle-shaped cells often express CD34 and type I (immature) procollagen (22, 24, 59).

**Pathogenesis**

**Role of fibrocytes, both bone marrow derived and resident.** The histology of gadolinium-associated systemic fibrosis-affected skin is remarkable for an increase in cellularity, with CD34- and procollagen I-expressing spindle cells, occasional histiocytes, and factor XIIIa+ dendritic cells (22, 59). Among markers of hematopoietic stem cells, CD34 is one of the first identified and commonly used as such (32). It can be expressed by vascular endothelia and mesenchymal cells of myeloid origin. CD34- and collagen type I-positive cells that are recruited from the circulation have been termed “fibrocytes” (13). Fibrocytes are unique in that they are peripheral blood cells that have the potential to generate matrix and may be important in the proliferative stages of wound repair (84). 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 healing), and these cellular markers, it has been hypothesized that the fibrosis is mediated by circulating fibrocytes (20, 51, 84).

**Effect of gadolinium-based contrast on fibroblasts in vitro.** The expression of α-smooth muscle actin stress fibers is an indication of an activated myofibroblast (94). When quiescent human foreskin fibroblasts are treated with a clinically relevant dose of gadodiamide (0.2 mM), they exhibit α-smooth muscle actin stress fibers in addition to an accumulation of fibronectin, a correlate of extracellular matrix accumulation. These doses of contrast do not elicit a very robust proliferative or toxic effect (26).

Are myeloid cells present in gadolinium-based contrast-induced fibrotic lesions? As there was no experimental proof that the cellularity of the lesions was even partially due to circulating, bone marrow-derived fibrocytes, we conducted an experiment to test the hypothesis (101). Rats with 5/6 nephrectomies (to model renal insufficiency) underwent lethal irradiation, followed by salvage bone marrow transplantation from...
tagged donors (Fig. 2A). These donors expressed an antigen, the human placental alkaline phosphatase, that permitted the differentiation of myeloid from host cells in the recipients. After an engraftment period, animals were treated with gadolinium-based contrast over a 4-wk period (corresponding to the treatment duration published by Sieber and colleagues in the first rat model of gadolinium-associated systemic fibrosis) (90). Both the control and contrast-treated groups tolerated the entire procedure. On occasion, animals demonstrated signs of stress (e.g., periorbital porphyrin staining), and sometimes even gross skin changes (Fig. 2B). Histologically, skin from the contrast-treated animals demonstrated greater epidermal thicknesses, severe dermal fibrosis, and an increase in dermal cellularity (Fig. 2C). Immunofluorescent staining of the dermis revealed a large percentage of myeloid cells in the diseased skin from the contrast-treated animals (Fig. 2D). These cells also demonstrated a rich expression of α-smooth muscle actin stress fibers. Levels of fibronectin were increased in skin from the contrast-treated animals (Fig. 2E). Finally, the common fibrocyte markers CD34 and procollagen type I were both markedly increased in the skin from the contrast-treated animals, and each very much expressed by the myeloid cells (Fig. 2F). These experiments proved that bone marrow-derived fibroblasts comprise the lesion of gadolinium contrast-induced systemic fibrosis. However, the increase in cellularity was not entirely from myeloid cells; there is an increase in both bone marrow-derived and host/resident cells in the dermis (101).

Comparison of “low” with “high” thermodynamic stability gadolinium-based contrast agents on severity of fibrotic lesions. As mentioned, there are a number of different formulations of gadolinium-based contrast, and these have slightly different properties. The volumes of distribution are essentially

