

## Pathophysiology of nephrogenic systemic fibrosis: A review of experimental data

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### Abstract

Since the association between nephrogenic systemic fibrosis (NSF) and gadolinium contrast agents (Gd-CAs) was suggested in 2006, several experimental studies have been published to elucidate the role of these agents in the pathogenesis of NSF. Low stability Gd-CAs have a stimulant effect on human skin and fibroblasts in culture and modulate the production of collagen by these cells. Low stability agents have also induced NSF-like skin changes in a rat model with normal renal function after multiple repeat administrations. The role of the 5/6 subtotal nephrectomy rat model in investigating NSF remains under evaluation.

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**Key words:** Experimental models; Gadolinium-based contrast agents; Nephrogenic systemic fibrosis; Pathophysiology

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### INTRODUCTION

Extracellular gadolinium contrast agents (Gd-CAs) are chelates containing the gadolinium ion ( $Gd^{3+}$ ) which has been associated with the development of nephrogenic systemic fibrosis (NSF) in patients with a marked reduction in renal function<sup>[1,2]</sup>. The epidemiology of NSF associated with Gd-CAs suggests that the stability of the Gd-chelate is an important factor in the pathogenesis of this condition<sup>[3]</sup>. Stability refers to the relative tendency of the  $Gd^{3+}$  to remain coordinated (attached) to the chelating ligand. Free  $Gd^{3+}$  is highly toxic and can cause splenic degeneration, central lobular necrosis of the liver, enzyme inhibition, calcium channel blockade and a variety of haematological abnormalities<sup>[3]</sup>. Therefore, it is crucially important that  $Gd^{3+}$  should be strongly attached to a ligand to avoid its toxic effects. The configuration of the Gd-CA molecule is either linear (gadopentetate, gadobenate, gadodiamide, gadoversetamide, gadofosveset and gadoxetate) or cyclic in which the  $Gd^{3+}$  ion is "caged" in the pre-organized cavity of the ligand (gadoterate, gadobutrol and gadoteridol), and are available as ionic (where the remaining carboxyl groups are saltified with meglumine or sodium) or non ionic (in which the number of carboxyl groups is reduced to three, neutralizing the three positive charges of the  $Gd^{3+}$ ) preparations. There are differences in the chemical stability of these agents and, therefore, liability to release free gadolinium ions. The binding to  $Gd^{3+}$  is relatively weak in the non ionic linear molecule, whereas the cyclic molecule offers better protection and binding to  $Gd^{3+}$ <sup>[3]</sup>. The physicochemical characteristics of Gd-CA available for clinical use are summarized in Table 1.

Molecules of low stability are prone to undergo transmetallation with endogenous ions, leading to the release of free Gd ions ( $Gd^{3+}$ ) which may deposit in tissues and initiate the process of fibrosis that characterises the condition NSF<sup>[3]</sup>. Transmetallation is likely to occur if the elimination of low stability Gd-CAs molecules from the body is delayed. All Gd-CAs are eliminated from the body through the kidneys by glomerular filtration and the excretion half life is prolonged in patients with reduced renal function, enhancing the opportunity for both transmetallation and release of free  $Gd^{3+}$ <sup>[4]</sup>. Although the pathogenesis of NSF remains uncertain, data from experimental studies are accumulating to support the hypothesis of a causal relation between low stability Gd-CAs and NSF. In this chapter, experimental studies which have investigated the stability of Gd-CAs and their effects on fibroblasts and skin *in vitro* and *in vivo* will be critically evaluated.

## STABILITY OF GD-CAS

### Thermodynamic stability

The stability of a metal chelate is described as the equilibrium between the metal (M), its ligand (L) and the complex (ML) of the type:  $[M] + [L] \rightleftharpoons [ML]$ . The thermodynamic stability of Gd-CA is typically expressed as the thermodynamic stability constant ( $\log K_{therm}$ ) measured at very basic condition (pH 11). The conditional thermodynamic stability constant ( $\log K_{cond}$ ) is more appropriate than  $\log K_{therm}$  to describe thermodynamic stability as it is measured at the physiological pH 7.4. As the thermodynamic and conditional stability constants reflect the affinity of gadolinium for its ligand, the higher these stability constants, the more stable the complex and less free  $Gd^{3+}$  ions and free ligand are present when sufficient time is allowed to reach thermodynamic equilibrium.

