Biometals (2008) 21:469–490
DOI 10.1007/s10534-008-9135-x

Efficiency, thermodynamic and kinetic stability of marketed gadolinium chelates and their possible clinical consequences: a critical review

Marc Port · Jean-Marc Idée · Christelle Medina · Caroline Robic · Monique Sabatou · Claire Corot

Received: 21 November 2007 / Accepted: 13 February 2008 / Published online: 15 March 2008

Abstract Gadolinium-based contrast agents are widely used to enhance image contrast in magnetic resonance imaging (MRI) procedures. Over recent years, there has been a renewed interest in the physicochemical properties of gadolinium chelates used as contrast agents for MRI procedures, as it has been suggested that dechelation of these molecules could be involved in the mechanism of a recently described disease, namely nephrogenic systemic fibrosis (NSF). The aim of this paper is to discuss the structure-physicochemical properties relationships of marketed gadolinium chelates in regards to their biological consequences. Marketed gadolinium chelates can be classified according to key molecular design parameters: (a) nature of the chelating moiety: macrocyclic molecules in which Gd^{3+} is caged in the pre-organized cavity of the ligand, or linear open-chain molecules, (b) ionicity: the ionicity of the complex varies from neutral to tri-anionic agents, and (c) the presence or absence of an aromatic lipophilic residue responsible for protein binding. All these molecular characteristics have a profound impact on the physicochemical characteristics of the pharmaceutical solution such as osmolality, viscosity but also on their efficiency in relaxing water protons (relaxivity) and their biodistribution. These key molecular parameters can also explain why gadolinium chelates differ in terms of their thermodynamic stability constants and kinetic stability, as demonstrated by numerous in vitro and in vivo studies, resulting in various formulations of pharmaceutical solutions of marketed contrast agents. The concept of kinetic and thermodynamic stability is critically discussed as it remains a somewhat controversial topic, especially in predicting the amount of free gadolinium which may result from dechelation of chelates in physiological or pathological situations. A high kinetic stability provided by the macrocyclic structure combined with a high thermodynamic stability (reinforced by ionicity for macrocyclic chelates) will minimize the amount of free gadolinium released in tissue parenchymas.

Keywords Magnetic resonance imaging · Contrast agents · Gadolinium · Osmolality · Viscosity · Relaxivity · Thermodynamic stability · Kinetic stability · Nephrogenic systemic fibrosis

M. Port (✉) · J.-M. Idée · C. Medina · C. Robic · M. Sabatou · C. Corot
Guerbet, Research Division, Aulnay-sous-Bois, France
e-mail: marc.port@guerbet-group.com

Introduction

Over recent years, there has been a renewed interest in the physicochemical properties of gadolinium chelates used as contrast agents for magnetic resonance imaging (MRI) procedures.

It has been suggested (Morcos 2007a, b; Perazella 2007) that dechelation of these molecules could be involved in the mechanism of a new disease,

nephrogenic systemic fibrosis (NSF). NSF was first recognized in 1997 in 15 dialyzed patients and described in 2000 (Cowper et al. 2000). This rare and highly disabling disorder is characterized by extensive thickening and hardening of the skin associated with skin-coloured to erythematous papules that coalesce into erythematous to brawny plaques with a *peau d'orange* appearance.

NSF always occurs in patients with severe or end-stage renal failure (Cowper and Boyer 2006). Importantly, the systemic nature of NSF has recently been emphasized by several authors, who have reported that a considerable subset of patients have involvement of multiple organ systems, especially lungs, but also heart, skeletal muscles, oesophagus and diaphragm, which can eventually lead to death (Galan et al. 2006; Ting et al. 2003).

In 2006, two European teams independently suggested a link between the administration of gadolinium chelates and development of NSF in patients with renal failure (Grobner 2006; Marckmann et al. 2006). In these studies, a direct temporal relationship was found between injection of a Gd^{3+} chelate and subsequent onset of NSF. The vast majority of published cases clearly appeared to be associated with the open chain, nonionic Gd^{3+} chelate gadodiamide (Gd-DTPA-BMA). As of January 17, 2007, 85 of the more than 100 cases reported to the US Food and Drug Administration MedWatch reporting system were associated with Gd-DTPA-BMA, 21 were associated with Gd-DTPA, 6 were associated with Gd-DTPA-BMEA, one was associated with Gd-BOPTA (although this same patient had also received Gd-DTPA-BMA 5 days after his Gd-BOPTA MR examination), and none was associated with Gd-HP-DO3A (Kanal et al. 2007).

These compounds are administered intravenously and excreted unchanged by glomerular filtration. The plasma elimination half-life is about 1.5 h in healthy human volunteers, but is dramatically increased in patients with renal insufficiency and may then exceed 30 h (Bellin et al. 2003; Joffe et al. 1998). Among the numerous hypotheses proposed to explain this disease, the most widely supported (Morcos 2007a, b; Perazella 2007) postulates that, in the setting of renal failure, the remanence of a Gd^{3+} chelate in the body is dramatically increased, thus enhancing the chance for dissociation of Gd^{3+} from its chelate especially in the case of nonionic, linear open-chain molecules such as Gd-DTPA-BMA.

The objective of this review is to exhaustively and critically review the available data regarding all of the physicochemical properties of marketed gadolinium chelates in terms of their biological consequences. For this purpose, the physicochemical characteristics (osmolality, viscosity) of the pharmaceutical injected solution, the efficiency (and consequently the dose administered), and thermodynamic and kinetic stability assessed by in vitro or in vivo studies of all marketed gadolinium chelates will be compared and discussed. Structure-physicochemical properties relationships will be reviewed in order to underline the main structural parameters governing each physicochemical characteristic. Importantly, the concepts of kinetic and thermodynamic stability will be discussed, as this remains a somewhat controversial topic, especially to predict the amount of free gadolinium which may be released in vivo following dechelation of gadolinium chelates.

Chemical structure

The contrast of an MR image results from a complex interplay of various factors such as T_1 and T_2 relaxation rates, proton density, and MRI sequences. MRI contrast agents are used in MR imaging as they modify the longitudinal (T_1) and transverse (T_2) relaxation rate of water protons present in the tissues. The efficacy of an MRI contrast agent is measured in terms of relaxivity, i.e. the longitudinal and transverse relaxation rate of the water protons observed at a millimolar concentration of contrast agent. MR contrast agents are composed of paramagnetic ions that increase the relaxation rates of protons in the body. In terms of contrast agent design, attention has been essentially focused on gadolinium (III) both because of its high paramagnetism (due to the seven unpaired electrons of Gd(III)) and its very slow electronic relaxation rate (due to the asymmetric S-state of Gd^{3+}) and nine contrast agents based on gadolinium have now been marketed: Table 1 and Scheme 1.

However, free gadolinium is highly toxic (Idée et al. 2006), as the ionic radius of Gd^{3+} (107.8 pm) is close to that of Ca^{2+} (114 pm) and this element is an inorganic blocker of many types of voltage-gated calcium channels at nano- to micromolar concentrations. Gadolinium inhibits the activity of some

Table 1 General characteristics of currently marketed gadolinium chelates used for magnetic resonance imaging (Idée et al. 2006; Caravan et al. 1999; Brücher and Sherry 2001)

Name	Acronym Generic Name Trade Name	Gd-DTPA Gadopentetate dimeglumine Magnevist®	Gd-DTPA-BMA Gadodiamide Omniscan®	Gd-DTPA-BMEA Gadoversetamide OptiMARK®	Gd-BOPTA Gadobenate dimeglumine MultiHance®	Gd-EOB-DTPA Gadoxetic acid disodium salt Primovist®	MS325 Gadofosveset trisodium salt Vasovist®	Gd-DOTA Gadoterate meglumine Dotarem®	Gd-HP-DO3A Gadoteridol ProHance®	Gd-BT-DO3A Gadobutrol Gadovist®
Company		Bayer-Schering	GE-Healthcare	Covidien	Bracco	Bayer-Schering	Bayer-Schering	Guerbet	Bracco	Bayer-Schering
Chemical structure		Open-chain	Open-chain	Open-chain	Open-chain	Open-chain	Open-chain	Macrocyclic	Macrocyclic	Macrocyclic
Charge		Di-ionic	Nonionic	Nonionic	Di-ionic	Di-ionic	Tri-ionic	Ionic	Nonionic	Nonionic
Dissociated particles per molecule		3	1	1	3	3	4	2	1	1
Log *P* BuOH/H_2O		−3.16	−2.13	ND	−2.33	−2.11	−2.11	−2.87	−1.98	−2
Concentration (M)		0.5	0.5	0.5	0.5	0.25	0.25	0.5	0.5	1.0
Standard dose (mmol/kg)		0.1	0.1	0.1	0.1[a]	0.025	0.03	0.1	0.1	0.1
Osmolality at 37°C (mOsm/kg H_2O)		1960	789	1110	1970	688	825	1350	630	1603
Osmotic load[b] (mOsmol/l)		2	0.67	0.67	2	0.5	0.8	1.33	0.67	0.67
Relaxivity (r_1/r_2) $mM^{-1}s^{-1}$ at 37°C, 1.5 T in water[c]		3.3/3.9	3.3/3.9	3.6/4.1	3.8/4.4	4.6/5.3	5.0/5.9	3.0/3.5	2.9/3.4	3.3/3.9
Viscosity (mPa.s) at 37°C		2.9	1.4	2.0	5.3	1.19	2.1[c]	2.0	1.3	4.96
Formulation		Free DTPA 0.2% (1 mmol/l)	Ca-DTPA-BMA (Na^+ salt) 5% (25 mmol/l)	Ca-DTPA-BMEA (Na^+ salt) (50 mmol/l)	No formulation	Ca-EOB-DTPA (trisodium salt)[d]	Fosvest ligand (0.325 mmol/l)[e]	No formulation	$[Ca\text{-}HP\text{-}DO3A]_2$ (Ca^{2+} salt) 0.1% (0.5 mmol/l)	Ca-BT-DO3A (Na^+ salt) (1 mmol/l)
Log K_{therm}		22.1	16.9	16.6	22.6	23.46	22.1[f]	25.6[g]	23.8	21.8
Log K_{cond}		17.7	14.9	15.0	18.4[h]	18.7[i]	18.9[f]	19.3[g]	17.1	14.7[j]

[a]Dose for liver imaging: 0.05 mmol/kg

[b]osmotic load (mOsm/l) $= \frac{\text{dose(mmol/kg)} * 70}{\text{V}_{\text{distribution}}(l)} *$ number dissociated ions (values are calculated on the assumption that the agents distribute homogeneously in the interstitial space (10.5 l for a patient weighing 70 kg)

[c]Guerbet measurement on commercial solution; Incertainty on relaxometric measurement: ±0.3 mM^{-1} s^{-1} for relaxicity measurements

[d](concentration not disclosed)

[e]Steger-Hartmann et al. (2006)

[f]Caravan et al. (2001)

[g]Moreau et al. (2004) and Guerbet calculations

[h]Uggeri et al. (1995)

[i]Schmitt-Willich et al. (1999)

[j]Bellin et al. (2003)

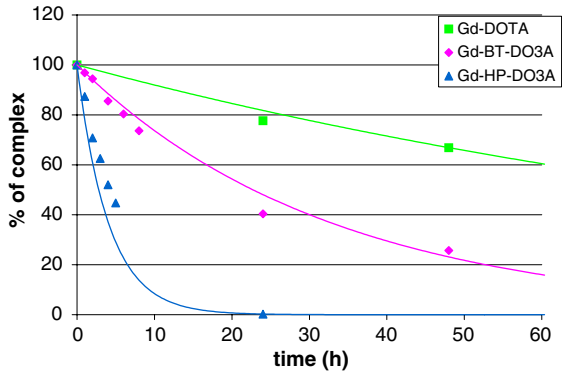


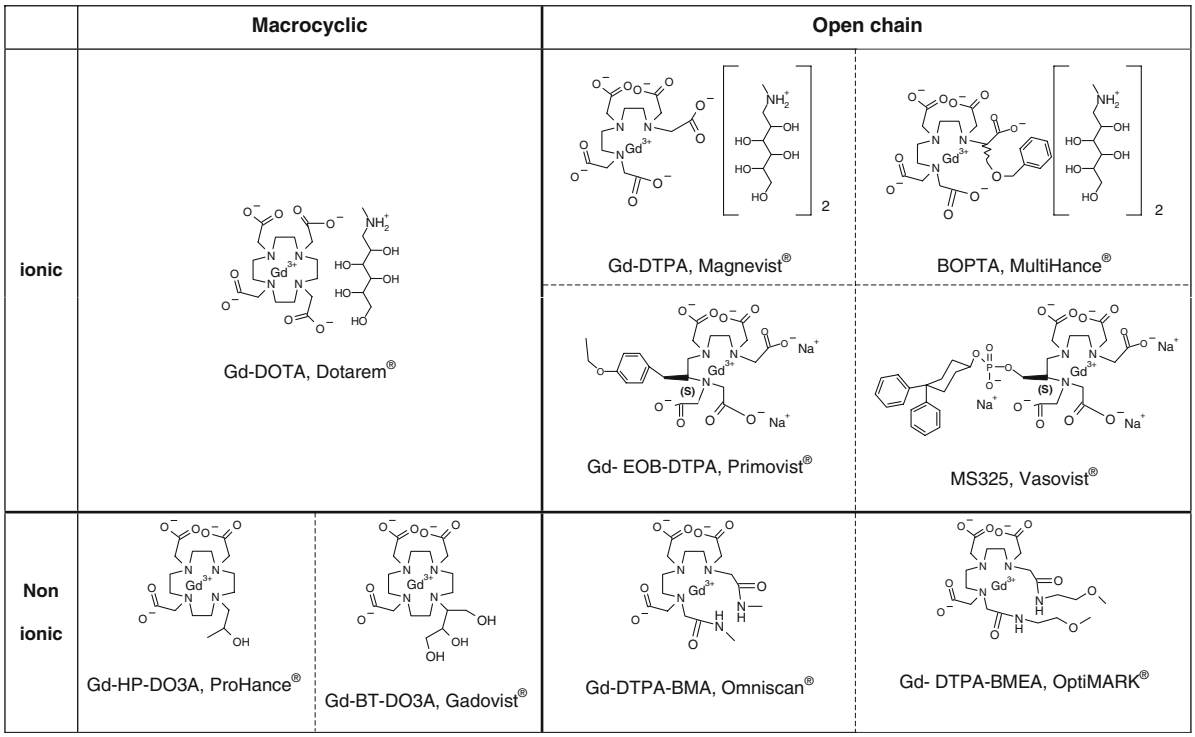
Fig. 1 Dechelation kinetics at pH 1.2, 37°C, of marketed macrocyclic gadolinium contrast agents: percentage of remaining gadolinium chelate over time

