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The ProHance story: the making of a novel MRI contrast agent

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Abstract. The four gadolinium chelates currently in clinical use as magnetic resonance imaging (MRI) contrast agents differ in two structural features: linear vs. macrocyclic cores, and ionic vs. nonionic charge types. While all are equivalent in relaxation effectiveness, the nonionic molecules have lower osmolality and viscosity and may be formulated safely at greater concentrations, and delivered confidently at greater doses and as a faster bolus. The macrocyclic molecules are more stable and show less tendency to dissociate free Gd. ProHance was conceived well over a decade ago, based upon a unique structure. It was first marketed in the USA in 1992, and was the first nonionic agent. It remains today still the only commercial MRI agent that is both macrocyclic and nonionic. To date it has been used safely in over a million patients.

Key words: Magnetic resonance imaging – Contrast media – Gadoteridol – Physico-chemical characteristics – Stability

Introduction

Physicians have enhanced clinical magnetic resonance imaging (MRI) with gadolinium (Gd) chelates throughout most of the 1990's. But what we take for granted now, was anything but obvious in early 1983, when gadoteridol, the active ingredient in ProHance, was conceived. By 1983 stable nonionic X-ray agents had only just been made broadly available for intravenous use in Europe, and were still in clinical trials in the USA. But the inherent value of nonionic agents was already appreciated by radiologists, even if many experts were decidedly negative on the very idea of contrast agents in MRI. Hence, a nonionic formula, and the expected high osmotolerance, became an early design criterion for our MRI agent. The intravenous dose was expected to be relatively high: several grams of the heavy metal, gadolinium, per patient. Consequently, maximizing tolerance was an overriding concern. A pure extracellular biological tissue distribution and complete excretion through glomerular filtration were identified as critically important for insuring high acute tolerance. Long term retention of gadolinium by the body was to be minimized as a safeguard against possible chronic toxicity. Water solubility had, of course to be high enough to formulate the product for a bolus injection while leaving viscosity as low as possible.

Chemists had long known that the gadolinium ion, Gd^{3+}, was the most efficient water relaxation catalyst for nuclear magnetic resonance (NMR), due to its high magnetic moment and very labile water coordination but it possessed exactly the opposite of each of the other properties. It is largely insoluble above pH 6, where it hydrolyzes to produce insoluble oxides and hydroxides. It also readily precipitates with carbonate and phosphate ions, both ubiquitous in blood and tissues. Gd ion alone is acutely toxic due in part to these precipitation tendencies and it deposits in liver and macrophagic tissues, and finally in bones with a biological half-life of months in mice. Chelation (wrapping the Gd ion in a tightly bound organic "ligand" framework) was an obvious necessity. All of the above requirements, therefore had to be incorporated into an organic ligand framework which simultaneously bound the Gd ion tenaciously and yet permitted some water coordination to allow Gd to behave as a powerful relaxation catalyst in MRI [1].

Design of the structure

Gd chelates enhance MRI by catalytic relaxation of bulk water protons in the tissues. In the MRI, a radiofrequency burst on the order of a microsecond excites protons on the water molecules in the tissue. These excited water protons slowly return to their ground (pre-excited) state in about 0.1 to 1 s, depending on the tissue. The MRI measures, voxel by voxel, the rate at which