### Kinetic stability

Kinetic stability reflects the speed of dissociation of the metal ligand complex and provides insight into the potential of *in vivo* dissociation<sup>[5]</sup>.

The kinetic stability of Gd-CAs, can be assessed by measuring the dissociation half life ( $T_{1/2}$ ) under very acidic conditions (pH 1)<sup>[5]</sup>. However, dissociation half life data of different Gd-CAs has been obtained from different laboratories making direct comparison between these agents rather difficult. A recent study evaluated the dissociation half life ( $T_{1/2}$ ) of Gd-CAs under the same laboratory conditions, confirming the following order for kinetic stability: linear chelates < non-ionic macrocyclic chelates  $\leq$  ionic macrocyclic chelates (Table 2)<sup>[6]</sup>.

### Transmetallation in vitro

Transmetallation of Gd-CAs leads to release of free gadolinium through replacement of the  $Gd^{3+}$  within the chelate molecule by another cation such as iron, copper, zinc or calcium<sup>[3]</sup>. Transmetallation of Gd-CAs would be difficult if the attachment between the Gd ion and the chelate were strong. *In vivo*, zinc might have the potential to displace an amount of  $Gd^{3+}$  because its concentration

in the blood is relatively high (55-125  $\mu\text{mol/L}$ ) whereas, copper is present in low concentration (1-10  $\mu\text{mol/L}$ ) and calcium ions have low affinity to organic ligands<sup>[3,7]</sup>. Iron ions are tightly bound by the storage proteins, ferritin and haemosiderin, and only a small amount is available for transmetallation with  $Gd^{3+}$ <sup>[7]</sup>.

A validated and reliable *in vitro* relaxometric method has been used to evaluate the release of  $Gd^{3+}$  from gadolinium chelates<sup>[8,9]</sup> in the presence of  $Zn^{2+}$  at pH 7.4. Three classes of Gd-CA were determined using this approach: (1) Macrocyclic chelates were characterized by very high kinetic stability; (2) Ionic linear chelates for which a moderate kinetic stability leads to significant decomplexation in the presence of  $Zn^{2+}$  (Gd-DTPA, Gd-EOB-DTPA, Gd-BOPTA); and (3) Non-ionic linear chelates which exhibited poor kinetic stability and the highest extent of decomplexation.

Clinical studies also demonstrated transmetallation between Gd-CAs and endogenous zinc ions. The non-ionic linear chelate, Gd-DTPA-BMA (Omniscan<sup>®</sup>, GE Healthcare, USA), caused a large increase in zinc excretion that was 3-fold higher than the zincuria induced by the ionic linear molecule, Gd-DTPA (Magnevist<sup>®</sup>, Bayer Healthcare, Germany), in patients undergoing contrast-enhanced MRI examinations. The presence of free ligand in Omniscan may have also contributed to the observed zincuria. No effect on zinc excretion was observed with the ionic macrocyclic, Gd-CA Gd-DOTA (Dotarem<sup>®</sup>, Guerbet, France)<sup>[10]</sup>.

### Stability of Gd-CA in human serum

A recent study measured the release of free  $Gd^{3+}$  after addition of different Gd-CAs to human serum incubated at 37°C over 15 d. A highly sensitive high pressure liquid chromatography connected to mass spectrometry (HPLC-ICP-MS) method was used to differentiate between dissociated and complex-bound Gd. Early release of Gd was observed with the linear chelates which increased with time<sup>[11]</sup>. The release induced by non-ionic linear chelates was 10-fold higher in comparison to ionic chelates. Marked release of  $Gd^{3+}$  from gadodiamide and gadoversetamide without excess ligand was observed from day one. The excess ligand in formulated Omniscan<sup>®</sup> and OptiMark<sup>®</sup> (Covidien, USA) offered early protection and chelated the released  $Gd^{3+}$  in the first 2 d, but by day 3 the level of free  $Gd^{3+}$  increased about 5-fold from < 0.5% at day one to almost 2.5%. The amount of Gd released from Omniscan<sup>®</sup> and OptiMark<sup>®</sup> at day 15 was almost 20% of the dose added compared with 2% for Magnevist<sup>®</sup>.