enzymes (Ca^{2+}-activated-ATPase in the sarcoplasmic reticulum of skeletal muscle fibres, some dehydrogenases and kinases, glutathione S-transferases, etc.) (Evans 1990; Itoh and Kawakita 1984) and has a remarkable capacity to depress the reticuloendothelial system (Evans 1990). Gadolinium inhibits phagocytosis in normal and activated Kupffer cells and increases the expression of several cytokines.

Gadolinium must therefore be chelated with a coordinating ligand as chelation of gadolinium by appropriate highly kinetic and thermodynamically stable polyamino-polycarboxylic ligands dramatically reduces its acute toxicity. For example, intravenous LD_{50} of Gd-DOTA in mice was 10.6 mmol/kg, whereas LD_{50} of $GdCl_3$, tested under similar conditions, was only 0.35 mmol/kg (Bousquet et al. 1988).

In order to understand the key features involved in the physicochemical properties of gadolinium chelates, several chemical structural aspects must be taken into account:

- The nature of the chelating moiety: either macrocyclic chelates such as Gd-DOTA, Gd-HP-DO3A, Gd-BT-DO3A where Gd^{3+} is "caged" in the pre-organized cavity of the ligand which perfectly matches the size of Gd^{3+}, or " linear open-chain" chelates such as Gd-DTPA or Gd-DTPA-BMA, Gd-DTPA-BMEA, Gd-BOPTA, Gd-EOB-DTPA. All these ligands form



Scheme 1 Structure of currently marketed gadolinium chelates used for magnetic resonance imaging

octa-coordinated gadolinium chelates, and the coordination sphere is completed with a water molecule in the inner sphere, achieving a total coordination of nine. As discussed below, this structural difference between macrocyclic and linear open-chain chelates mainly has an impact on the stability of the gadolinium chelate.

- The ionicity of the chelate: some ligands such as Gd-DTPA, Gd-BOPTA, Gd-EOB-DTPA chelates are di-ionic whereas Gd-DOTA is a mono-ionic chelate. MS325 is the only tri-ionic agent. In the pharmaceutical formulation, the charge of the ionic chelates is neutralized at physiological pH by a stoichiometric quantity of protonated counter cation, meglumine, except for Gd-EOB-DTPA and MS325 marketed as a disodium and trisodium salts, respectively. On the contrary, ligands such as HP-DO3A, BT-DO3A, DTPA-BMA, DTPA-BMEA form nonionic complexes with Gd(III), as the charges on the ligand and metal ion are equal (Chang et al. 1992).
- The hydrophilicity of the chelate: the high solubility of gadolinium chelates correlates with a pronounced hydrophilicity of all gadolinium chelates as demonstrated by their negative log *P* values measured between butanol and water. However, ionic chelates are significantly more hydrophilic (log *P* one order of magnitude higher) than nonionic chelates (Caravan et al. 1999) Gd-DOTA, Gd-HP-DO3A, Gd-BT-DO3A, Gd-DTPA, Gd-DTPA-BMA and Gd-DTPA-BMEA are considered to be nonspecific extracellular agents which means that, after IV injection, they are distributed non-specifically in plasma and interstitial fluid of the body and are eliminated almost exclusively by renal glomerular filtration with a elimination half-life of about 90 min in patients with normal renal function but which can be dramatically increased in patients with renal failure (Joffe et al. 1998). However, three linear open-chain gadolinium chelates are characterized by the presence of aromatic residues on the scaffold of the DTPA ligand: MS-325, Gd-EOB-DTPA and Gd-BOPTA. The introduction of an aromatic lipophilic group on the DTPA backbone decreases the overall hydrophilicity of the chelates, as demonstrated by lower log *P* values in comparison to Gd-DTPA (log *P* for MS325 = −2.11 vs. −3.16 for Gd-DTPA) and modifies the biodistribution and pharmacokinetics of these contrast agents, as the presence of the aromatic group can lead to hepatocellular uptake and excretion into the bile duct, gallbladder and intestines (Gd-EOB-DTPA and Gd-BOPTA) (Vander Elst et al. 2001) or can cause strong but reversible albumin binding thereby confining the contrast agent to the vascular compartment, allowing blood pool imaging (MS325) (Caravan et al. 1999).

Physicochemistry of the pharmaceutical solution: osmolality and viscosity

Nonionic chelates were originally designed to lower the osmolality of 0.5 M injected solutions which is higher than that of plasma, as, based on the experience of X-ray iodinated contrast agents, it was hypothesized that lowering osmolality could reduce adverse events such as sensation of warmth or heat at the injection site or vascular endothelium damage (Cohan et al. 1991). However, all marketed gadolinium chelate formulations are hypertonic in comparison to blood (the osmolality of blood and body fluids is about 280–300 mOsmol/kg) and the global increase in plasma osmolality after injection of the standard clinical dose is insignificant, as demonstrated by the value of osmotic load (Table 1) and does not have impact on the body's osmotic balance. As osmolality is a colligative property, and therefore depends on the total number of particles in solution, regardless of their charge, Gd-BT-DO3A solutions formulated at 1 M do not conserve the characteristic of reduced osmolality in comparison to 0.5 M nonionic gadolinium chelate solutions. Interestingly, the experimental value of osmolality of gadolinium chelates is higher than predicted by the theory, which has been explained by a high hydration number of the gadolinium chelates, reducing the amount of free water and increasing the apparent concentration of the gadolinium chelate (Gries and Miklautz 1984). The negative charge of ionic chelates may result in a slower rate of diffusion into disk cartilage which contains negatively charged proteoglycans compared to a nonionic chelate, as demonstrated in an MR study in rabbits after intravenous administration of equimolar doses of Gd-DTPA and Gd-DTPA-BMA (Ibrahim et al. 1994).

The gadolinium concentration has an obvious impact on viscosity, as the 1 M Gd-BT-DO3A

solution is more viscous than 0.5 M solutions of gadolinium chelates, which are more viscous than 0.25 M MS-325 and Gd-EOB-DTPA solutions. The introduction of an aromatic group into the structure of Gd-BOPTA also significantly increases the viscosity of the pharmaceutical solution in comparison with the other 0.5 M formulations of gadolinium chelates. However, the viscosity of all these pharmaceutical solutions does not preclude bolus IV administration.

Relaxivity

According to the Solomon Bloembergen-Morgan equations (Caravan et al. 1999), various structural parameters influence the relaxivity of gadolinium chelates such as correlation time, with contributions derived from the rotational correlation time, the electronic relaxation time and the water residence life time but also the distance between Gd^{3+} and the coordinated water hydrogen.

The NMRD profiles of the marketed gadolinium chelates recorded at 37°C in water were recently published (Laurent et al. 2006; Adzamli et al. 1999; Vander Elst et al. 1997; Muller et al. 1999). When measured in water, the r_1 and r_2 relaxivities obtained for non-protein-binding chelates are fairly similar, in the field range from 0.47 T to 4.7 T. Gd-DOTA is nevertheless characterized by a higher low field relaxivity (0.005–0.05 T) in comparison to other non-protein-binding chelates due to its longer electronic relaxation time related to the high symmetry and rigidity of this chelate (Benmelouka et al. 2006). The water residence time of bisamide chelate (Gd-DTPA-BMA and Gd-DTPA-BMEA) has also been demonstrated to be longer than that of other chelates (Caravan et al. 1999; Adzamli et al. 1999). However, the relaxivity of these chelates at 37°C is not limited by this increase of the water residence time. The relaxivities obtained in water for protein-binding chelates are slightly higher than those of non-protein-binding chelates, partly due to the higher molecular weight of these molecules which lengthens the rotational correlation time, but it has also been proposed that the distance between Gd^{3+} and the coordinated water hydrogen could be reduced due to the lipophilic residue grafted on the C4 position of the DTPA backbone of EOB-DTPA-Gd and MS325 (Laurent et al. 2006; Muller et al. 1999; Vander Elst et al. 1997).

In a recent comparative study, the r_1 and r_2 relaxivities in bovine plasma of all commercially available gadolinium chelates were measured at 37°C (Rohrer et al. 2005). When measured in bovine plasma, the r_1 and r_2 relaxivities obtained for non-protein-binding chelates are again fairly similar in the field range from 0.47 to 4.7 T (Rohrer et al. 2005) and higher than those measured in water due to a slightly higher viscosity of plasma resulting in lengthening of the rotational correlation time. Due to the reversible interaction of the gadolinium chelate with plasmatic proteins, which further lengthens the rotational correlation time of the bound chelate, the relaxivities of protein-binding chelates are significantly increased in the presence of plasma.

However, it is noteworthy that the value of the relaxivities of these protein-binding chelates reported in the literature is highly dependent on the experimental conditions used, especially the concentration of contrast agent (typically from 0.25 to 0.5 mmol/l) and plasma protein (Cavagna et al. 1997; Caravan et al. 2002, De Haen et al. 1999) and are not easily comparable to the well-defined relaxivity of non-protein-binding chelates. Physicochemical measurements of relaxivity in plasma performed in vitro at a fixed concentration of gadolinium therefore do not necessarily accurately reflect the efficiency of the contrast agent in vivo (De Haen et al. 1999; Port et al. 2005; Corot et al. 2003), as, in vivo, the proportion of free and bound forms of protein-binding contrast agents varies according to the pharmacokinetic profile, and the relaxivities of protein-bound and free contrast agents are different. The concept of dynamic relaxivity was therefore introduced (Port et al. 2005) to compare the in vivo efficiency of protein-binding chelates in a protocol mimicking an angiographic procedure. A significant variation of dynamic relaxivities between the bolus and post-bolus phase was demonstrated for Gd-BOPTA, as its relaxivity varies from 6.7 to 5.2 mM^{-1} s^{-1} and for MS325 (relaxivity variation from 17.3 to 8.6 mM^{-1} s^{-1}).

Chelate stability

- The integrity of the gadolinium chelate must be maintained in vivo in order to ensure good tolerability. Dissociation of Gd^{3+} from an MRI contrast agent is undesirable, as both the free

metal and unchelated ligands are generally more toxic than the chelate itself. This stability issue is discussed, in particular, in the context of NFS (Morcos 2007a, b; Perazella 2007). Two sometimes confusing concepts have been proposed to describe the stability of gadolinium chelates: thermodynamic and kinetic stability.

Thermodynamic stability

Two thermodynamic concepts have been proposed in the literature to explain the thermodynamic stability of Gd chelates:

(1) Thermodynamic stability constants log K_{therm} and log K_{cond} reflecting the affinity of gadolinium for its ligand (at high basic pH and 7.4, respectively) These two thermodynamic constants are useful to quantify the amount of free Gd^{3+} or free ligand in equilibrium in a water solution.
(2) Calculation based on thermodynamic models in an attempt to predict the amount of free Gd^{3+} released from gadolinium chelates in the presence of endogenously available ions, e.g., Ca^{2+}, Cu^{2+}, Zn^{2+}, Fe^{3+}, H^{+}, OH^{-}, CO_3^{2-} and PO_4^{3-} etc, once thermodynamic equilibrium has been achieved.

