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Involvement of the Renin Angiotensin System in the Development of Nephrogenic Systemic Fibrosis-like Lesions in the RenTag Mouse Model

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Abstract

Background—Renin-angiotensin system (RAS) activation increases angiotensin II production which stimulates profibrotic factors especially in the setting of chronic kidney disease.

Nephrogenic systemic fibrosis (NSF) is associated with gadolinium (Gd) exposure and renal failure. RAS involvement in NSF is unclear compared to transforming growth factor beta and Smad. RenTag mice were chosen to investigate the role of RAS in NSF-like dermal fibrosis because they demonstrated dermal fibrosis at birth, perturbations of RAS in subcutaneous tissue, and renal failure within 4 weeks of age.

Methods—Wild-type and RenTag mice were injected weekly with intravenous gadodiamide (3.0mmol/kg body weight) and sacrificed at 12 weeks of age for skin and kidney histology.

Results—RenTag mice had elevated BUN levels, pitted kidneys, and glomerular damage. RenTag mice skin revealed an increased density of fibroblasts, no mucopolysaccharide deposits, and increased collagen fibril density regardless of Gd exposure. Skin and kidney histopathology of wild-type mice were normal regardless of Gd exposure. CD34 positivity was higher in RenTag compared to wild-type.

Conclusions—Since RenTag's dermal lesions remained unchanged after gadolinium exposure in the setting of renal failure this animal model suggests perturbations of subcutaneous RAS may be involved in Gd-naïve dermal fibrosis.

Keywords

Renin; Renin-Angiotensin System; Transgenic; Nephrogenic Systemic Fibrosis; Gadolinium; Animal model

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CONFLICT OF INTEREST

The authors state no conflict of interest.

INTRODUCTION

There is accumulating evidence in support of a relationship between renin-angiotensin system (RAS) activation and systemic fibrosis especially in the setting of chronic kidney disease (CKD) [1, 2]. Pharmacologic blockage of RAS improves prognosis in chronic nephropathies [3–6]. In renal fibrosis, angiotensin converting enzyme (ACE) and angiotensin II levels are increased which activates transforming growth factor- β (TGF- β) which stimulates matrix accumulation and inflammation [7–14].

Nephrogenic systemic fibrosis (NSF) is a disorder of widespread tissue fibrosis and has been associated with end stage renal disease (ESRD) as well as gadolinium-based contrast agent (GBCA) exposure [15]. NSF histology demonstrates hypercellularity with an increase in number of fibroblasts, increase in collagen density in the skin and the subcutis, with or without mucin deposits which clinically results in a thickening and hardening of the skin of the extremities with a ‘wooden’ appearance, and joint contractures [15]. Patients with NSF may improve with restoration of renal function either spontaneously or with renal transplantation. Although our understanding of NSF continues to evolve its pathogenesis remains unclear.

The current pathogenetic model for NSF supports the role of aberrantly functioning circulating fibroblasts coupled with the elaboration of fibrogenic factors.

Immunohistochemical studies show that a dual positive CD34/procollagen spindle cell is the dominant cell type induced in NSF [15, 16]. CD34 is a marker for dermal stromal cells including dendrocytes and fibroblasts derived from peripheral blood that are positive for procollagen I [17–19]. CD34 positive cells are also found in the vascular endothelium. It is likely that a circulating fibroblasts which has the capacity of synthesizing collagen I/III and transforming growth factor- β 1 (TGF- β 1) is recruited, activated, and proliferated from the circulation to the dermis in response to either gadolinium deposition in tissues or with the concomitant use of erythropoietin [17–19].

The renin promoter SV40 T-antigen transgenic mouse model RenTag was employed to investigate the underlying pathogenesis of nephrogenic systemic fibrosis-like dermal lesions [20].

MATERIALS AND METHODS

The transgenic mouse model was created by fusing 4.6 Kilobases (Kb) of the 5′ flanking region of the mouse Ren2 gene to the SV40 T antigen structural gene and deriving transgenic founders by pronuclear injection [21]. The line was maintained on a Ren1c genetic background of C57BL and C3H origin and constructed with the intent of deriving renin-expressing cell lines by directed oncogenesis. This was successfully accomplished and a cell line, the AS4.1 cell line (ATCC CRL2193), established from a tumor which arose in kidney, has been in culture for over 15 years [21]. T antigen was found to accurately report the known expression pattern for renin temporally and spatially. This reflects that fact that the 4.6 Kb of 5′ flanking sequence utilized has been shown to harbor the key evolutionarily

conserved cis-acting transcriptional regulatory elements required to promote renin transcription [21].