The addition of phosphate to serum markedly increased the release of Gd at day one by a factor of 100 with the non-ionic linear preparations and by a factor of 30 with the ionic linear chelates. At day 15, phosphate increased the amount of released  $Gd^{3+}$  from 20% to around 35% of the total dose of Omniscan<sup>®</sup> and OptiMark<sup>®</sup>. For Magnevist<sup>®</sup> the total amount of released  $Gd^{3+}$  did not change and remained at 2%, only the speed of the release was increased in day one to 2% and remained at this level up to day 15. No release of  $Gd^{3+}$  was observed with the

Table 1 General characteristics of currently marketed gadolinium chelates used for magnetic resonance imaging<sup>[6]</sup>

Name	Acronym	Gd-DTPA	Gd-EOB-DTPA	Gd-BOPTA	MS325	Gd-DTPA-BMA	Gd-DTPA-BMEA	Gd-HP-DO3A	Gd-BT-DO3A	Gd-DOTA
Generic name		Gadopentetate dimeglumine	Gadoxetic acid, disodium salt	Gadobenate dimeglumine	Gadofosveset, trisodium salt	Gadodiamide	Gadoverse-tamide	Gadoteridol	Gadobutrol	Gadoterate meglumine
Trade name		Magnevistâ	Primovist®	MultiHanceâ	Vasovist®	Omniscanâ	OptiMARKâ	ProHanceâ	Gadovistâ	Dotaremâ
Company		Bayer-Schering	Bayer-Schering	Bracco	EPIX	GE-Healthcare	Mallinckrodt	Bracco	Bayer-Schering	Guerbet
Chemical structure		Open-chain	Open-chain	Open-chain	Open-chain	Open-chain	Open-chain	Macrocytic	Macrocytic	Macrocytic
Charge		Ionic	Ionic	Ionic	Ionic	Nonionic	Nonionic	Nonionic	Nonionic	Ionic
Concentration (M)		0.5	0.25	0.5	0.25	0.5	0.5	0.5	1	0.5
Osmolality at 37°C (mOsm/kg H <sub>2</sub> O)		1960	688	1970	825	789	1110	630	1603	1350
Viscosity (mPa.s) at 37°C		2.9	1.19	5.3	2.06	1.4	2	1.3	4.96	2
Formulation		Free DTPA (1 mmol/L)	Ca-EOB-DTPA (trisodium salt) (1.5 mmol/L)	No formulation	Fosveset (0.325 mmol/L)	Ca-DTPA-BMA (caldiamide) (Na <sup>+</sup> salt) (25 mmol/L)	Ca-DTPA-BMEA (Na <sup>+</sup> salt) (50 mmol/L)	[Ca-HP-DO3A] <sub>2</sub> (Ca <sup>2+</sup> salt) (0.5 mmol/L)	Ca-BT-DO3A (Na <sup>+</sup> salt) (1.0 mmol/L)	No formulation
Hydrophilicity (log P Butanol/water)		-3.16	-2.11	-2.33	-2.11	-2.13	N/A	-1.98	-2	-2.87
log K <sub>therm</sub>		22.1	23.5	22.6	22.06	16.9	16.6	23.8	21.8	25.6
log K <sub>cond</sub>		17.7	18.7	18.4	18.9	14.9	15	17.1	14.7	19.3
Kinetic Stability <sup>a</sup>		Low	Medium	Medium	Medium	Low	Low	High	High	High

<sup>a</sup>Low: long-time index < 0.3, Medium: Long-time index 0.49 to 0.86; High: Long-time index > 0.95. N/A: Not available.

macrocytic Gd-CAs even after the addition of phosphate to the serum<sup>[11]</sup>.

These data support the high stability of the macrocytic Gd-CAs and that the non-ionic linear chelates have the lowest stability. The ionic linear chelates have a moderately higher stability in comparison to the non-ionic linear preparations as they released only a small amount of free Gd<sup>3+</sup> throughout the observation period<sup>[11]</sup>.