Thermodynamic stability constant K_{therm} and conditional stability constant K_{cond}

By definition, as gadolinium is chelated, a thermodynamic equilibrium exists between the metal [M], the ligand [L] and the chelate [ML]:

$$[\text{M}] + [\text{L}] \rightleftarrows [\text{ML}] \quad (1)$$

The stability of gadolinium chelates is expressed in terms of log K_{therm} (sometimes called log K or log K_{GdL}), where K_{therm} is the thermodynamic stability constant, defined as:

$$K_{\text{therm}} = [\text{ML}]/([\text{M}] * [\text{L}]) \quad (2)$$

As the value of log K_{therm} is valid at high basic pH, the conditional thermodynamic stability constant log K_{cond} (sometimes called log K' or log K'_{GdL}) is calculated at pH 7.4 on the basis of log K_{therm} values and protonation constants of the ligand. Consequently, log K_{cond} describes the position of the equilibrium at physiological pH 7.4. It should be noted that the values for log K_{therm} and log K_{cond} reported in the literature can differ slightly as they depend on the methods used to determine these constants (potentiometric measurement or competition experiments) and the conditions used (ionic force, nature of the electrolyte, temperature) (Moreau et al. 2004).

The conditional thermodynamic stability constant is defined as:

$$K_{\text{cond}} = K_{\text{therm}} * [\text{L}]/\text{L}_T \quad (3)$$

where L_T is the total concentration of the unchelated ligand, i.e. $\{\text{L} + [\text{HL}] + [\text{H}_2\text{L}] + \cdots\}$ where [HL], $[\text{H}_2\text{L}]$ are the protonated forms of the free ligand species.

As the thermodynamic stability constants reflects the affinity of gadolinium for its ligand (at high basic pH for log K_{therm} and pH 7.4 for log K_{cond}), the higher the thermodynamic stability constants (log K_{therm} or log K_{cond}), the more stable is the chelate and the less free gadolinium ion and free ligand are present when given enough time to reach thermodynamic equilibrium.

As there is a significant proton competition at pH 7.4 depending on the basicity of the ligand, which differs between ligands, the conditional thermodynamic stability constant log K_{cond} at pH 7.4 is always substantially lower than the overall thermodynamic constant log K_{therm} as shown in Table 1.

Thermodynamic stability constants and conditional stability data are undoubtedly useful in terms of formulation, where formulated solutions have weeks to equilibrate at the pH of the formulation (the shelf-life of marketed gadolinium contrast agents is 3 years) (Tweedle 1992). Because of their relatively low conditional stability, pharmaceutical solutions of some gadolinium chelates include a variable amount of free ligand, or sodium or calcium salt (Table 1). These excipients are intended to ensure the absence of free Gd^{3+} cations in the pharmaceutical solution throughout their shelf-life (Idée et al. 2006); in the case of Gd-BT-DO3A, the calcium chelate was introduced to avoid certain metallic impurities appearing during the sterilization process (Schmitt-Willich 2007).

However, the need for free ligand or calcium chelate in the formulation of less stable gadolinium chelates can have an impact in terms of in vivo

biocompatibility. Corot et al. (1998) demonstrated that Gd-DTPA and Gd-DTPA-BMA significantly inhibit angiotensin-converting enzyme (ACE) activity at the clinical dose of 0.1 mmol/kg, whereas no significant effect was observed for the two macrocyclic chelates Gd-DOTA and Gd-HP-DO3A. This effect were mainly due to the presence of free ligand and calcium chelate in the formulation of Gd-DTPA and Gd-DTPA-BMA, respectively, because the addition of Zn^{2+} in the same quantities suppressed their inhibitory effects. Puttagunta et al. (1996a) compared the effects of a clinical dose (0.1 mmol/kg) of Gd-DTPA, Gd-HP-DO3A and Gd-DTPA-BMA on serum and urine levels of Cu^{2+} and Zn^{2+} in 31 healthy volunteers (by using inductively-coupled AES). No significant difference was observed between the three chelates with respect to serum and urine Cu^{2+} levels (although a slight increase in Cu^{2+} excretion was found for the three molecules). Conversely, Gd-DTPA-BMA induced a drop in serum Zn^{2+} concentration (but not significant, due to the large inter-individual variability). Gd-DTPA-BMA caused the greatest increase in urinary zinc excretion (post-injection urinary zinc amount: 27.4 ± 9.2 μmol), followed by Gd-DTPA (5.9 ± 1.9 μmol) and Gd-HP-DO3A (1.2 ± 0.5 μmol). The excess ligand present in the formulation of the contrast agents (Table 1) could at least partly explain the high urinary zinc excretion reported. In the case of Gd-DTPA-BMA and Gd-HP-DO3A, Zn^{2+} may have replaced Ca^{2+} on the Ca-ligand in excess and would subsequently be excreted in urine in the form of Zn-DTPA-BMA or Zn-HP-DO3A. For Gd-DTPA, the additional free DTPA may have chelated blood Zn^{2+}. More recently, the influence of gadolinium chelates on urinary excretion of Zn^{2+} and Cu^{2+} (assayed by atomic absorption spectrometry) was studied in a prospective randomised trial where patients were divided into three groups receiving either Gd-DTPA-BMA, or Gd-DTPA or Gd-DOTA. A fourth group of patients, undergoing MRI but without gadolinium chelate, was used as a control (Kimura et al. 2005). The linear open chain chelate Gd-DTPA-BMA caused the greatest increase in urinary zinc excretion among the three agents tested, whereas the macrocyclic agent Gd-DOTA had no effect on zinc excretion and Gd-DTPA induced an intermediate effect. No significant difference in Cu^{2+} excretion was observed between the three gadolinium chelate. This result could be explained by a higher blood concentration of Zn^{2+} (10–50 μmol/l) vs. Cu^{2+} (1–10 μmol/l) (Mann 1993). This study is fully consistent with the data reported in rats by Corot et al. (1998), where an increase in urinary Zn^{2+} excretion was observed after injection of Gd-DTPA and Gd-DTPA-BMA due to the presence of free ligand and calcium chelate in the respective formulations.

Structure/thermodynamic relationships

All teams that developed contrast agents for imaging applications endeavoured to increase log K_{therm} and log K_{cond} to reduce the risk of dissociation of the chelate into free gadolinium and free ligand. However, it is difficult to achieve high thermodynamic stability with the gadolinium ion because its valency *f* orbitals are inner orbitals with no directing effects. The stability of lanthanide chelates is therefore essentially derived from electrostatic interactions between the gadolinium ion considered to be a "spherical" hard acid ion and the donor groups of the chelating agent (such as carboxylate considered to be a hard base). Since chemical bonds in gadolinium chelates are predominantly ionic, stable chelates are formed with multidentate polyaza-carboxylate chelates and three main structural factors influencing the thermodynamic stability of gadolinium chelates have been described:

(1) The basicity of the polyaza-carboxylate scaffold, which can be evaluated by calculating the sum of the protonation constants of each donor atom of the ligand. This overall basicity (ΣpK_a) is directly correlated with the intensity of electrostatic interactions between the metal and the donor atoms of the ligand, and therefore with the stability of the chelate. This assertion is supported by a linear correlation between log K_{therm} vs. ΣpK_a observed for linear open chain and also macrocyclic polyamino-carboxylates, which form five-membered chelate rings (Kumar et al. 1994, 1995).

(2) The number of five-membered rings (N–Gd–N and N–Gd–O) (Mann 1993) formed by the chelate between the metal and the various donor atoms of the ligand as five-membered rings minimizes the steric strain in the chelate (Meyer et al. 1988).

(3) The macrocyclic effect (Desreux 1980), which is related to the cavity size of the chelate ring and also to the preorganization, rigidity and conformation of the ligand. Macrocyclic ligands appeared particularly well suited for chelation of "spherical" gadolinium because these ligands feature an internal cavity with oxygen and/or nitrogen binding sites and are able to wrap themselves around the gadolinium. NMR experiments have demonstrated that DOTA ligand is a highly preorganized structure encouraged by the stability of the [3333] conformation of the macrocycle (Desreux 1980). To illustrate this macrocyclic effect, the spider-like DOTA ring very tightly incorporates the gadolinium ion, as demonstrated by molecular calculation of binding cavities using Connolly surfaces (Fossheim et al. 1991) and forms a tightly packed chelate with marked rigidity (Kumar 1997).

These structural aspects have major consequences on the thermodynamic stability of the available chelates:

(1) Macrocyclic chelates are characterized by quite different thermodynamic constants which can be correlated with their ionic or nonionic chemical structure, as the electrostatic interactions between the metals and ligands would be drastically decreased if the macrocyclic rings were substituted with nonionizable pendant arms. This point is clearly illustrated by the fact that the stability constants of Gd-DOTA are 2–4 orders of magnitude higher than those of Gd-HP-DO3A and Gd-BT-DO3A. This difference is a direct consequence of the higher negative charge of the $DOTA^{4-}$ ligand, which results in a stronger electrostatic metal ligand interaction than that in nonionic macrocyclic chelates. The difference of stability between the two nonionic macrocyclic chelates, Gd-HP-DO3A and Gd-BT-DO3A, is due to a weaker metal-alcoholic OH interaction in Gd-BT-DO3A. As coordination of the bulky group containing the three OH groups of Gd-BT-DO3A is sterically hindered, the coordinated alcoholic OH group is more acidic in Gd-BT-DO3A ($pK_{OH} = 9.48$) than in Gd-HP-DO3A ($pK_{OH} = 11.36$) and this factor leads to a significant loss of thermodynamic stability for Gd-BT-DO3A (Toth et al. 1996).

(2) The linear open-chain ligands do not benefit from the macrocyclic effect and the number of five-membered rings (N–Gd–N and N–Gd–O) is reduced (seven) in comparison to the macrocyclic series (eight). These two factors explain the difference in terms of thermodynamic stability between the ionic linear open-chain chelates (Gd-DTPA, Gd-EOB-DTPA, MS325, Gd-BOPTA) and the macrocyclic ionic chelate Gd-DOTA. The BOPTA, EOB-DTPA and MS325 ligands possess the same number and type of donor atoms as DTPA and the resulting stabilities of these linear open-chain gadolinium chelate are fairly similar. As nonionic donor atoms are less basic and consequently bind more weakly than ionic donor atoms, replacement of the two anionic carboxylate groups of Gd-DTPA by a nonionic amide group coordinating gadolinium via the neutral carbonyl oxygen in Gd-DTPA-BMA and Gd-DTPA-BMEA results in a marked (threefold) decrease in the stability of gadolinium chelates (Brücher and Sherry 2001).

Equilibrium calculation and thermodynamic transmetallation: is thermodynamic selectivity useful?

Blood and other body fluids are relatively complex milieux containing a very large number of ligands or endogenous metal ions. Consequently, various competitive reactions can occur in vivo. For example, in vivo, endogenous cations (Fe^{3+}, Ca^{2+}, Zn^{2+}, Cu^{2+}) can react with gadolinium chelates by displacing Gd^{3+} in a metal-metal transmetallation exchange. The free Gd^{3+} is then complexed by metal-binding proteins, endogenous ions (citrate, glutaminate) or endogenous anionic precipitants (CO_3^{2-}, OH^-, PO_4^{3-}), whereas the free ligand reacts with various endogenous metal ions (Idée et al. 2006). In order to understand the in vivo fate of gadolinium chelates, some authors (Cacheris et al. 1990; Sarka et al. 2000; Jackson et al. 1990; Chang 1993) have tried to quantify the release of free Gd^{3+} using a broad thermodynamic approach using calculations based on a simplified biospeciation plasma model. For this purpose, they defined the thermodynamic selectivity constant (K_{sel}) which addresses the possibility of thermodynamic transmetallation according to the following equilibrium:

$$GdL + [M]^{2+} \rightleftarrows Gd^{3+} + [ML]^{-} \quad (4)$$

where M is the endogenous bivalent cation which exchanges with Gd^{3+} in the chelate.

In fact, different and confusing definitions of the selectivity constant have been reported in the literature (Caravan 1999).