RenTag and wild-type mice were divided into several groups. In the RenTag and wild-type control groups no treatment was given nor any gadolinium administered. The remaining RenTag and wild-type mice were administered a weekly intravenous dose of gadodiamide at 3.0 mmol/kg body weight beginning at ages 6 or 10 weeks (Table 1). All mice were sacrificed at 12 weeks of age and skin biopsies were sent to an independent analysis laboratory for histology and biochemical staining. The skin biopsies were stained with Hematoxylin and Eosin for fibroblasts, Alcian blue for mucopolysaccharide deposits, and Picrosirius red for collagen density to identify exacerbations of NSF-like lesions after gadodiamide exposure. Immunohistochemistry was performed to detect the presence of CD34 positive dermal spindle cells which are abundant in NSF and involved in dermal fibrosis [22, 23].

RESULTS

Wild type mice without Gd had a normal average BUN of 23.7 mg/dl $\pm$ 3.5 (normal BUN 23.8–32.5mg/dl)[24]. All RenTag mice had elevated BUN levels. RenTag without Gd had an average BUN value of 49.8 mg/dl $\pm$ 10.4 (Table 2). RenTag mice exposed to intravenous Gd starting at 6 weeks of age had an average BUN of 45.7mg/dl $\pm$ 17.2. RenTag mice treated with Gd beginning at 10 weeks had an average BUN of 81.3 mg/dl $\pm$ 19.2. Histopathology of the RenTag kidney revealed increased mesangial cell proliferation and mild interstitial fibrosis compared to wild-type mice (Figure 1). RenTag mice were also found to have more glomerular damage, tubular damage, tubular regeneration, pitting, spindle cell proliferation, and basement membrane thickening compared to wild-type mice.

RenTag mice treated with and without Gd demonstrated an increased density of fibroblasts and elastic fibers in the dermis, increased collagen fibril density, and no mucin deposition consistent with histopathology found in NSF (Figure 2). There was no observable exacerbation of the histopathological skin findings in RenTag mice treated with intravenous Gd compared to pretreatment histology. RenTag mice had stronger CD34 antibody positivity compared to wild-type mice (Figure 3). Skin biopsies for wild-type mice with and without gadodiamide treatment were negative for histopathological features of NSF (Table 3).

DISCUSSION

RenTag mice have dermal fibrosis which correlates with nephrogenic systemic fibrosis (NSF) on gross examination and histology. Contrary to NSF, RenTag skin lesions were not exacerbated after supratherapeutic dosages of gadodiamide in the presence of renal failure. These findings suggest that perturbations in the renin-angiotensin system, perhaps initiated in utero, may have profound effects on fibroblasts stimulating a significant increase in fibroblast growth, spindle cell proliferation, increased collagen density, and induction of a myofibroblast phenotype, which may be driving the underlying mechanism of dermal fibrosis in the RenTag mouse [25].

RenTag Phenotype, Histology, and Immunohistochemistry

RenTag mice were found to have more glomerular damage, tubular damage, tubular regeneration, pitting, spindle cell proliferation, and basement membrane thickening compared to Wild-type mice. Our study was consistent with other studies which demonstrated RenTag mice with severe vascular lesions and marked atypical hyperplasia of vascular smooth muscle [26]. RenTag had cellular transformation and overt tumor formation in renin-expressing cells of the kidney and subcutaneous tissues as well as endocrine glands such as the submandibular, adrenals, and testes [26]. Furthermore, kidneys of RenTag mice without parenchymal tumors appeared pale and ischemic and weighed one-third that of negative littermates kidneys [26]. Narrowing and occlusion of lumen vessels were also noted in RenTag arterial walls presumed to be due to marked proliferation of smooth muscle cells in the medial layer of the arterial wall with mild to moderate secondary intimal fibrosis [26]. These changes were noted along interlobar, arcuate, and interlobular branches of the renal arteries, as well as afferent arterioles often surrounded by inflammatory cells with focal obstruction of the collecting tubules and dilatation and degeneration of proximal tubules. Glomeruli were noted to be hypercellular with nuclear polymorphism and hyperplastic cells were located in the mesangial stalk. The endothelial cells generally appeared unaffected [26].