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**Fig. 2.** Myeloid cells comprise much of the cellularity of gadolinium-based, contrast-induced lesions. A: it has been presupposed that bone marrow-derived circulating cells are the mediators of gadolinium contrast-induced systemic fibrosis. Transgenic rats “tagged” with a human placental alkaline phosphatase antigen served as bone marrow donors to lethally irradiated recipients with 5/6 nephrectomies (as a model of chronic kidney disease). After an engraftment period, one group was treated with gadolinium-based contrast (2.5 mmol/kg intraperitoneally daily, for a total of 20 doses over 4 wk) (101). B: this type of experiment has been conducted several times in our laboratory. Occasionally, animals will demonstrate gross dermatological changes, ranging from hyperpigmentation to punctate ulcerations and eschars. C: histologically, skin changes in rats resembles that found in humans; there is an increase in epidermal thickness, disorganized collagen bundles in the dermis, and an increase in dermal cellularity. D: dermis from the contrast-treated animals demonstrate a marked increase in myeloid cellularity. These cells often express α-smooth muscle actin-positive stress fibers. Immunofluorescence is shown of frozen skin (original magnification ×40). E: immunoblot of fibronectin, a marker of fibrosis, which is increased in the skin from contrast-treated animals. F: the fibrocyte markers procollagen type I (procol) and CD34 are increased in the dermis from contrast-treated animals. This pattern resembles what has been reported in humans afflicted with gadolinium contrast-induced systemic fibrosis. Note that some of the myeloid cells express these markers. DAPI, 4,6-diamidino-2-phenylindole; HPAP, human placental alkaline phosphatase; α-SMA, α-smooth muscle actin.
the same, and they are excreted by the kidney. It does appear that the pinocytosis of gadolinium-bound ligands relates to the thermodynamic stability (in vitro, demonstrated in rat hepatoma and glioma cells) (14). From a nephrologist’s perspective, many of us are concerned about administering gadolinium-based compounds, regardless of what narrative reviews and editorials champion, particularly with regard to the safety of one agent over another. The theories concerning why one ligand may be safer than another are entirely grounded in observational case reports and have not been subjected to a prospective experimental testing. Therefore, we treated rats with 5/6 nephrectomies with either a “low” or “high” thermodynamic stability contrast agent (i.e., gadodiamide and gadoteridol, respectively) for 4 wk (26). Again, contrast-treated animals rarely developed gross lesions. Microscopically, though, the skin from the gadodiamide-treated group again showed epidermal thickening, dermal fibrosis, and increased dermal cellularity. The increase in dermal cellularity and the markers of fibrosis were less in the animals treated with gadoteridol. However, the skin of the gadoteridol-treated animals did manifest an increase in transforming growth factor-β1 (TGF-β1). Perhaps the degree of fibrosis would have been similar if longer durations of treatment were conducted, or if a recovery period was added to the end of the experiment. Both gadodiamide and gadoteridol induced greater dermal procollagen I, CD34, and factor XIIIa levels with respect to the untreated controls. Gadolinium was found in a number of organs in addition to the skin, yet by histology and immunofluorescence, fibrosis was not detected in

Fig. 3. Reactive oxygen species mediate gadolinium contrast-induced systemic fibrosis. A: tempol, a superoxide dismutase mimetic, was concomitantly administered (in the drinking water) to rats in a model of contrast-induced fibrosis (101). B: skin from rats treated with magnetic resonance imaging contrast showed dermal fibrosis and an increase in dermal cellularity. This effect was abrogated in the group where tempol was coadministered. Hematoxylin and eosin: top, ×10 objective; bottom, ×40 objective. Calibration bar = 0.05 mm. C: gadolinium contrast-induced skin fibronectin is normalized by coadministration of tempol. D: tempol suppresses the gadolinium-based contrast increases of dermal fibrocyte marker CD34 and α-smooth muscle actin (α-SMA) positive stress fibers. E: magnetic resonance imaging contrast increases skin NADPH oxidase isoform 4 (Nox4) levels. F: animals that received tempol demonstrated less oxidative stress than those subjected to magnetic resonance contrast alone.
many of the metal-laden organs (other than the kidney) (26). Because the liver should be a sink for liberated gadolinium, yet remains histologically intact, perhaps there is an antifibrotic mechanism that is specific to this and other organs.

**Reactive oxygen species and gadolinium-based contrast-induced fibrosis.** It has been documented that reactive oxygen species are required for the fibrogenic processes and myofibroblast activation taking place in numerous organs undergoing injury, including the kidney, the heart, the lung, and the liver (7, 57, 60, 89). In general, fibrosis is a pathological response typically preceded by damage to the endothelial/epithelial barriers, the release of TGF-β, the recruitment of inflammatory cells, the induction of reactive oxygen species, and the deposition of collagen and extracellular matrix (58). The generation of reactive oxygen species may mediate the transdifferentiation of myofibroblast progenitor cells (46). Profibrotic pathways are redox dependent in numerous cell types, including fibroblasts (39, 100). Therefore, we examined the effect of a superoxide dismutase mimetic, tempol (104), on gadolinium-induced systemic fibrosis (Fig. 3A) (101). Again, when comparing the contrast-treated with the control animals, the former demonstrated thickening of the epidermal layers and a great increase in dermal cellularity (Fig. 3B). When contrast-treated animals were given tempol, the fibrosis and dermal cellularity were greatly attenuated. The same effect was apparent with skin fibronectin quantity (Fig. 3C): tempol abrogated the contrast-induced increase. Similarly, contrast-induced increases in dermal CD34 and cells expressing α-smooth muscle actin-containing stress fibers were reduced by tempol coadministration (Fig. 3D).