## TISSUE RETENTION OF GD-CA

There are several factors *in vivo* such as endogenous ions, enzymes and other biological elements that may work simultaneously to dissociate the Gd-CA with unpredictable effects<sup>[12]</sup>. Therefore, it has been suggested that *ex-vivo* data are not reliable to predict the behaviour of Gd-CA *in vivo* as the conditions under which these measurements are obtained are disparate from those found *in vivo*<sup>[12]</sup>.

Retention of gadolinium in tissues has been used to assess the stability of Gd-CAs *in vivo*. Once Gd<sup>3+</sup> is dissociated from its ligand, it can deposit in body tissues and may persist for long periods of time. On the other hand, virtually all the injected water soluble undissociated chelate is eliminated from the body after 3 d in the presence of normal renal function. Therefore, most of the gadolinium detected in the body 5 d after administration of a Gd-CA is likely to be released from the chelate<sup>[13]</sup>. Thus, the lower the stability of the Gd-CA, the higher the retention of gadolinium in tissues.

Tweedle *et al.*<sup>[13]</sup> compared the percent injected doses (%ID)/g-tissue of <sup>153</sup>Gd-labelled contrast agents in sev-

eral organs in mice and rats, and found that Gd retention in tissues 2 wk after injection was 3 times greater following Omniscan compared to the ionic linear chelate, Magnevist®. Gd retention in tissues was minimal with the macrocytic agents, Dotarem® and ProHance® (Bracco, Italy). The long-term retention of gadolinium in the skin after intravenous administration of commercially available Gd-CAs has also been investigated in the normal rat. The contrast agents were injected daily at the dose of 2.5 mmol/kg bodyweight for 5 consecutive days. Skin biopsies were taken at various time points up to a year after the last injection to measure total gadolinium concentration using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Retention of gadolinium in the skin was found throughout the observation period which correlated with the Gd-CA stability. The amount of gadolinium retention in the skin followed the order: Omniscan® > OptiMark® > Magnevist®. Only a minimal amount of gadolinium retention was observed with the macrocytic agents<sup>[14]</sup>.

## IN VITRO STUDIES

Edward *et al.*<sup>[15]</sup> found that Omniscan® (10-500 µmol/L) stimulated proliferation of normal human skin fibroblasts in culture. However, since no proliferation was seen with Gd chloride, the response to Omniscan® was attributed to the Gd-chelate molecule rather than free Gd<sup>3+</sup>. However, the same group in a recent study demonstrated that Gd chloride can stimulate the proliferation of fibroblasts confirming a role for free Gd<sup>3+</sup> in this effect<sup>[16]</sup>. Serum from NSF patients stimulated the proliferation of normal

**Table 2** Dissociation half life ( $T_{1/2}$ ) of gadolinium contrast agent under the same laboratory conditions<sup>[6]</sup>

Gd-CA	$T_{1/2}$ , pH 1.2	$T_{1/2}$ , pH 1	$T_{1/2}$ , pH 1	$T_{1/2}$ , pH 1 (Temp 250)
Temperature (°C)	370	370	250	
Dotarem <sup>®</sup> (Guerbet, France)	85 h	23 h	338 h	338 h
Gadovist <sup>®</sup> (Bayer Schering Pharma AG, Germany)	18 h	7 h	43 h	43 h
ProHance <sup>®</sup> (Bracco, Italy)	4 h	1.6 h	3.9 h	3.9 h
All linear chelates	ND	ND	< 5 s	< 5 s

Gd-CA: Gadolinium contrast agent.

human skin fibroblasts but had no effect on fibroblasts derived from NSF patients, suggesting that once activated, these fibroblasts can not be activated further<sup>[15]</sup>.

A subsequent study by Varani *et al*<sup>[17]</sup>, confirmed the stimulant effect of Omniscan<sup>®</sup> on the proliferation of human fibroblasts over a lower concentration range (0.5-25  $\mu\text{mol/L}$ ) with no proliferative effect on human keratinocytes. However, a proliferative response to Gd chloride was detected, an effect inhibited in the presence of the free ligand, DTPA. More stable Gd-CAs also induced proliferation but at a higher minimum concentration, 50  $\mu\text{mol/L}$  for Magnevist<sup>®</sup> and Multihance<sup>®</sup> (Bracco, Italy) and 25 mmol/L for the macrocyclic Prohance<sup>®</sup> (Bracco, Italy) a 100 000 higher concentration than Omniscan<sup>®</sup>. These data taken as a whole would support a role for released  $\text{Gd}^{3+}$  ions rather than the entire Gd chelate molecule in the proliferative effect of Gd-CA. An experiment to establish whether the response to Omniscan<sup>®</sup> can be inhibited in the presence of a large amount of free ligands to chelate released  $\text{Gd}^{3+}$  ions is warranted.