There was a strong correlation of NSF-like histopathological features in RenTag mice regardless of Gd treatment. RenTag NSF-correlating features in histology and immunohistochemistry included increased background spindle cells in the dermis as shown in hematoxylin and eosin staining (H&E), thickened collagen bundles, no mucin deposits, and CD34 positivity. When comparing RenTag skin lesions to NSF lesions it is important to note that in some cases of NSF mucin is absent [15].

Elevated renin levels in fibrosis

Elevated renin levels are involved in fibrosis of the heart, liver, and kidneys [27]. Activation of the renin-angiotensin system causes an increase in AII, which stimulates profibrotic factors including connective tissue growth factor (CTGF), endothelin-1, plasminogen activator inhibitor-1, matrix metalloproteinase-2 (MMP-2), and tissue inhibitor of metalloproteinase-2 [28–30], all contributing to renal damage and progressive fibrosis. In renal fibrosis, activated fibroblasts may proliferate and invade the periglomerular and peritubular spaces contributing to matrix deposition in the tubulointerstitial area [28–30]. AII also has cytokine-like properties that can allow it to regulate inflammation and fibrosis via the production of growth factors such as tumor necrosis factor alpha, interleukin 6, monocyte chemotactic protein-1, and nuclear factor B [28–30]. Therefore, it is possible that both angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARB) may exhibit their anti-fibrotic effects via the down regulation of these inflammatory markers.

There is accumulating evidence in support of the relationship between the RAS and fibrosis in cutaneous diseases. Patients with systemic sclerosis have elevated angiotensinogen in their skin and their scar tissue has increased ACE activity and increased angiotensin receptors [27]. It is possible that skin lesions are mediated by a cutaneous specific RAS [31].

Experiments with murine fetal and newborn mice revealed the existence of processed renin mRNA in fetal and newborn subcutaneous tissue [31] which indicates there is a renin-expressing cell population in fetal and newborn subcutaneous tissue.

Mice with AII infused into skin have increased dermal fibrosis, collagen synthesis/deposition, and TGF- β activation, number of myofibroblasts, skin inflammation, and infiltration of fibroblastic cells in the upper dermis as well as endothelial-to-mesenchymal transition [27] which is similar to our findings in the RenTag mice. Moreover, AII skin infusion induced inflammation and fibrosis via MCP1 upregulation and accumulation of activated fibroblasts suggesting that populations of fibroblasts originate from circulating blood cells [27]. Since II infusion induced dermal fibrosis it is likely that perturbations in the renin-angiotensin system are involved in the pathogenesis of NSF-like lesions in RenTag mice.

Clinical implications for the RenTag model and evidence of gadolinium-naïve NSF

Although there is a strong correlation of gadodiamide exposure in renal failure patients to the development of NSF, a causative relationship has yet to be established. Studies in Wistar rats failed to demonstrate NSF-like lesions after exposure to exponentially high doses of gadodiamide. Langer *et al.* conducted experiments with naïve Wistar rats treated with intraperitoneal injections of gadodiamide and several other gadolinium-based contrast agents and found that none of the rats developed NSF-like skin lesions [33]. Similarly, Grant *et al.* conducted experiments exposing naïve and 5/6 nephrectomized Wistar rats to high doses of intravenous gadodiamide and gadopentetate dimeglumine [34]. After treatment, the rats failed to show any increase in fibroblasts, collagen thickening, or mucin deposition. These animal models failed to show a relationship between gadodiamide exposure and NSF-like lesions.

RenTag mice had renal failure, widespread skin fibrosis, and increased fibroblasts/spindle cell proliferation, increased collagen bundle density/fibril thickness, and increased CD34 antibody positivity. NSF occurs with and without mucin deposition [15] and RenTag mice had no increase in mucin deposition. Since the RenTag mice developed skin lesions similar to those of NSF in utero and these lesions were not exacerbated by gadodiamide we conclude in accordance with others [33,34] that gadolinium may not be the only etiology for NSF development and other factors may be involved. Also, we cannot rule out that perturbation of the RAS maximally activated the fibrotic process so the additional exposure to gadodiamide had no further effect.