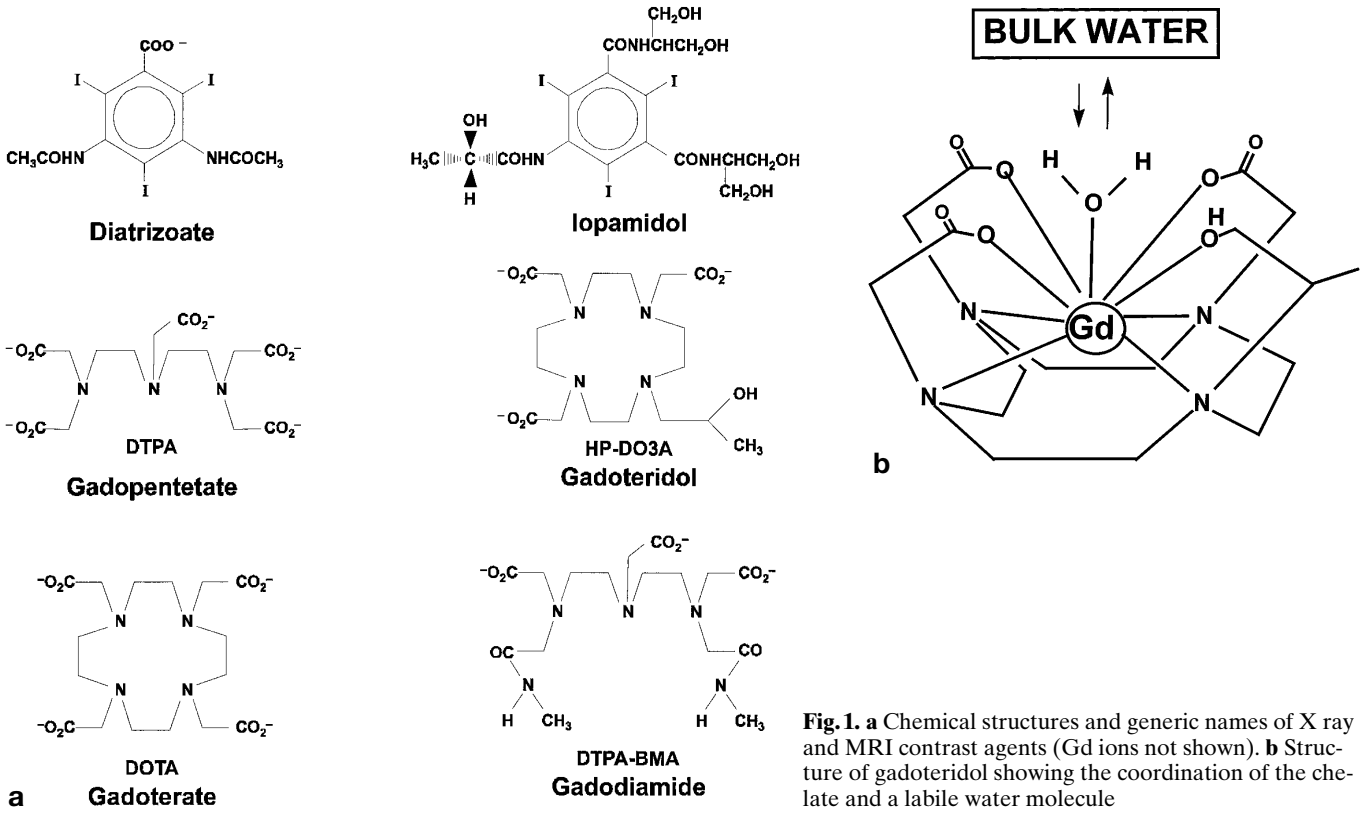

Fig. 1. a Chemical structures and generic names of X ray and MRI contrast agents (Gd ions not shown). **b** Structure of gadoteridol showing the coordination of the chelate and a labile water molecule

they return to their ground state, translates those rates to a gray scale, and uses the gray scale data to create an image dominated by water proton relaxation rates. (Images contain some proton concentration and some other parameters, as well.) Certain paramagnetic ions, like Gd, are very efficient catalysts for the process of water proton relaxation. When a water molecule comes very close to a Gd^{3+} ion (i.e. becomes chemically bound to it), the water proton relaxation occurs in about 10^{-6} s. Because Gd^{3+} ions are very labile for water binding, meaning that they trade water molecules with their surroundings at rates greater than 10^6 s^{-1}, each Gd^{3+} ion can relax the protons of $> 10^6$ water molecules per second. One design challenge of a Gd-based relaxation agent was to create an organic ligand framework which left a water binding site open on the Gd ion.

Largely due to the desire for keeping a nonionic molecule while maintaining stability, we chose a macrocyclic core structure. This structure type was expected to create particularly rigid molecules with high stability and inertia to dissociation of the metal [2]. Because Gd^{3+} must be exactly neutralized to form a nonionic molecule, we made unsymmetrical macrocyclic structures such as gadoteridol, shown in Figure 1 together with the three other MRI agents currently marketed, and examples of ionic and nonionic X-ray agents.

The first candidate synthesized was not gadoteridol, but DO3A, which is a precursor to gadoteridol lacking the unique hydroxyl containing arm, and having only the unsubstituted amine in that position. As such it was the first example of an unsymmetrical, nonionic, macrocyclic Gd chelate [3]. Frankly, we were amazed that this molecule was so stable with $K_{eq} = 10^{20}$ M^{-1}, and equally surprised that it was remarkably water soluble at > 2 M. Gd(DO3A) was considered seriously for development, but was actually so soluble that its manufacture was impractical. In fact, it took the next ten years to finally crystallize it [4]. The hydroxy containing arm was added to DO3A as a refinement to further increase stability and address the manufacturing issues [5].