## MATRIX SUBSTANCES

The effect of Gd-CAs on matrix protein synthesis *in vitro* is somewhat controversial, but they appear to modulate the synthesis of the enzymes that control collagen synthesis<sup>[17]</sup>. Omniscan<sup>®</sup> has been shown to increase hyaluronan synthesis in cultured normal human dermal fibroblasts together with an enhanced immunostain for  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA)<sup>[15]</sup>. Human skin fibroblasts derived from patients with NSF released higher levels of hyaluronan and the procollagen metabolite, procollagen type-1 C-propeptide (CICP). They also showed positive immunostain for  $\alpha$ -SMA. Interestingly, serum from NSF patients stimulated both collagen (up to 3.3-fold) and hyaluronan synthesis (up to 7-fold) in healthy skin fibroblasts compared to serum from either healthy volunteers or dialysis patients. However, NSF serum had little effect on skin fibroblasts derived from NSF patients, suggesting these fibroblasts had already been maximally stimulated. Edward *et al*<sup>[15]</sup> concluded that the ability of Omniscan<sup>®</sup> to stimulate fibroblast growth, differentiation and matrix synthesis supported its role in the pathophysiology of NSF.

## ANIMAL STUDIES *IN VIVO*

Cytokine production was investigated in the normal rat

injected with 2.5 mmol/kg per day of gadodiamide (Omniscan<sup>®</sup> without excess chelate) for up to 8 d once, three or 8 times. Animals were sacrificed 6 h after the last injection<sup>[18]</sup>. Gadodiamide for up to 8 d increased vascular endothelial growth factor (VEGF), osteopontin (OPN) and TIMP-1 in serum collected 6 h after the last dose. VEGF enhances vascular permeability and promotes angiogenesis. OPN regulates macrophage activity in response to calcification, cell-matrix interaction and is a chemoattractant for dendritic cells and t-lymphocytes, while TIMP-1-inhibits extracellular matrix degradation.

Gadolinium was detected in the skin, liver and femur and the detected amount increased with the total injected dose of gadodiamide. Gadolinium deposition in tissues was proposed to initiate a physiological response similar to tissue calcification seen in patients with advanced chronic kidney disease. This includes upregulation of OPN, chemoattractants for macrophages and monocytes, and cytokines that increase the vascular permeability and modulate extra-cellular matrix synthesis<sup>[18]</sup>. All animals injected with gadodiamide over 8 d developed macroscopic skin lesions (reddening, ulceration and scab formation). Dermal microscopic changes included cellular infiltration and thickening of the collagen fibres<sup>[18]</sup>.

## MODELS OF NSF

Experimental studies using normal rats and rats with 5/6 subtotal nephrectomy have been carried out to investigate the association between Gd-CAs and NSF. The subtotal nephrectomy model was proposed to reproduce *in vivo* a comparable situation to that of patients with advanced chronic kidney disease.

### Rat with normal renal function

Gd-CAs at the dose of 2.5 mmol/kg per day administered intravenously 5 d a week over 4 wk has been proposed to induce skin lesions consistent with human NSF<sup>[19,20]</sup>. Skin lesions were observed as early as 8 d after starting non-formulated gadodiamide and 20 d after starting Omniscan<sup>®</sup> (with excess chelate). No skin lesions were observed with either the Omniscan<sup>®</sup> ligand, caldiumide (Ca-DTPA-BMA) or with Magnevist<sup>®</sup>. The incidence of skin lesions (ulceration, fibrosis, CD34+ cell infiltration and increased cellularity) was qualitatively associated with gadolinium concentrations in tissues. The highest Gd concentrations were found in animals that received either gadodiamide or

the less stable Gd-EDTA. These data support the concept that NSF-like skin lesions are associated with gadolinium release from low stability chelates<sup>[19,20]</sup>.