One definition assumes that the logarithmic selectivity constant S (sometimes called log K_{sel}), of the ligands for Gd^{3+} over other metal ions is the difference between log K_{therm} and the stability constant of the endogenously available metal ion chelate, log K_{therm} (ML) (where M = Ca^{2+}, Cu^{2+}, Mn^{2+}, Fe^{3+} and Zn^{2+}), individually (Table 2) (Kumar 1997; Kumar et al. 1995). This selectivity constant can also be determined at pH 7.4 (called S*), (De Haen et al. 1999) and can be calculated using log K_{cond} and log K_{cond} (ML) (where M = Ca^{2+}, Cu^{2+}, Mn^{2+}, Fe^{3+} and Zn^{2+}). Another definition of selectivity constant log K_{sel} (sometimes called log K'_{sel} (Kumar 1997; Kumar et al. 1995) was also introduced and represents an overall selectivity, which is a hybrid calculated constant that takes into account all of the competition reaction (Gd^{3+}, H^+, OH^-, Ca^{2+}, Zn^{2+}, Cu^{2+}, etc) for the ligand studied. K'_{sel} is defined according to equation 5 (where K_1, K_2, K_3 are the consecutive ligand protonation constants) with Ca^{2+}, Zn^{2+} and Cu^{2+} concentrations corresponding to physiological plasmatic concentration i.e. 2.5 mM, 1 μM and 50 μM, respectively (Kumar et al. 1995):

$$K'_{sel} = K_{(ML)}\left(\alpha_H^{-1} + \alpha_{CaL}^{-1} + \alpha_{CuL}^{-1} + \alpha_{ZnL}^{-1}\right) \quad (5)$$

with

$$\alpha_H^{-1} = 1 + K_1[H^+] + K_1K_2[H^+]^2 + K_1K_2K_3[H^+]^3 + \cdots$$
$$\alpha_{CaL}^{-1} = 1 + K_{CaL}[Ca^{2+}]$$
$$\alpha_{CuL}^{-1} = 1 + K_{CuL}[Cu^{2+}]$$
$$\alpha_{ZnL}^{-1} = 1 + K_{ZnL}[Zn^{2+}]$$

With the initial goal to try to predict relationships between thermodynamic constants and toxicity, Cacheris et al. (1990) used an eight-component plasma model to try to simulate the equilibria occurring in plasma and incorporating the endogenous cations H^+, Ca^{2+}, Cu^{2+} and Zn^{2+} (iron was not considered), citrate, amino acids and albumin. According to Cacheris' calculations based on the selectivity constant log K'_{sel}, Gd-DTPA-BMA releases approximately one half as much as Gd^{3+} as Gd-DTPA and Gd^{3+} release was predicted to mainly result in Gd-citrate (phosphate was not taken into account although $GdPO_4$ precipitates) (Jackson et al. 1990). According to their biospeciation model, the overall selectivity constants log K'_{sel} were situated in the order Gd-DTPA-BMA > Gd-DTPA which parallels the order observed for LD_{50} for these two linear open-chain chelates (and two other linear open-chain chelates). In contrast, no correlation was observed between K_{therm} values and their rodent LD_{50} values (intravenous LD_{50}, injected in divided doses in the case of Gd-DTPA-BMA, in contrary with the classical method and therefore not comparable with other published LD_{50}). Consequently, Cacheris et al. suggested that an increase in selectivity for Gd^{3+} over endogenous cations substantially contributes to the high LD_{50} of Gd-DTPA-BMA (it should be noted that other parameters can also contribute to acute toxicity, such as osmolality, viscosity, injection rate, strain of animals) and, strictly speaking, only compounds that have been tested under rigorously similar conditions should be compared in terms of LD_{50}. Furthermore, LD_{50} values reflect immediate toxicity and therefore cannot be used to compare molecules in terms of their potential to trigger long-term toxic effects such as those supposed to be associated with dechelation. Despite this calculated increase in selectivity of Gd-DTPA-BMA, in the same article, the authors attempted to minimize transmetallation of this

Table 2 Thermodynamic stability constant log K_{therm} (ML) and log K_{cond} (ML) of several chelates, according to the cation (Mn^{2+}, Ca^{2+}, Cu^{2+}, Zn^{2+}, Fe^{3+}) (De Haen et al. 1999; Caravan et al. 1999)

Ligand L	Log K_{therm} (CaL)	Log K_{therm} (CuL)	Log K_{therm} (ZnL)	Log K_{cond} (MnL)	Log K_{cond} (CuL)	Log K_{cond} (ZnL)	Log K_{cond} (FeL)
DTPA	10.75	21.38	18.29	11.0	16.8	13.8	23.4
DTPA-BMA	7.17	13.03	12.04	ND	11.1	10.1	ND
DOTA	17.23	22.63	21.05	13.2	15.7	13.8	22.7
HP-DO3A	14.83	22.84	19.37	ND	ND	ND	ND
BOPTA	ND	ND	ND	11.1	17.3	13.9	23.4
MS325	10.45	21.3	17.82	ND	ND	ND	ND

gadolinium chelate. For this purpose, they studied the influence of the formulation of Gd-DTPA-BMA on acute toxicity LD_{50} (once again determined after injection in divided doses). Acute toxicity of non-formulated Gd-DTPA-BMA was found to be 14.8 mmol/kg in mice. The addition of 5 mol% Na[Ca-DTPA-BMA] to the Gd-DTPA-BMA solution dramatically reduced this toxicity (LD_{50} of the formulated solution = 38.3 mmol/kg) (Cacheris et al. 1990). The authors proposed a possible mechanism: displacement of non-toxic Ca^{2+} from Na[Ca-DTPA-BMA] by endogenous Zn^{2+} which, under these conditions, is no longer available for Gd^{3+} transmetallation. This hypothesis was confirmed in a subchronic toxicity study of Gd-DTPA-BMA (Cacheris et al. 1990), in which animals receiving a dosage of 5 mmol/kg showed signs of zinc depletion. Following injection of Na[Ca-DTPA-BMA] to the rats, 7% of the initial molecule was excreted in the urine in the form of Zn^{2+} complex (and 1% in the form of Cu^{2+} complex) (Okazaki et al. 1996), thereby confirming that the previously hypothesized displacement probably occurs in vivo and that despite high overall selectivity constants log K'_{sel} (100 times higher than for Gd-DTPA), Gd-DTPA-BMA is transmetallated in vivo by Zn^{2+}.

The experimental results reported by Puttagunta et al. (1996b) contradict the prediction of Cacheris' biospeciation model and demonstrate that the selectivity between Gd^{3+} and Zn^{2+} in an in vitro model is substantially different from that calculated from thermodynamic data. In an in vitro transmetallation assay, in which solutions of gadolinium chelates were mixed with an equimolar solution of zinc citrate in 1:1 ratio at pH 7.4, these authors found that the selectivity of Gd-DTPA-BMA for Zn^{2+} was more than tenfold lower than that calculated by Cacheris (Puttagunta et al. 1996b). This contradiction can be explained by the fact that the equilibrium model proposed by Cacheris neglected the formation of binuclear chelates. In reality, DTPA-BMA (like DTPA (Tweedle et al. 1988) presumably forms binuclear complexes with Zn^{2+}, which decrease the selectivity for Gd^{3+} over Zn^{2+} (Brücher 2002). Moreover, an additional limitation in the thermodynamic model of blood pool plasma used by Cacheris is that it does not consider iron as a gadolinium competitor and, above all, phosphate as a gadolinium precipitant.

In another study, Sarka et al. (2000) used a simplified plasma model in which only competition between Gd^{3+} and Zn^{2+} was considered to study the release of gadolinium from Gd-DTPA. This equilibrium calculation indicated partial displacement of Gd^{3+} by Zn^{2+} and a large amount of Gd^{3+} displaced in the form of Gd-citrate (9.2%), as only 90.7% of gadolinium was still chelated in the form of Gd-DTPA.

The practical application of this type of equilibrium model based on selectivity constants is limited by several main factors at the present time (Sarka et al. 2000; Bianchi et al. 2000):

- The ability to simulate all of the equilibria that are expected to occur in vivo and then to define a relevant plasma model.
- In addition, these thermodynamic models do not consider the kinetic aspects related to dechelation and transmetallation. As mentioned in the initial paper (Cacheris et al. 1990; Chang 1993), the biospeciation model is not adapted to gadolinium chelates with high kinetic stability such as Gd-DOTA as the thermodynamic equilibrium cannot be reached due to the kinetic inertia of this macrocycle chelate. Therefore, according to the authors themselves, log K'_{sel} has no physiological meaning for Gd-DOTA. Numerous experimental data have now demonstrated that the kinetic stability of gadolinium chelates is also an important parameter to predict the fate of gadolinium chelates in vivo. It has been suggested that the relative tolerance of gadolinium chelates can be explained not only by selectivity constants, but also by kinetic inertia, acid-assisted dissociation and metal exchange rates (Kumar et al. 1995) and that, like plasma equilibrium models, kinetic models taking into account excretion rates and distribution rates in the extracellular space of gadolinium chelates need to be developed in order to predict the amount of free gadolinium that dissociates in vivo (Brücher 2002).

Kinetic stability

High thermodynamic stability constants measured in water are neither necessary nor sufficient conditions for in vivo stability (Tweedle et al. 1991), as kinetic stability is also clearly an important parameter for

understanding relative in vivo dissociation by describing:

- Either the dechelation kinetics corresponding to the reaction:

$$GdL \rightleftarrows Gd^{3+} + L \quad (6)$$

.

This reaction can occur via two mechanisms: spontaneous or proton-assisted dissociation of the Gd^{3+} chelate possibly catalysed by endogenous metals (Sarka et al. 2000; Robic et al. 2007, unpublished results)

- or the transmetallation kinetics of the gadolinium chelate by endogenous metals, particularly Ca^{2+}, Cu^{2+} and Zn^{2+}) corresponding to the reaction:

$$GdL + [M]^{2+} \rightleftarrows Gd^{3+} + [ML]^{-} \quad (7)$$

.

This metal exchange reaction can occur via two mechanisms (Brücher 2002)

– Spontaneous or proton-assisted dissociation of the Gd^{3+} chelate followed by rapid reaction of the free ligand with endogenous metal ions.
– Direct attack of the endogenous ion on the chelate via formation of a dinuclear intermediate GdLM followed by the formation of ML.

Once the importance of kinetic stability of the Gd^{3+} chelates was recognized, various types of studies were performed to describe the structural aspect driving the dechelation and transmetallation kinetics, which are significantly different according to each marketed gadolinium chelate.

Kinetic stability: in vitro *studies*

The dissociation rates of gadolinium chelates are low at pH 7.4 and the chelates dissociate much more rapidly in acidic solutions (Brücher 2002). Consequently, the rate of spontaneous or proton-assisted dissociation of the Gd^{3+} chelate was studied by measuring the rate of Gd^{3+} dissociation in the presence of 0.1 M HCl (pH 1.0) at 25°C (Tweedle 1992). In this type of acidic medium, dissociation of the chelate is a pseudo-first order reaction, with:

$$\text{Dissociation rate} = k_{obs}\,[GdL] \quad (8)$$

with

$$k_{obs} = k_0 + k_1[H^+] + k_2[H^+]^2 \quad (9)$$

Neglecting k_0 and k_2, the k_{obs} constant has been determined for several chelates by calculating the slope of the curves $\ln[GdL]_t = f(t)$ by Tweedle (1992). The results obtained demonstrated that the half-lives dramatically differed and were considerably longer for macrocyclic chelates ($T_{1/2} > 1$ month for Gd-DOTA, 3 h for Gd-HP-DO3A) than for linear open chain chelates ($T_{1/2} = 10$ min for Gd-DTPA and ~35 s for Gd-DTPA-BMA). However, these authors reported different k_{obs} constant for the same experiment in successive publications (Tweedle 1992; Kumar et al. 1993; Wedeking et al. 1992; Kumar and Tweedle 1993) that were also different to that measured by Pulukkody et al. (1993). Schmitt-Willich reported Bayer Schering's dissociation half-life $T_{1/2}$ values at pH 1.0 for Gd-HP-DO3A and Gd-BT-DO3A, but unfortunately not for Gd-DOTA (Schmitt-Willich 2007). As these $T_{1/2}$ values depend on the experimental conditions used (pH, temperature, ionic strength, metal concentration) and the assay method (free gadolinium, free ligand, proton consumption due to protonation of the released ligand), in an attempt to be exhaustive, we measured the k_{obs} and $T_{1/2}$ values of the three macrocyclic compounds by using a strict comparative protocol in our laboratory (arzenazo dosage) (Table 3). This comparative study demonstrated that, under the experimental conditions used (pH 1.0 and 1.2, temperature 37°C and 25°C), the kinetic stability of macrocyclic gadolinium chelates can be classified in the following order: Gd-DOTA > Gd-BT-DO3A >

Table 3 $T_{1/2}$ values of gadolinium chelates

	$T_{1/2}$ pH = 1.2, 37°C (h)	$T_{1/2}$ pH = 1.0, 37°C (h)	$T_{1/2}$ pH = 1.0, 25°C (h)
Gd-DOTA	85	26.4	338
Gd-HP-DO3A	4	2.0	3.9
Gd-BT-DO3A	18	7.9	43
Linear open-chain chelates	ND	ND	<5 s

Gd-HP-DO3A and that, as expected, the kinetics are more rapid at 37°C in comparison to 25°C. This is clearly illustrated by Fig. 1 showing the decrease of the remaining percentage of chelate as a function of time at pH 1.2, 37°C. Under these experimental conditions, the $T_{1/2}$ for all linear open-chain chelates was <5 s (Table 3), clearly demonstrating their lower kinetic stability in comparison to that of macrocyclic chelates.

The kinetic stability of linear open-chain chelates such as MS325 (Caravan et al. 2001), Gd-DTPA (Sarka et al. 2000), Gd-DTPA-BMEA (Rothermel et al. 1997) and Gd-DTPA-BMA (Sarka et al. 2002) was assessed by characterizing the rates of the exchange reactions with Eu^{3+}. From a mechanistic point of view, the dissociation rate of the bisamide linear open-chain gadolinium chelates Gd-DTPA-BMA and Gd-DTPA-BMEA is higher because Gd^{3+}-amide oxygen bonds are weaker than Gd^{3+}-carboxylate oxygen bonds (White et al. 1991) and consequently the protonated intermediate, which is important for the rate-controlling step of dissociation, can be more easily formed (Sarka et al. 2002).