The reactive oxygen species generated by the NADPH oxidases of the Nox family have been involved as key mediators of renal, cardiac, lung and liver fibrosis (7, 37, 41, 65). To date, the Nox family comprises seven members: Nox1–Nox5, dual oxidase (Duox) 1, and Duox2 (36, 37, 40). We noted that the expression of NADPH oxidase isoform Nox4 in the skin, but not that of Nox2 and Nox1 isoforms, paralleled the increase of fibronectin when animals were treated with gadolinium contrast (Fig. 3E). Notably, evidence of oxidative stress in the dermis, measured by dihydroethidium staining, was suppressed in the contrast-treated animals given tempol (with respect to the animals given contrast only, Fig. 3F). Therefore, the generation of reactive oxygen species, possibly by Nox4, may be an early mediator of the fibrotic process.

Interestingly, a wide array of evidence shows that Nox4-derived reactive oxygen species play a major role in the pathogenesis of renal, cardiac, lung, and liver fibrosis (9, 36, 37, 40, 45, 49, 63, 65). Importantly, Nox4 has also been reported to be a primary target of TGF-β and a critical mediator of its profibrotic actions in various cell types (7, 36, 40, 45). It should be pointed out that pharmacological inhibitors of Nox4 are available and have undergone preclinical studies in animal models of fibrotic diseases, where they successfully attenuated the pathological changes observed in renal complication of diabetes, atherosclerosis, liver fibrosis and idiopathic pulmonary fibrosis (5, 9, 36, 37, 40, 49, 50, 63). Therefore, Nox4 may represent an attractive therapeutic target for prophylaxis or the treatment of gadolinium-associated systemic fibrosis.

Overall, we have demonstrated that gadolinium-based contrast administration in rats can reproduce the pathology of systemic fibrosis witnessed in humans. Perhaps decreased renal elimination of the gadolinium-ligand complex promotes the liberation of gadolinium as an initial step, but this still has yet to be experimentally proven. The next conjecture is that gadolinium deposition in tissue initiates the release of profibrotic chemokines. Regardless, we do have the experimental proof that there is a recruitment of myeloid spindle-shaped cells concomitant with increases in CD34, α-smooth muscle actin stress fibers, and procollagen I (as well as collagen types I and IV, and factor XIIIa) (101). Our hypothesis is that the generation of reactive oxygen species is involved in gadolinium-
associated systemic fibrosis. The fact that dermal Nox4 is concomitantly increased (unlike other Nox isoforms), together with its status as key player in organ fibrosis, implicates this protein not only as an early mediator of the disease, but also as a therapeutic target. There are small molecular inhibitors specific to Nox4 that successfully passed phase I clinical trials and are currently being tested for the mitigation of diabetic nephropathy in phase II studies (38) that may prove to be prophylactic or therapeutic for this condition, too. Figure 4 depicts the possible molecular mechanisms implicated in gadolinium-based contrast induced fibrosis.

Conclusions

Gadolinium-associated systemic fibrosis is a rare condition. From 1997 to 2009, the number of cases increased from 15 to 304 (18, 21). The incidence of contrast-induced fibrosis is declining, as patients with renal disease are being excluded from gadolinium-enhanced studies. In one retrospective study of patients referred to an academic nephrology subspecialty group, a remarkable 18% were diagnosed with the disorder (69).

The discovery that gadolinium-based magnetic resonance imaging contrast, combined with renal impairment, is a strong risk factor for systemic fibrosis has led to drastic changes in how diagnosticians approach patients. Gadolinium-based contrast-induced systemic fibrosis is a chronic, incurable, and ghastly disease that leads to extreme suffering, increased morbidity, and increased mortality. Those at risk are a subset already burdened by chronic disease, compounding the tragedy. It is estimated that over 20 million United States citizens are afflicted with renal disease. That patients with renal insufficiency will be exposed to gadolinium-based contrast remains a risk. Because so little is known about this new and iatrogenic disorder, clinicians need to be guarded. How gadolinium accelerates and directs skin fibrosis only in the milieu of renal insufficiency will provide us an opportunity to design better gadolinium-based contrast agents, provide information about the physiology of scarring and the pathophysiology of other sclerotic conditions, and could lead to improvements in the understanding of wound healing: this is still a knot that needs unraveling.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

B.W. conception and design of research; B.W. performed experiments; B.W. analyzed data; B.W. interpreted results of experiments; B.W. and Y.G. prepared figures; B.W. drafted manuscript; B.W., V.D., and Y.G. edited and revised manuscript; B.W., V.D., and Y.G. approved final version of manuscript.

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