The induction of skin lesions by low stability Gd-CAs in this model, despite their short half-life (20 min) and rapid elimination, suggests that some dissociation of the Gd-CA and release of free Gd<sup>3+</sup> may occur even in the presence of normal renal function. Frenzel *et al*<sup>[11]</sup> showed that the dissociation of Omniscan<sup>®</sup> occurs from day one in human serum and the release of free Gd<sup>3+</sup> increases with time. It is reasonable to conclude from these studies that multiple repeat injections of low stability Gd-CAs in the absence of renal impairment could lead to gradual accumulation of Gd<sup>3+</sup> in tissues until it reaches a threshold level that triggers the fibrotic process. A previous study in man has shown that Gd<sup>3+</sup> deposition in bone occurs in patients with normal renal function. In this study, the Gd<sup>3+</sup> retention in bone with Omniscan<sup>®</sup> was 2-4 times more than ProHance<sup>®</sup>, a non-ionic macrocyclic Gd-CA<sup>[21]</sup>.

### Rats with partial nephrectomy

Grant *et al*<sup>[22]</sup> investigated the effects of Gd-CAs in partially nephrectomised rats, with a reported serum creatinine level twice that of healthy animals. Rats received a dose of 5 mmol/kg body weight of Omniscan<sup>®</sup>, Magnevist<sup>®</sup>, 1 mmol/kg body weight of gadodiamide or Gd chloride or 25 µmol/kg body weight of gadolinium citrate daily except weekends (total of 10 doses). Rats were sacrificed on day 15, 3 d after the last injection. Both gadodiamide (1 mmol/kg per day) and Omniscan<sup>®</sup> (5 mmol/kg per day) induced macroscopic and histological skin lesions. Skin lesions were greater and developed more rapidly in animals which received gadodiamide in comparison to those which received Omniscan<sup>®</sup> (gadodiamide + excess ligand) confirming initial beneficial effects of the excess ligand, caldiumide, in the Omniscan<sup>®</sup> preparation. No macroscopic or histological skin lesions were observed with Magnevist<sup>®</sup> or with the gadolinium salts, chloride or citrate. Grant *et al*<sup>[22]</sup> concluded that the skin lesions detected did not represent NSF, due to absence of fibrosis and CD34 positive cells. The authors indicated that the ulcerative skin lesions were produced secondary to scratching precipitated by an allergic response since there was some elevation of plasma histamine and an increase in dermal mast cells. The possibility that the dermatological changes could be a reaction to Gd deposition in the skin was not considered. Interestingly, itching and macroscopic skin lesions were only observed in animals with a high skin Gd content and no acute inflammatory response was observed when the skin Gd content was low. However, we would agree only with the statement of Grant *et al*<sup>[22]</sup> that the model described was not an animal model of NSF since: (1) Ulcerative skin lesions are not a feature of NSF in man and should not be used as the endpoint of detection in an animal model. Their presence may modify or obscure the microscopic and immunohistochemical features of NSF in the skin; (2) The development of NSF in the rat requires time and allowing only 3 d following

cessation of Gd-CA administration is too short for the detection of typical histological changes of NSF; and (3) The only immutable epidemiological feature of human NSF is renal impairment. Marked reduction in GFR was not demonstrated in the study by Grant *et al*<sup>[22]</sup>.

Grant *et al*<sup>[22]</sup> also commented that Gd salts are not suitable for investigating the role of free Gd<sup>3+</sup> *in vivo*. Free Gd<sup>3+</sup> ions within the intravascular compartment following intravenous administration of Gd chloride/citrate will form insoluble salts with body anions such as phosphate and hydroxide. These insoluble Gd salts would be attached to plasma proteins forming colloid micro-emboli which in turn will be phagocytosed by the reticuloendothelial system, particularly in the liver and spleen causing toxic effects in these organs. According to Grant *et al*<sup>[22]</sup>, for Gd<sup>3+</sup> to be delivered to the skin or other organs it has to be in a soluble form i.e. while it is still in the chelated form. We agree with this suggestion and believe that delay in excretion of the Gd-chelate molecules would allow time for their dissociation in the extravascular extracellular fluid leading to deposition of insoluble gadolinium salts in the skin and other organs. These salts could be phagocytosed by local macrophages precipitating the release of varieties of profibrotic cytokines. Boyd *et al*<sup>[23]</sup> employing scanning electron microscopy and energy dispersive x-ray spectroscopy (SEM/EDS) demonstrated gadolinium deposition in association with calcium phosphate in the skin of patients affected with NSF. It is also probable that the released Gd<sup>3+</sup> may penetrate local fibroblasts causing direct activation of these cells as shown in *in vitro* studies<sup>[16,17]</sup>. It is less likely that the entire Gd-CA molecule, which is highly hydrophilic, would enter the cell.