Various other studies have addressed the kinetic stability of the three macrocyclic gadolinium chelates (Kumar et al. 1993; Wang et al. 1992), but only two studies have used the same protocol for Gd-DOTA (Toth et al. 1994), Gd-BT-DO3A, and Gd-HP-DO3A (Toth et al. 1996). The kinetic stability of the three macrocyclic gadolinium chelates was studied under the same conditions by UV spectroscopy by following the exchange reactions between high concentration of gadolinium chelates and very low concentration of Eu^{3+}. Under these conditions, the exchange rate does not depend on the europium concentration and exchange only occurs via proton-assisted dissociation of the gadolinium chelate. Interestingly, the dissociation rate was studied in the pH range 3.2–5.3 (at 37°C for Gd-DOTA and at 25°C for Gd-BT-DO3A and Gd-HP-DO3A), which is obviously more relevant in terms of physiology compared to the studies discussed above, performed at pH 1 or 1.2. Under these conditions, the exchange is considered to be a pseudo-first order reaction characterized by the constant k_{obs}:

$$d[GdL]/dt = k_{obs}[GdL] \quad (10)$$

In the pH range studied, the first order constant k_{obs} is directly proportional to the H^+ concentration

$$k_{obs} = k_0 + k_1 [H^+] \quad (11)$$

The rate constants k_0 and k_1 are characteristic of spontaneous and proton-assisted dissociation of gadolinium chelates (Brücher 2002). The dissociation of these macrocyclic chelates, which is much less important than that of linear open-chain chelates (Varadarajan et al. 1994), is also a proton-assisted process and the observed linear dependence of the exchange rate (k_{obs}) on the H^+ concentration has been interpreted in the same way for the three macrocyclic chelates. In weakly acidic solutions ($3.2 < pH < 5$), the exchange rate is linearly proportional to the concentration of a monoprotonated species. In this monoprotonated chelate, the protonation of a carboxylate oxygen reduces the kinetic stability of the chelate, but, prior to dissociation the proton is transferred to a nitrogen atom of the ligand and Gd^{3+} steps out of the coordination cage. This proton transfer, which proceeds via an intramolecular rearrangement of the protonated chelate, is probably the rate-controlling step and very slow due to the rigid structure of macrocyclic chelates. After or during this rearrangement, a second proton is immediately protonated to another nitrogen atom forming a diprotonated intermediate, while the Gd^{3+} ion leaves the coordination cage due to the electrostatic repulsion between the protons and Gd^{3+}. As previously shown at pH 1 and 1.2, the data obtained between pH 3.2 and 5 (Table 4) demonstrate that the sequence of kinetic stability of the gadolinium macrocyclic chelates is as follows: Gd-DOTA > Gd-BT-DO3A (the dechelation kinetics are accelerated when the temperature increases: Table 3).

Several studies have been performed to determine the dechelation or transmetallation kinetics in physiological milieu (or a milieu mimicking biological fluids).

As described above, in vivo, several endogenous cations such as Fe^{3+}, Cu^{2+}, Zn^{2+} or Ca^{2+} can compete with the Gd^{3+} cation for the ligand and a number of other anions such as PO_4^{3-}, CO_3^{2-}, OH^-, can compete with the ligand for the Gd^{3+} (Tweedle et al. 1988). An in vitro test was performed, in which precipitated $GdPO_4$ was measured 15 min after mixing 25 mM of macrocyclic and linear open chain gadolinium chelates with 25 mM of endogenous Cu^{2+} or Zn^{2+} cations in a phosphate medium (at pH 7.0 and at room temperature) (Tweedle 1992; Tweedle et al. 1991). The formation of insoluble $GdPO_4$ is the

Table 4 Rate constants characterizing the proton-assisted dissociation of the macrocyclic chelates

Gadolinium chelates	Temperature (°C)	k_0 (s^{-1})	k_1 (M^{-1} s^{-1})	Reference
Gd-DOTA	37	5×10^{-10}	2×10^{-5}	Toth et al. (1994)
Gd-HP-DO3A	25	-7×10^{-10}	2.6×10^{-4}	Toth et al. (1996)
Gd-BT-DO3A	25	2×10^{-10}	2.8×10^{-5}	Toth et al. (1996)

driving force of this in vitro stress (Chang 1991). The experimental data showed that macrocyclic chelates, Gd-DOTA and Gd-HP-DO3A, are kinetically inert unlike linear open-chain chelates. Dechelated Gd^{3+} was not observed for the macrocyclic chelates Gd-DOTA and Gd-HP-DO3A for Cu^{2+} or Zn^{2+} cations, whereas a significant release of Gd^{3+} was observed for the linear open-chain chelates Gd-DTPA-BMA and Gd-DTPA (35% and 25% for Cu^{2+} and 25% and 21% for Zn^{2+}, respectively).

In another in vitro transmetallation assay, solutions of various gadolinium chelates were mixed with an equimolar solution of zinc citrate in a 1:1 ratio at pH 7.4 (Puttagunta et al. 1996b). Kinetic stability was higher for the macrocyclic molecule Gd-HP-DO3A than for Gd-DTPA. The lowest kinetic stability in the presence of Zn^{2+} was observed for Gd-DTPA-BMA. In a second experiment, the transmetallation kinetics were assessed by measuring the changes in the concentration of Gd-chelates at various time-points following mixing with equimolar zinc citrate. Interestingly, formulated Gd-DTPA-BMA demonstrated the immediate appearance of Gd citrate when mixed with equimolar Zn^{2+} citrate (Puttagunta et al. 1996b).

Magerstät et al. (1986) performed serum stability studies for DOTA, DTPA chelates of ^{153}Gd incubated at 37°C. Loss of Gd^{3+} from the chelates was evidenced by measuring the radioactivity in the precipitates. Over the 150-h observation period, Gd-DOTA lost almost no radioactivity, whereas Gd-DTPA lost about 10–20% of free radioactive gadolinium. The observed difference between the two chelates in this study was most likely due to the difference in terms of dissociation kinetics between the macrocyclic and linear open-chain gadolinium chelates.

Sarka et al. assessed the kinetic stability of linear open-chain chelates Gd-DTPA (Sarka et al. 2000), and Gd-DTPA-BMA (Sarka et al. 2002) in an in vitro transmetallation assay by characterizing the rates of the exchange reactions with endogenous cations or Cu^{2+} or Zn^{2+}. Under physiological conditions, the exchange reaction mainly occurs via direct attack of Cu^{2+} and Zn^{2+} on the chelate for Gd-DTPA, but proton-assisted dissociation is still important for Gd-DTPA-BMA. During direct attack of Cu^{2+} and Zn^{2+}, a dinuclear intermediate is formed, in which the functional groups of the ligand are gradually and slowly transferred from Gd^{3+} to the attacking metal ion step by step. Although the plasma Cu^{2+} concentration is very low, Cu^{2+} has been found to be kinetically very active as Cu^{2+} is 10–80 times more effective than Zn^{2+} in this exchange reaction. The authors concluded that the contribution of proton-assisted dissociation is about tenfold higher for Gd-DTPA-BMA than for Gd-DTPA, but Zn^{2+}-assisted dissociation is about ten-fold faster for Gd-DTPA than for Gd-DTPA-BMA, which compensates for the difference observed for proton-assisted dissociation. Consequently, when all possible exchange reactions are taken into account (i.e. proton-assisted dissociation and Cu^{2+}-, Zn^{2+}-assisted dissociation), the two chelates Gd-DTPA and Gd-DTPA-BMA have similar overall dissociation rates. To explain these results, the authors hypothesized that although the binding strength of the amide group is weaker than that of a carboxylate, its reactivity with H^+, Zn^{2+} or Cu^{2+} is lower. However, these conclusions are in contradiction with the results obtained in vivo and by the in vitro relaxometric method (see below).

The kinetic stability of Gd-DOTA, Gd-HP-DO3A, Gd-DTPA, Gd-DTPA-BMA and Gd-EOB-DTPA in solutions containing metabolites such as adenosine triphosphate (ATP), phosphocreatine (PCr), inorganic phosphate (Pi) found at the relatively high concentrations observed in the cytosol was studied by measuring the longitudinal P-31 relaxation rate enhancements of phosphate groups (Vander Elst et al. 1994, 1997). No kinetic dechelation of gadolinium ion was observed for macrocyclic chelates, but the effects observed for the two linear open-chain

gadolinium chelates indicate that ATP, which is known to be an extremely good chelator of metals, competes with DTPA, DTPA-BMA and EOB-DTPA for chelation of Gd^{3+} ions, indicating a poor kinetic inertia of these chelates. Under these experimental conditions, Gd-DTPA-BMA was shown to be less stable than Gd-DTPA which was also less stable than Gd-EOB-DTPA. Gd-BOPTA was not studied in this protocol. However, another study demonstrated that ATP interacts with Gd-BOPTA at physiological pH (Bianchi et al. 1999).

An easy and reliable in vitro relaxometric method has recently been validated to quantitatively evaluate the release of Gd^{3+} from gadolinium chelates (Laurent et al. 2001) in the presence of Zn^{2+} at pH 7.4. This method is based on the following principle: if dechelation of a soluble gadolinium chelate by diamagnetic Zn^{2+} ions were to occur in a phosphate-buffered solution, then the released Gd^{3+} would react to form precipitated $GdPO_4$ which has a negligible influence on the relaxation rate of water. Therefore, a decrease of relaxation rates $R_1^P(t)$ would occur with time and the magnitude of this decrease would be related to the proportion of precipitated $GdPO_4$. The time-course of the $R_1^P(t)/R_1^P(t = 0)$ ratio of macrocyclic and linear open-chain chelates is presented in Fig. 2 as recently published (Laurent et al. 2006) and completed by values obtained with MS325, Gd-EOB-DTPA and Gd-DTPA-BMEA under strictly similar conditions (Robic et al. 2007, unpublished results). As previously proposed (Laurent et al. 2006), 3 classes of gadolinium contrast agents can be determined using a "long time index" equal to the ratio of the paramagnetic relaxation rates after 50 h (Fig. 2):

- Macrocyclic chelates, Gd-DOTA or Gd-HP-DO3A and Gd-BT-DO3A, characterized by very high kinetic inertia stability: long-term index >0.95.
- Ionic linear open-chain chelates, Gd-DTPA, EOB-DTPA, Gd-BOPTA and MS325, with moderate kinetic inertia leading to significant dechelation in the presence of Zn^{2+} under these pseudo-physiological conditions (long-term index: 0.49 to 0.85).
- Nonionic linear open-chain chelates Gd-DTPA-BMA and Gd-DTPA-BMEA which exhibit poor kinetic stability and the highest degree of dechelation (long-term index <0.3) of all commercially available gadolinium chelates.

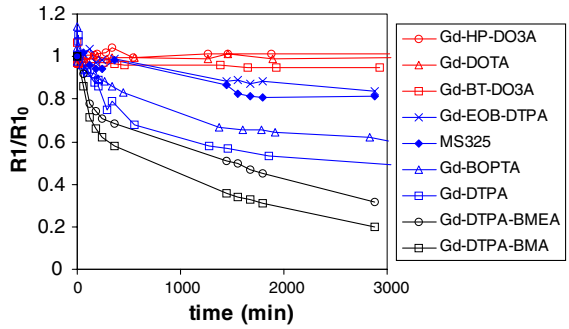


Fig. 2 Time-course of the $R_1^P(t)/R_1^P(t = 0)$ ratio for Gd-DTPA, Gd-DTPA-BMA, Gd-DTPA-BMEA, Gd-BOPTA, Gd- EOB-DTPA, MS325, Gd-DOTA, Gd-BT-DO3A, Gd-HP-DO3A. Concentration of Gd chelates and $ZnCl_2$ are 2.5 mM in phosphate buffer at pH 7.0 and 310 K ($B_0 = 0.47$ T)

Whether the reaction studied under these experimental conditions corresponds to transmetallation or dechelation catalysed by Zn^{2+} is still an unresolved question that is currently under investigation.

Kinetic inertia: structural aspect driving the kinetic stability or structure/stability relationships

Overall, these physicochemical and in vitro studies demonstrate that:

- All linear open-chain gadolinium chelates are significantly less kinetically stable than macrocyclic polyaminocarboxylates.
- The order of kinetic stability of macrocyclic gadolinium chelates is Gd-DOTA > Gd-BT-DO3A > Gd-HP-DO3A.