The development of gadolinium naïve NSF is rare. Wahba *et al.* reported two NSF patients without prior exposure to gadolinium [35]. The first patient was a 19-year-old woman who developed end stage renal disease secondary to cardiogenic shock and cardiorenal syndrome immediately after orthotopic heart transplantation. Three months after the heart transplantation she developed NSF plaques on her lower extremities. Extensive review of the records failed to show any exposure to gadolinium. The second patient was a 22-year-old woman with end stage renal disease secondary to systemic lupus erythematosus. Six weeks after renal transplantation she developed macules papules and a bosselated peau d'orange

appearance of the upper and lower extremities consistent with NSF. Review of medical records also did not reveal any evidence of gadolinium exposure [35].

Limitations of our study

The main limitation of the present study is that the RenTag mouse model is an artificial system and RAS expression may have occurred very early in development. In contrast, in a study of eleven NSF patients it was found that NSF skin lesions were associated with TGF- β and Smad without evidence of RAS [36]. Furthermore, TGF- β and Smad were detected in eight of the eleven samples, ACE was minimally detected in two samples, and AT1 was not detected in any samples.

Conclusions

NSF is a debilitating disease whose etiology and pathogenesis is unclear. The RenTag model suggests that the activation of RAS and production of AII, and perhaps the recruitment of pericytes, may lead to the development of NSF-like lesions. RAS may play an integral role in the pathogenesis of gadolinium-naïve NSF since these lesions were not exacerbated by gadodiamide. Further studies to elucidate the role of the RAS in peripheral fibrosis are warranted so that therapeutic targets for the treatment of NSF and other fibrotic diseases can be established.

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Figure 1. Kidney histology for wild type mice without Gd treatment (a). RenTag without Gd (b). and RenTag with Gd beginning at 6 weeks (c) demonstrating increased interstitial fibrosis.

Figure 2.

Skin histology for wild type without Gd (a1–a3). RenTag without Gd (b1–b3). and RenTag with Gd at 6 weeks in H&E detecting increased fibroblasts and spindle cell proliferation. Aldan blue detecting no mucin deposits, and Picrosirius red detecting increased collagen bundles taken at 200X. Key: Collagen (C). Dermis (D), and Epidermis (E).

Figure 3. Immunohistochemical staining for CD34 in dermal lesions of wild type mice without Gd (a), RenTag without Gd (b), and RenTag with Gd beginning at 10 weeks (c).

Table 1
Experimental methodology for administration of Gd to RenTag and Wild type animals.

Mice per group (n)	GBCA	Weekly Dose of IV GBCA	Age of mice at first dose of IV GBCA (weeks)	Number of doses (n)	Endpoint (weeks)
RenTag (2)	–	–	–	–	12
RenTag (5)	Gadodiamide (Omniscan®)	3.0 mmol/kg BW	10	2	12
RenTag (4)	Gadodiamide (Omniscan®)	3.0 mmol/kg BW	6	6	12
Wild-type (2)	–	–	–	–	12
Wild-type (4)	Gadodiamide (Omniscan®)	3.0 mmol/kg BW	10	2	12
Wild-type (3)	Gadodiamide (Omniscan®)	3.0 mmol/kg BW	6	6	12

Table 2

Average BUN in mg/dl of RenTag and Wildtype mice.

Groups (n)	Average BUN (mg/dl)	Standard deviation
Wild type without Gd (3)	23.7	3.5
RenTag without Gd (5)	49.8	10.4
RenTag with Gd at 6 weeks (7)	45.7	17.2
RenTag with Gd at 10 weeks (4)	81.3	19.2

* Normal BUN values in mice: 23.8 – 32.5mg/dl.

Table 3

Histopathology of wild-type and RenTag skin biopsies at 12 weeks of age.

Mice per group (n)	Total GBCA treatments (n)	Fibroblast/Spindle cell proliferation	Collagen bundle density	Fibril thickness	Mucin/mucosubstance deposits	Alcian blue for fibrils	Alcian blue for matrix	CD34 antibody positivity
RenTag (2)	–	+	+	+	–	+	–	+++
RenTag (5)	2	++	++	+++	–	+	–	++
RenTag (4)	6	+	+	++	–	+	–	++
Wild-type (2)	–	–	–	–	–	+	–	+
Wild-type (4)	2	–	–	–	–	+	–	+
Wild-type (3)	6	–	–	+	–	+	–	+

Key: (–) = no change; (+) = mild increase; (++) = moderate increase; (+++) = marked increase.