Grant *et al*<sup>[22]</sup> did not adequately discuss the importance of the stability of Gd-CA although there are several observations in the study to indicate that released Gd from the low stability Gd-CA, Omniscan<sup>®</sup>, is likely to be the culprit of the observed skin lesions. The study showed that the excess ligand in Omniscan<sup>®</sup> provided an initial protective effect which is mainly due to reducing transmetallation with endogenous ions and chelating released free Gd<sup>3+</sup> ions. In addition, higher retention of gadolinium in the skin was found with gadodiamide and Omniscan<sup>®</sup> in comparison to the more stable Magnevist<sup>®</sup>. The authors indicated that analytical technique did not allow them to determine whether the gadolinium detected in tissue is released free Gd<sup>3+</sup> or the whole Gd-chelate molecule. However, recent work by Abraham *et al*<sup>[24]</sup> using SIMS ion microscopy has confirmed that gadolinium detected in skin is insoluble gadolinium precipitated with tissue anions.

## CONCLUSION

Since a causal relation between low stability Gd-CAs and NSF was suggested in 2006, several experimental studies to elucidate this possible association have been published. However, there has been no consensus on the requirements of the ideal animal model of NSF. Initial studies by Sieber *et al*<sup>[19,20]</sup> indicated that rats with normal

renal function receiving multiple large doses of a Gd-CA could be used as a suitable model for NSF. However, the model lacks an essential clinical feature of NSF which is a marked reduction in renal function. The animal model of a 5/6 subtotal nephrectomy was subsequently proposed to reproduce *in vivo* a comparable situation to that of patients with advanced chronic kidney disease. Reported experience with this model so far has been mixed, some authors concluded that it is not a suitable model of NSF<sup>[22]</sup>, while others have used the model to investigate long-term retention of Gd in tissues<sup>[25]</sup>. In the authors view a good animal model of NSF should possess the following features: (1) Histological changes of the skin similar to those seen in human NSF<sup>[26]</sup>: (a) dermal cellularity for monocytes and fibrocytes/fibroblasts; (b) thickness of collagen bundles with decreased interstitial space; and (c) fibroblast/fibrocyte markers including CD34, collagen and  $\alpha$ -SMA; (2) Absence of macroscopic skin lesions; (3) Reduction in GFR, equivalent to stage 5 CKD in man; (4) Adequate time allowed after Gd-CA injection and before sacrifice; and (5) Quantitative assessment of skin collagen.

We have considerable experience in the use of rats following 5/6 subtotal nephrectomy and have recently reported a positive correlation between the reduction in renal function, the retention of gadolinium in tissues and histological response of the skin following a single intravenous dose of Omniscan<sup>®</sup><sup>[27]</sup>. More recently, we reported a differential response of Gd retention in tissues and histological changes in the skin between Dotarem and Omniscan in rats with subtotal nephrectomy, with high retention of Gd in the skin associated with increase in cell cellularity and collagen of the dermis following Omniscan. These changes were absent with the highly stable Dotarem<sup>[28]</sup>. A recent report by other authors employing the subtotal nephrectomy model, found that the retention of Gd in tissues following Omniscan administration was mainly due to dechelated gadolinium<sup>[29]</sup>.

Finally, the role of lanthanides in stimulating fibrillogenesis was identified more than 20 years ago<sup>[30,31]</sup>. Current experimental data support the view that gadolinium released from low stability Gd-CAs is an important factor in promoting fibrosis, the predominant feature of NSF.

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