The low kinetic stability of linear open-chain chelates has been explained by the rapid unravelling of these chelates due to their conformational mobility (Kumar 1997). As donor atoms derived from nonionic amides are less basic, they bind more weakly to gadolinium than ionic carboxylate donor atoms (Brücher and Sherry 2001; Brücher 2002) probably introducing more flexibility and conformational mobility in gadolinium chelates and explaining the significant loss of kinetic stability for Gd-DTPA-BMA and Gd-DTPA-BMEA. The introduction of a functional group on the DTPA scaffold (EOB-DTPA, Gd-BOPTA and MS325) appears to slightly improve kinetic inertia. This may be explained by the steric effect of the bulky substituents which slightly hinders

unwrapping of the ligand around the metal ion (Caravan et al. 2001).

The kinetic inertia of macrocyclic chelates probably stems from their tight packing and their high conformational rigidity (Tweedle et al. 1991; Meyer et al. 1998; Wang et al. 1992). Their exceedingly slow dissociation rate is attributed to the peculiar structure of macrocyclic chelates: because of the high rigidity of the tetraaza ring, the inversion rate of nitrogen atoms and the dissociation rate of the gadolinium-oxygen bonds are considerably reduced by the steric requirements of the macrocyclic ligand. The dissociation of macrocyclic chelates probably involves the formation of a protonated species which undergoes a slow rearrangement into an intermediate chelate to transfer the proton to a nitrogen ring atom. Proton transfer via this mechanism is very slow, as entry of a proton into the macrocyclic cage is markedly hindered because of the rigid structure of the macrocyclic chelate. The acid-catalyzed reaction is therefore much less effective than for linear open chain chelates (Brücher and Sherry 2001). Gd-DOTA is the most kinetically stable chelate, as ionic carboxylate binds more strongly to gadolinium and therefore more efficiently limits the conformational motions of the chelate than the alcohol chain of Gd-HP-DO3A or Gd-BT-DO3A (Meyer et al. 1998; Desreux and Barthelemy 1988). Pollet and Marx (2007), using ab initio molecular dynamic simulation in aqueous solution at ambient temperature, recently demonstrated that the hydroxypropyl arms of Gd-HP-DO3A which break the symmetry hemisphere, is dynamically flexible in contrast with the four Gd-DOTA carboxylates. The proton-assisted dissociation rate of Gd-BT-DO3A is about tenfold lower than that of Gd-HP-DO3A. In this case, the greater kinetic inertia of Gd-BT-DO3A may be due to the large size of its functional group, containing three alcoholic OH groups. The bulkiness of the "butrol" group could slow down and restrict the rearrangement and dissociation of the protonated chelate for steric reasons (Toth et al. 1996).

Kinetic inertia of gadolinium chelates: in vivo *studies*

The relevance of in vitro studies demonstrating the significant difference in kinetic inertia between various marketed gadolinium chelates can be challenged, as the experimental conditions are very different from in vivo conditions, which is why many papers have addressed this issue of the in vivo inertia of gadolinium chelates.

A study demonstrated higher levels of ^{153}Gd in bones with Gd-DTPA than with Gd-DOTA 7 days and more after intravenous injection in mice (0.25 mmol/kg) (Wedeking and Tweedle 1988). The authors attributed this difference to either a different uptake and/or washout of intact gadolinium chelates by bone or bone marrow or higher in vivo dechelation for Gd-DTPA than for Gd-DOTA.

In vivo dissociation of gadolinium chelates was subsequently measured by quantifying the residual gadolinium in various organs of mice at different residence intervals (5 min to 14 days) following intravenous injection of 0.4 mmol/kg of radiolabelled gadolinium chelates (Tweedle 1992, Wedeking et al. 1992). At residence intervals $>$ 60 min, significant differences were observed, especially concerning long-term distribution of ^{153}Gd in the liver and femur. Significant amounts of residual gadolinium were found in the case of linear open-chain chelates, Gd-DTPA-BMA and to a lesser extent Gd-DTPA, indicating in vivo dissociation of gadolinium chelates, whereas the macrocyclic chelates Gd-DOTA and Gd-HP-DO3A resulted in much lower residual gadolinium levels. The amount of residual gadolinium at 7 days did not correlate with the thermodynamic conditional constant but a correlation was observed between acid dissociation rates measured at pH 1 and long-term deposition of ^{153}Gd in the whole body, liver and femur of mice (Wedeking et al. 1992). This correlation probably confirms the relevance of measuring acidic dissociation rates even though the experimental conditions are very different from physiological pH. Nevertheless, in another study (McMurry et al. 1998), no correlation was demonstrated between the release of lanthanides and the acidic dissociation of several chelates based on the DTPA scaffold. This indicates that more detailed kinetic studies, taking into account the major endogenous ions such as Cu^{2+} and Zn^{2+}, are probably needed to understand the release of free lanthanides in vivo. It should also be noted that the amount of residual gadolinium in the whole body found by Wedeking et al. (1992) was also correlated with the order of stability assessed by the relaxometric method in the presence of Zn^{2+} cations developed

by Laurent et al. (2006) under experimental conditions much closer to physiological conditions (pH 7.4).

Subsequently, Tweedle et al. (1995) injected ^{153}Gd-labelled formulated solutions of Gd-DTPA, Gd-DOTA, Gd-HP-DO3A and Gd-DTPA-BMA to mice and rats as well as the unformulated form of Gd-DTPA-BMA, and measured the residual ^{153}Gd at various time-points up to 14 days after intravenous administration (0.1 mmol/kg). They found that unformulated Gd-DTPA-BMA showed considerably greater residual ^{153}Gd at 7 and 14 days in whole body, liver and bone than the other chelates. The addition of 25 mmol/l Na[Ca-DTPA-BMA] (in the commercially available solution) to the Gd-DTPA-BMA solution reduced the residual ^{153}Gd in the mouse whole body (from 1% to 0.3% of the injected dose), thus suggesting that Gd-chelate dissociation is actually responsible for the higher levels of residual ^{153}Gd. Furthermore, this study clearly showed that the formulated solutions of the linear open chain chelates Gd-DTPA and especially Gd-DTPA-BMA led to higher amounts of residual ^{153}Gd in whole body, liver and femur than those of the macrocyclic chelates Gd-HP-DO3A and Gd-DOTA at late time-points and the authors concluded that the order of residual whole body gadolinium at 14 days (lowest to highest) was: Gd-HP-DO3A # Gd-DOTA $\leq$ Gd-DTPA << Gd-DTPA-BMA (Tweedle et al. 1995; Tweedle 2007; Port et al. 2008).

Similar studies were also conducted (Harrison et al. 1993) and tissue distribution of $^{157/153}$Gd chelates was measured at 5 min and 24 h after an intravenous injection of 0.1 μmol/kg, (much lower than the dose commonly used for MRI) in mice. At 24 h post-injection, <0.05% I.D was detected for Gd-DOTA in the skeleton whereas the percent I.D. retained in the skeleton of injected mice was higher for Gd-DTPA and Gd-BOPTA (0.3% I.D and 0.22%, respectively). In this study, the percent of gadolinium retained in the skeleton was greater than that reported for a high dose in mice at 24 h (Wedeking et al. 1992). An explanation for this difference proposed by the authors is related to the susceptibility of linear open-chain chelates to cation (particularly zinc and copper) promoted dissociation. The amount of chelate dissociated would be limited by the amount of cations present in the plasma so that a smaller fraction of the high dose would dissociate in the study performed at 0.4 mmol/kg of radiolabelled gadolinium chelates (Tweedle 1992; Wedeking et al. 1992).

In vivo gadolinium release was evaluated for Gd-BOPTA, Gd-DTPA-BMA and Gd-BT-DO3A estimating gadolinium content following single or repeated intravenous administrations to rats at a dose of 1 mmol/kg (cumulative dose: 6 mmol/kg). Under these experimental conditions, the order of residual gadolinium found in these organs was Gd-DTPA-BMA > Gd-BT-DO3A > Gd-BOPTA (Bussi et al. 2007). These results can be explained by the dual (urinary and biliary) route of elimination of Gd-BOPTA considering that biliary excretion of Gd-BOPTA is significantly higher in rats than in humans (Lorusso et al. 1999; Spinazzi et al. 1999).

The human relevance of these animal data was demonstrated by Gibby et al. (2004) and White et al. (2006) who measured Gd^{3+} levels in bone collected after administration of a clinical dose of either Gd-HP-DO3A or Gd-DTPA-BMA in patients undergoing total hip arthroplasty. Bone tissue retention of Gd^{3+} was significantly higher in patients who received Gd-DTPA-BMA than in those who received the macrocyclic agent Gd-HP-DO3A, as demonstrated by inductively coupled plasma (ICP) atomic emission spectroscopy (Gibby et al. 2004) and, more recently, by ICP mass spectroscopy. In the latter study, approximately 4 times more Gd^{3+} remained in femoral head bone after Gd-DTPA-BMA than after Gd-HP-DO3A (White et al. 2006). However, the analytical method used is unable to define the status of gadolinium (chelated or precipitated as $GdPO_4$).

Conclusion: what is the possible in vivo and clinical relevance of thermodynamic and kinetic data?

As mentioned above, thermodynamic stability has a practical relevance in terms of formulation, as formulated solutions have a shelf-life of several years to equilibrate at neutral pH (marketed gadolinium contrast agents have shelf-lives between 2 and 3 years).

In vivo, under physiological or pathological conditions, the amount of free gadolinium released will depend on either the dissociation rate (i.e. kinetic stability) and thermodynamic stability when the system cannot reach a thermodynamic equilibrium (kinetic phase) or mainly thermodynamic stability

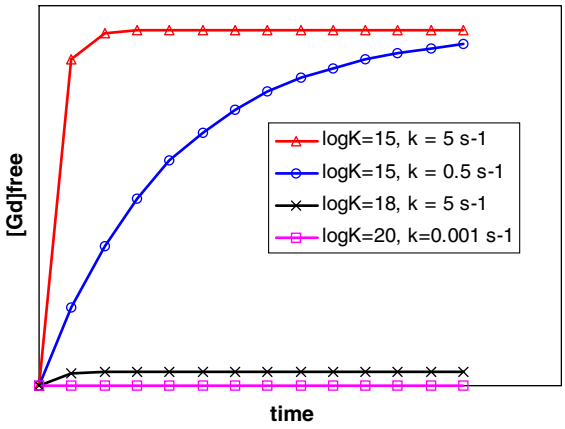


Fig. 3 Calculated percentage of free gadolinium released by various gadolinium chelates assuming a first-order mechanism

when the system is able to reach a thermodynamic equilibrium (thermodynamic state).

These points are illustrated in Fig. 3

- Red and blue curves illustrate the percentage of free gadolinium released by two gadolinium chelates with the same thermodynamic stabilities, but different dissociation kinetics. At the thermodynamic plateau, the same amount of free gadolinium is released, but in the kinetic phase (i.e. time dependant part of the curves), the slower the dissociation kinetics, the less free gadolinium is released.
- Red and black curves illustrate the percentage of free gadolinium released by two gadolinium chelates with the same kinetic constant, but different thermodynamic stabilities (log K_{cond}). At the thermodynamic plateau and kinetic phase, the amount of free gadolinium is significantly different and the amount of free gadolinium released decreases as log K_{cond} increases.
- Red and pink curves illustrate the percentage of free gadolinium released by two gadolinium chelates with different dissociation kinetics and different thermodynamic stabilities. At both the thermodynamic plateau and the kinetic phase, the amount of free gadolinium is significantly different and less free gadolinium is released for more kinetically stable (low dissociation rate) and more thermodynamically stable (high log K_{cond}) chelates.

No validated mechanism for NFS has yet been identified, but several hypotheses have been proposed to explain this highly disabling disease. They are summarized in Table 5. One of the most widely accepted hypotheses (Perazella 2007; Morcos 2007a, b) postulates that, in the setting of renal failure, remanence of a Gd^{3+} chelate in the body is dramatically increased, thereby increasing the chance for dissociation of Gd^{3+} from its chelate especially in the case of nonionic, linear open chain molecules such as Gd-DTPA-BMA. This would allow increased tissue exposure. This is the only hypothesis consistent with the higher incidence of NSF cases associated with Gd-DTPA-BMA.

Consequently, the question to be raised is then which criterion, thermodynamic or kinetic stability, is more important to ensure the lowest Gd release in vivo?

Table 5 Current hypotheses for the pathophysiology of NSF

- Possible involvement of an infectious agent or toxic contaminant, although none have been identified to date (Evenepoel et al. 2004);
- Treatment with calcineurin inhibitors such as cyclosporin which are known to increase the level of TGF-β (Khanna et al. 1997; Inigo et al. 2001). TGF-β may mediate the florid fibroblastic proliferation which characterizes NSF;
- Involvement of antiphospholipid and anticardiolipin antibodies (a marker of small-vessel thrombotic vasculopathy) and the HLA-A_2 allele (Evenepoel et al. 2004; Mackay-Wiggan et al. 2003);
- Early dermal infiltration with bone marrow-derived CD45RO+/CD34+ circulating fibrocytes in response to macrophages that have phagocytosed Gd^{3+} from Gd^{3+} chelate following dechelation (Morcos 2007a, b; Perazella 2007);
- Immune response of the host to noxious stimuli involving dendritic cells and synthesis of TGFβ (as part of the response of the dendritic cells to this noxious agent). The TGFβ produced by these dendritic cells would, in turn, precipitate both the fibrotic process and the expansion and enhancement or initiation of antigen-presenting functions of further dendritic cells establishing a vicious circle (Mendoza et al. 2006);
- Resistance to erythropoietin (Swaminathan et al. 2006);
- Metabolic acidosis as a cofactor (Dharnidharka et al. 2006).

Due to their relatively rapid dechelation or transmetallation kinetics, it has been argued that linear open-chain gadolinium chelates could reach a thermodynamic equilibrium (in contrast with macrocyclic chelates characterized by high kinetic stability) and in that case conditional stability and selectivity could be important physicochemical criteria to predict the amount of gadolinium released (as shown by the plateau in Fig. 3). However, this speculation should be relativised as, in physiological situations, fast rate of diffusion and fast rate of excretion of the gadolinium chelate do not guarantee that thermodynamic equilibrium is reached in vivo (Tweedle et al. 1991; Cacheris et al. 1990; Brücher 2002).

The situation is even more complex in vivo in pathological situations such as renal failure where the biodistribution is modified and excretion is slowed and where gadolinium chelates can be sequestrated in compartments characterized by a special biological composition in terms of proteins, ions and pH, etc as suggested in NSF (Thakral et al. 2007; Broome et al. 2007). In the case of compartmentalization, it can be speculated that a pseudo-equilibrium can be reached and consequently more thermodynamically stable chelates should be preferred in order to minimize any free gadolinium release.

Beyond this speculative debate on the possible achievement of a pseudo-equilibrium in vivo, it appears that high kinetic stability (i.e. macrocyclic chelates in the order of kinetic stability: Gd-DOTA > Gd-BT-DO3A > Gd-HP-DO3A) combined with high thermodynamic stability (i.e. macrocyclic chelates in the order of thermodynamic conditional stability: Gd-DOTA > Gd-HP-DO3A > Gd-BT-DO3A) will minimize the amount of free gadolinium released in vivo.

Acknowledgment The authors thank Prof. R.N. Muller and his group for the experimental data shown in Fig. 2 concerning Gd-BT-DO3A, Gd-DTPA, Gd-BOPTA.

References

Adzamli K, Periasamy MP, Spiller M, Koenig SH (1999) NMRD assessment of Gd-DTPA-bis(methoxyethylamide), (Gd-DTPA-BMEA), a nonionic MRI agent. Invest Radiol 34:410–414

Bellin MF, Vasile M, Morel-Precetti S (2003) Currently used non-specific extracellular MR contrast media. Eur Radiol 13:2688–2698

Benmelouka M, Van Tol J, Borel A, Port M, Helm L, Brunel LC, Merbach AE (2006) A high-frequency EPR study of frozen solutions of Gd(III) complexes: straightforward determination of the zero-field splitting parameters and simulation of the NMRD profiles. J Am Chem Soc 128:7807–7816

Bianchi A, Calabi L, Foresti M, Losi P, Palaeri L, Rodriguez A, Valtancoli B (1999) Interaction of ATP with a Gd^{3+} complex employed as paramagnetic contrast agent in NMR imaging. Inorganica Chim Acta 288:244–248

Bianchi A, Calabi L, Giorgi C, Losi P, Mariani P, Paoli P, Rossi P, Valtancoli B, Virtuani M (2000) Thermodynamic and structural properties of Gd^{3+} complexes with functionalized macrocyclic ligands based upon 1,4,7,10-tetraazacyclododecane. J Chem Soc Dalton Trans 697–705

Bousquet JC, Saini S, Stark DD, Hahn PF, Nigam M, Wittenberg J, Ferrucci JT (1988) Gd-DOTA: characterization of a new paramagnetic complex. Radiology 166:693–698

Broome DR, Cottrell AC, Kanal E (2007) Response to "Will dialysis prevent the development of nephrogenic systemic fibrosis after gadolinium-based contrast administration?". Am J Roentgenol 189:W234–W235

Brücher E (2002) Kinetic stability of gadolinium (III) chelates used as MRI contrast agents. Top Curr Chem 221: 103–122

Brücher E, Sherry AD (2001) Stability and toxicity of contrast agents. In: Merbach AE, Toth E (eds) The chemistry of contrast agents in medical magnetic resonance imaging. Wiley, New York

Bussi S, Fouillet X, Morisetti A (2007) Toxicological assessment of gadolinium release from contrast media. Exp Toxicol Pathol 58:323–330

Cacheris WP, Quay SC, Rocklage SM (1990) The relationship between thermodynamics and the toxicity of gadolinium complexes. Magn Reson Imaging 8:467–481

Caravan P, Ellison JJ, McMurry TJ, Lauffer RB (1999) Gadolinium(III) chelates as MRI contrast agents: structure, dynamics, and applications. Chem Rev 99:2293–2352

Caravan P, Comuzzi C, Crooks W, McMurry TJ, Choppin GR, Woulfe SR (2001) Thermodynamic stability and kinetic inertness of MS325, a new blood pool agent for magnetic resonance imaging. Inorg Chem 40:2170–2176

Caravan P, Cloutier NJ, Greenfield MT, McDermid SA, Dunham SU, Bulte JW, Amedio JC, Looby RJ, Supkowski RM, Horrocks WD, McMurry TJ, Lauffer RB (2002) The interaction of MS-325 with human serum albumin and its effect on proton relaxation rates. J Am Chem Soc 27;124:3152–3162

Cavagna FM, Maggioni F, Castelli PM, Dapra M, Imperatori LG, Lorusso V, Jenkins BG (1997) Gadolinium chelates with weak binding to serum proteins. A new class of high-efficiency, general purpose contrast agents for magnetic resonance imaging. Invest Radiol 32:780–796

Chang CA (1991) Lanthanide magnetic resonance imaging contrast agents: thermodynamic, kinetic, and structural properties of lanthanide (III) macrocyclic complexes. Eur J Solid State Inorg Chem 28:237–244

Chang CA (1993) Magnetic resonance imaging contrast agents design and physicochemical properties of gadodiamide. Invest Radiol 28(suppl 1):S21–S27

Chang CA, Sieving PF, Watson AD, Dewey TM, Karpishin TB, Raymond KN (1992) Ionic versus nonionic MR imaging contrast media: operational definitions. J Magn Reson Imaging 2:95–98

Cohan RH, Leder RA, Herzberg AJ, Hedlund LW, Wheeler CT, Beam CA, Nadel SN, Dunnick NR (1991) Extravascular toxicity of two magnetic resonance contrast agents. Preliminary experience in the rat. Invest Radiol 26:224–226

Corot C, Idée JM, Hentsch AM, Santus R, Mallet C, Goulas V, Bonnemain B, Meyer D (1998) Structure-activity relationship of macrocyclic and linear gadolinium chelates: investigation of transmetallation effect on the zinc-dependent metallopeptidase angiotensin-converting enzyme. J Magn Reson Imaging 8:695–702

Corot C, Violas X, Robert P, Gagneur G, Port M (2003) Comparison of different types of blood pool agents (P792, MS325, USPIO) in a rabbit MR angiography-like protocol. Invest Radiol 38:311–319

Cowper SE, Boyer PJ (2006) Nephrogenic systemic fibrosis: an update. Curr Rheumatol Rep 8:151–157

Cowper SE, Robin HS, Steinberg SM, Su LD, Gupta S, LeBoit PE (2000) Scleromyxoedema-like cutaneous diseases in renal-dialysis patients. Lancet 356:1000–1001

De Haen C, Cabrini M, Akhnana L, Ratti D, Calabi L, Gozzini L (1999) Gadobenate dimeglumine 0.5 M solution for injection (MultiHance) pharmaceutical formulation and physicochemical properties of a new magnetic resonance imaging contrast medium. J Comput Assist Tomogr 23(Suppl 1):S161–S168

Desreux JF (1980) Nuclear magnetic resonance spectroscopy of lanthanide complexes with a tetraacetic tetraaza macrocycle. Unusual conformation properties. Inorg Chem 19:1319–1324

Desreux JF, Barthelemy PP (1988) Highly stable lanthanide macrocyclic complexes: in search of new contrast agents for NMR imaging. Nucl Med Biol 1:9–15

Dharnidharka VR, Wesson SK, Fennell RS (2006) Gadolinium and nephrogenic fibrosing dermopathy in pediatric patients. Pediatr Nephrol 22:1395

Evans CH (1990) Biochemistry of the lanthanides. Plenum Press, London

Evenepoel P, Zeegers M, Segaert S, Claes K, Kuypers D, Maes B, Flamen P, Fransis S, Vanrenterghem Y (2004) Nephrogenic fibrosing dermopathy: a novel, disabling disorder in patients with renal failure. Nephrol Dial Transplant 19:469–473

Fossheim R, Dugstat H, Dahl G (1991) Structure-stability relashionships of Gd(III) ion complexes for magnetic resonance imaging. J Med Chem 34:819–826

Galan A, Cowper SE, Bucala R (2006) Nephrogenic systemic fibrosis (nephrogenic fibrosing dermopathy). Curr Opin Rheumatol 18:614–617

Gibby WA, Gibby KA, Gibby WA (2004) Comparison of Gd-DTPA-BMA (Omniscan) versus Gd-HP-DO3A (ProHance) retention in human bone tissue by inductively coupled plasma atomic emission spectroscopy. Invest Radiol 39:138–142

Gries H, Miklautz H (1984) Some physicochemical properties of gadolinium-DTPA complex, a contrast agent for IRM. Physiol Chem Phys Med NMR 16:105–112

Grobner T (2006) Gadolinium: a specific trigger for the development of nephrogenic fibrosing dermopathy and nephrogenic systemic fibrosis? Nephrol Dial Transplant 21:1104–1108

Harrison A, Walker CA, Pereira KA, Parker D, Royle L, Pulukkody K, Norman TJ (1993) Hepato-biliary and renal excretion in mice of charged and neutral gadolinium complexes of cyclic tetra-aza-phosphinic and carboxylic acids. Magn Reson Imaging 11:761–770

Ibrahim MA, Haughton VM, Hyde JS (1994) Enhancement of intervertebral disks with gadolinium complexes: comparison of an ionic and a nonionic medium in an animal model. AJNR Am J Neuroradiol 15:1907–1910

Idée JM, Port M, Raynal I, Schaefer M, Le Greneur S, Corot C (2006) Clinical and biological consequences of transmetallation induced by contrast agents for magnetic resonance imaging: a review. Fundam Clin Pharmacol 20:563–576

Inigo P, Campistol JM, Lario S, Piera C, Campos B, Bescos M, Oppenheimer F, Rivera F (2001) Effects of losartan and amlodipine on intrarenal hemodynamics and TGF-beta(1) plasma levels in a crossover trial in renal transplant recipients. J Am Soc Nephrol 12:822–827

Itoh N, Kawakita M (1984) Characterization of Gd^{3+} and Tb^{3+} binding sites on Ca^{2+}, Mg^{2+}-adenosine triphosphatase of sarcoplasmic reticulum. Tokyo J Biochem 95:661–669

Jackson GE, Wynchank S, Woudenberg M (1990) Gadolinium(III) complex equilibria: the implications for Gd(III) MRI contrast agents. Magn Reson Med 16:57–66

Joffe P, Thomsen HS, Meusel M (1998) Pharmacokinetics of gadodiamide injection in patients with severe renal insufficiency and patients undergoing hemodialysis or continuous ambulatory peritoneal dialysis. Acad Radiol 5:491–502

Kanal E, Barkovich AJ, Bell C, Borgstede JP, Bradley WG Jr, Froelich JW, Gilk T, Gimbel JR, Gosbee J, Kuhni-Kaminski E, Lester JW Jr, Nyenhuis J, Parag Y, Schaefer DJ, Sebek-Scoumis EA, Weinreb J, Zaremba LA, Wilcox P, Lucey L, Sass N (2007) ACR guidance document for safe MR practices. Am J Roentgenol 188:1–27

Khanna A, Kapur S, Sharma VK, Li B, Suthanthiran M (1997) In vivo hyperexpression of transforming growth-factor-beta 1 in mice: stimulation by cyclosporine. Transplantation 63:1037–1039

Kimura J, Ishiguchi T, Matsuda J, Ohno R, Nakamura A, Kamei S, Ohno K, Kawamura T, Murata K (2005) Human comparative study of zinc and copper excretion via urine after administration of magnetic resonance imaging contrast agents. Rad Med 23:322–326

Kumar K (1997) Macrocyclic polyamino carboxylate complexes of Gd(III) as magnetic resonance imaging contrast agents. J Alloys Comp 249:163–172

Kumar K, Tweedle MF (1993) Macrocyclic polyaminocarboxylate complexes of lanthanides as magnetic resonance imaging contrast agents. Pure Appl Chem 65:515–520

Kumar K, Chang CA, Tweedle MF (1993) Equilibrium and kinetic studies of lanthanide complexes of macrocyclic polyamino carboxylates. Inorg Chem 32:587–593

Kumar K, Jin T, Wang X, Desreux JF, Tweedle MF (1994) Effect of ligand basicity on the formation and dissociation equilibria and kinetics of Gd^{3+} complexes of macrocyclic polyamino carboxylates. Inorg Chem 33:3823–3829

Kumar K, Tweedle MF, Malley MF, Gougoutas JZ (1995) Synthesis, stability, and crystal structure studies of some Ca^{2+}, Cu^{2+}, and Zn^{2+} complexes of macrocyclic polyamino carboxylates. Inorg Chem 34:6472–6480

Laurent S, Vander Elst L, Copoix F, Muller RN (2001) Stability of MRI paramagnetic contrast media. A proton relaxometric protocol for transmetallation assessment. Invest Radiol 36:115–122

Laurent S, Vander Elst L, Muller RN (2006) Comparative study of the physicochemical properties of six clinical low molecular weight gadolinium contrast agents. Contrast Media Mol Imaging 1:128–137

Lorusso V, Arbughi T, Tirone P, de Haen C (1999) Pharmacokinetics and tissue distribution in animals of gadobenate ion, the magnetic resonance imaging contrast enhancing component of gadobenate dimeglumine 0.5 M solution for injection (MultiHance). J Comput Assist Tomogr 23(Suppl 1):S181–S194

Mackay-Wiggan JM, Cohen DJ, Hardy MA et al. (2003) Nephrogenic fibrosing dermopathy (scleromyxoedema-like illness of renal disease). J Am Acad Dermatol 48: 55–60

Magerstät M, Gansow OT, Brechiel MW, Colcher D, Baltzer L, Knop RH, Girton ME, Naegle M (1986) GdDOTA: an alternative to GdDTPA as $T_{1,2}$ relaxation agent for NMR imaging or spectroscopy. Magn Reson Imaging 3: 808–812

Mann JS (1993) Stability of gadolinium complexes in vitro and in vivo. J Comput Assist Tomogr 17(suppl 1):S19–S23

Marckmann P, Skov L, Rossen K, Dupont A, Damholt MB, Heaf JG, Thomsen HS (2006) Nephrogenic systemic fibrosis: suspected causative role of gadodiamide used for contrast-enhanced magnetic resonance imaging. J Am Soc Nephrol 17:2359–2362

McMurry TJ, Pippin CG, Wu C, Deal KA, Brechbiel MW, Mirzadeh S, Gansow OA (1998) Physical parameters and biological stability of yttrium(III) diethylenetriaminepentaacetic acid derivative conjugates. J Med Chem 27;41:3546–3549

Mendoza FA, Artlett CM, Sandorfi N, Latinis K, Piera-Velazquez S, Jimenez SA (2006) Description of 12 cases of nephrogenic fibrosing dermopathy and review of the literature. Semin Arthritis Rheum 35:238–249

Meyer D, Schaefer M, Bonnemain B (1988) Gd-DOTA, a potential MRI contrast agent. Current status of physicochemical knowledge. Invest Radiol 23:S232–S235

Morcos SK (2007a) Nephrogenic systemic fibrosis following the administration of extracellular gadolinium-based contrast agents: is the stability of the contrast agent molecule an important factor in the pathogenesis of this condition ? Br J Radiol 80:73–76

Morcos SK (2007b) Reply to Schmitt-Willich and Tweedle MF. Br J Radiol 80:584–585

Moreau J, Guillon E, Pierrard JC, Rimbault J, Port M, Aplincourt M (2004) Complexing mechanism of the lanthanide cations Eu^{3+}, Gd^{3+}, and Tb^{3+} with1,4,7,10–tetrakis(carboxymethyl)-1,4,7,10-tetraazacyclododecane (dota)-characterization of three successive complexing phases: study of the thermodynamic and structural properties of the complexes by potentiometry, luminescence spectroscopy, and EXAFS. Eur J Chem 10:5218–5232

Muller RN, Radüchel B, Laurent S, Platzek J, Piérart C, Mareski P, Vander Elst L (1999) Physicochemical characterization of MS-325, a new gadolinium complex, by multinuclear relaxometry. Eur J Inorg Chem 11: 1949–1955

Okazaki O, Kurata T, Yoshioka N, Hakusui H (1996) Pharmacokinetics and stability of caldiamide sodium in rats. Arzneimittelforschung 46:79–83

Perazella MA (2007) Nephrogenic systemic fibrosis, kidney disease and gadolinium: is there a link? Clin J Am Soc Nephrol 2:200–202

Pollet R, Marx D (2007) Ab initio simulation of a gadolinium-based magnetic resonance imaging contrast agent in aqueous solution. J Chem Phys 14:126:181102

Port M, Corot C, Violas X, Robert P, Raynal I, Gagneur G (2005) How to compare the efficiency of albumin-bound and nonalbumin-bound contrast agents in vivo: the concept of dynamic relaxivity. Invest Radiol 40(9):565–573

Port M, Idée JM, Medina C, Dencausse A, Corot C (2008) Stability of gadolinium chelates and their biological consequences: new data and some comments. Br J Radiol 81:258–259

Pulukkody KP, Norman TJ, Parker D, Royle L, Broan CJ (1993) Synthesis of charged and uncharged complexes of gadolinium and yttrium with cyclic polyazaphosphinic acid ligands for in vivo applications. J Chem Soc Perkin Trans 2:605–620

Puttagunta NR, Gibby WA, Puttagunta VL (1996a) Comparative transmetallation kinetics and thermodynamic stability of gadolinium-DTPA bis-glucosamide and other magnetic resonance imaging contrast media. Invest Radiol 10: 619–624

Puttagunta NR, Gibby WA, Smith GT (1996b) Human in vivo comparative study of zinc and copper transmetallation after administration of magnetic resonance imaging contrast agents. Invest Radiol 31:739–742

Rohrer M, Bauer H, Mintorovitch J, Requardt M, Weinmann HJ (2005) Comparison of magnetic properties of MRI contrast media solutions at different magnetic field strengths. Invest Radiol 40:715–724

Rothermel GL, Rizkalla EN, Choppin GR (1997) The kinetic of exchange between a lanthanide ion and the gadolinium complex of N,N″-bis(2-methoxyethylamide-carbamoylmethyl)-diethylenetriamine-N,N′,N″-triacetate. Inorganic Chim Acta 262:133–138

Sarka L, Burai L, Brücher E (2000) The rates of the exchange reactions between $[Gd(DTPA)]^{2-}$ and the endogenous ions Cu^{2+} and Zn^{2+}: a kinetic model for the prediction of the in vivo stability of $[Gd(DTPA)]^{2-}$, used as a contrast agent in magnetic resonance imaging. Chem Eur J 6(4):719–724

Sarka L, Burai L, Kiraly R, Zekany L, Brücher E (2002) Studies on the kinetic stabilities of the Gd(3+) complexes formed with theN-mono(methylamide), N′-mono(methylamide) and N,N″-bis(methylamide) derivativesof diethylenetriamine-N,N,N′,N″,N″-pentaacetic acid. J Inorg Biochem 25;91:320–326

Schmitt-Willich H (2007) Stability of linear and macrocyclic gadolinium based contrast agents. Br J Radiol 80:581–582

Schmitt-Willich H, Brehm M, Ewers CL, Michl G, Müller-Fahrnow A, Petrov O, Platzek J, Radüchel B, Sülzle D

(1999) Synthesis and physicochemical characterization of a new gadolinium chelate: the liver-specific magnetic resonance imaging contrast agent Gd-EOB-DTPA. Inorg Chem 22;38:1134–1144

Spinazzi A, Lorusso V, Pirovano G, Kirchin M (1999) Safety, tolerance, biodistribution, and MR imaging enhancement of the liver with gadobenate dimeglumine: results of clinical pharmacologic and pilot imaging studies in non-patient and patient volunteers. Acad Radiol 6:282–291

Steger-Hartmann T, Graham PB, Müller S, Schweinfurth H (2006) Preclinical safety assessment of Vasovist (Gadofosveset trisodium), a new magnetic resonance imaging contrast agent for angiography. Invest Radiol 41:449–459

Swaminathan S, Ahmed I, McCarthy JT, Albright RC, Pittelkow MR, Caplice NM, Griffin MD, Leung N (2006) Nephrogenic fibrosing dermopathy and high-dose erythropoietin therapy. Ann Intern Med 145:234–235

Thakral C, Alhariri J, Abraham JL (2007) Long-term retention of gadolinium in tissues from nephrogenic systemic fibrosis patient after multiple gadolinium-enhanced MRI scans: case report and implications. Contrast Media Mol Imaging 2(4):199–205

Ting WW, Seabury Stone M, Madison KC, Kurtz K (2003) Nephrogenic fibrosing dermopathy with systemic involvement. Arch Dermatol 139:903–906

Toth E, Brücher E, Lazar I, Toth I (1994) Kinetics of formation and dissociation of lanthanide(III)-DOTA complexes. Inorg Chem 33:4070–4076

Toth E, Kiraly R, Platzek J, Radüchel B, Brücher E (1996) Equilibrium and kinetic studies on complexes of 10-[2,3-dihydroxy-(1-hydroxymethyl)-propyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triacetate. Inorganica Acta 249: 191–199

Tweedle MF (1992) Physicochemical properties of gadoteridol and other magnetic resonance contrast agents. Invest Radiol 27(Suppl 1):S2–S6

Tweedle MF (2007) "Stability" of gadolinium chelates. Br J Radiol 80:583–584

Tweedle MF, Eaton SM, Eckelman WC, Gaughan GT, Hagan JJ, Wedeking P, Yost FJ (1988) Comparative chemical structure and pharmacokinetics of MRI contrast agents. Invest Radiol 23:S236–S239

Tweedle MF, Hagan JJ, Kumar K, Mantha S, Chang CA (1991) Reaction of gadolinium chelates with endogenously available ions. Magn Reson Imaging 9:409–415

Tweedle MF, Wedeking P, Kumar K (1995) Biodistribution of radiolabeled, formulated gadopentetate, gadoteridol, gadoterate, and gadodiamide in mice and rats. Invest Radiol 30:372–380

Uggeri F, Aime S, Anelli PL, Botta M, Brocchetta M, de Haën C, Ermondi G, Grandi M, Paoli P (1995) Novel contrast agents for magnetic resonance imaging. Synthesis and characterization of the ligand BOPTA and its Ln(III) complexes (Ln = Gd, La, Lu). X-ray structure of disodium (TPS-9–145337286-C-S)-[4-Carboxy-5,8,11-tris (carboxymethyl)-1-phenyl-2-oxa-5,8,11-triazatridecan-13-oato(5-)]gadolinate(2-) in a mixture with its enantiomer. Inorg Chem 34:633–642

Vander Elst L, Van Haverbeke Y, Goudemant JF, Muller RN (1994) Stability assessment of gadolinium complexes by P-31 and H-1 relaxometry. Magn Reson Med 31:437–444

Vander Elst L, Maton F, Laurent S, Seghi F, Chapelle F, Muller RN (1997) A multinuclear MR study of Gd-EOB-DTPA: comprehensive preclinical characterization of an organ specific MRI contrast agent. Magn Reson Med 38(4):604–614

Vander Elst L, Chapelle F, Laurent S, Muller RN (2001) Stereospecific binding of MRI contrast agents to human serum albumin: the case of Gd-(S)-EOB-DTPA (Eovist) and its (R) isomer. J Biol Inorg Chem 6:196–200

Varadarajan JA, Crofts SP, Carvalho JF, Fellmann JD, Kim SH, Chang CA, Watson AD (1994) The synthesis and evaluation of macrocyclic gadolinium-DTPA-bis(amide) complexes as magnetic resonance imaging contrast agents. Invest Radiol 29(Suppl 2):S18–S20

Wang X, Jin T, Comblin V, Lopez-Mut A, Merciny E, Desreux JF (1992) A kinetic investigation of the lanthanide DOTA chelates. Stability and rates of formation and of dissociation of a macrocyclic gadolinium (III) polyaza polycarboxylic MRI contrast agent. Inorg Chem 31: 1095–1099

Wedeking P, Kumar K, Tweedle MF (1992) Dissociation of gadolinium chelates in mice: relationship to chemical characteristics. Magn Reson Imaging 10:641–648

Wedeking P, Tweedle M (1988) Comparison of the biodistribution of ^{153}Gd-labeled, $Gd(DTPA)^{2-}$, $Gd(DOTA)^{-}$ and Gd(acetate)n in mice. Nucl Med Biol 15:395–402

White DH, de Learie LA, Moore DA, Wallace RA, Dunn TJ, Cacheris WP, Imura H, Choppin GR (1991) The thermodynamics of complexation of lanthanide (III) DTPA-bisamide complexes and their implication for stability and solution structure. Invest Radiol 26(suppl 1): S226–S228

White GW, Gibby WA, Tweedle MF (2006) Comparison of Gd(DTPA-BMA) (Omniscan) versus Gd(HP-DO3A) (ProHance) relative to gadolinium retention in human bone tissue by inductively coupled mass spectroscopy. Invest Radiol 41:272–278