The structures of the chelating agents now used clinically are based on either linear (DTPA and DTPA-BMA) or macrocyclic (DOTA and HP-DO3A) cores. DTPA relies on 5 negatively charged carboxylic acids for stability, which more than neutralize the Gd^{3+} ion making the linear ionic molecule, $Gd(DTPA)^{2-}$. DOTA relies on a macrocycle and 4 acids to make the ionic macrocycle, $Gd(DOTA)^{-}$. In the linear nonionic, Gd(DTPA-BMA), two charged acids are replaced by uncharged amides, which bind Gd more weakly than acids through the carbonyl oxygen atoms. In Gd(HP-DO3A) the macrocyclic structure is the core, but unlike DOTA one position is a coordinated hydroxyl, making a nonionic Gd chelate. Thus, $Gd(DTPA)^{2-}$ is an ionic linear chelate, Gd(DTPA-BMA) is a nonionic linear chelate, $Gd(DOTA)^{-}$ is an ionic macrocycle, and Gd(HP-DO3A) is the nonionic macrocycle. These features, ionic or nonionic and linear or macrocyclic, lead to differences in the physical chemical properties which result in greater osmotolerance, dosing and formulation flexibility for nonionics, and greater stability for the macrocyclics [6]. Gd(HP-DO3A) is the only molecule having both properties.

Summary of experimental methods

DTPA, DTPA-BMA and DOTA, were purchased from Aldrich, Strem and Parish Chemical companies, respectively. Details of the purifications and chelate preparations have been published [5, 7]. Preparative high-pressure liquid chromatography was used for the purification of radiolabeled samples [8, 9]. Free Gd and free ligand in radiolabeled samples were < 0.002 %. All of the data presented herein were generated from measurements made on routinely available, analytical quality instruments, and the data are abstracted from the referenced publications. The acid dissociation data under the reported conditions is newly acquired for the linear chelators and still under refinement, though the order of magnitudes are accurate. The uncertainties are included by the significant figures method; the last digit contains the uncertainty. Precise uncertainties may be found in the original referenced articles. Differences mentioned in the text among chelates are significant at 95 % confidence or better, unless otherwise stated.

Physical properties

Relaxivity

Table 1 contains relaxivity data and the most important structural parameters which govern relaxivity for Gd chelates which do not bind proteins or tissues. τ_r (the rotational correlation time), Q (number of simultaneously coordinated water molecules), and Gd-OH_2 bond distances largely determine any observable differences in relaxivity among the Gd chelates. These parameters are all very similar, as are the relaxivities at 20 MHz and across the spectrum of MRI field strengths in use clinically (0.02–1.5 T). The slight increase in Q for Gd(HP-DO3A) is probably due to the coordinated hydroxyl [9, 10], which is expected to contribute to the luminescence quenching from which Q is determined, but not to the relaxivity of Gd(HP-DO3A), which requires proton exchange ($k_{ex} > 10^6 s^{-1}$) faster than that of the hydroxyl protons. The slight differences in T_1 relaxivity determined from repeated measurements at 20 MHz, $^{20}r_1$, though significant for $Gd(DOTA)^{-1}$, are unlikely to result in observable signal enhancement differences in MRI. $^{20}r_2$ values (not shown) are also similar.

Solution properties

Nonionic molecules behave substantially different from ionic molecules in aqueous solution. Colligative properties such as osmolality are governed by the total number of particles in solution. Nonionic molecules like Gd(HP-DO3A) exist independently, i.e. there is one particle (the Gd(HP-DO3A) chelate) per active Gd^{3+} in solution. Ionic molecules such as $Gd(DTPA)^{2-}$ and Gd(DOTA)$^-$ depend on positively charged ions to neutralize their excess negative charge. Hence when NMG_2 [Gd(DTPA)] is dissolved, there are three particles per active $Gd(DTPA)^{2-}$ in solution: 2 inactive NMG^+ ions and 1 $Gd(DTPA)^{2-}$. The extra particles inevitably raise the osmolality and viscosity of solutions of the ionic Gd chelates.

Proof of the charge status of the chelates was obtained by measuring the molar conductivity in aqueous solutions, and is shown in Table 2. To conduct electricity through distilled water, a dissolved solute must dissociate into ions. The process is referred to as ionization. The near zero molar conductivities are diagnostic for nonionizing (i.e., nonionic) substances, such as Gd(DTPA-BMA) and Gd(HP-DO3A). The terms "neutral" and "zero net charge" are also technically correct [11], although the latter term is unconventional and unnecessarily long, while the former term is sometimes confused with "pH neutral". $Gd(DTPA)^{2-}$ and Gd(DOTA)$^-$ are clearly ionic.

The osmolalities of 0.5 M solutions (the standard commercial concentration) of the contrast agents are shown in Table 2 [12]. A wide range is obvious with the nonionic molecules showing lower osmolalities than the ionics, and Gd(HP-DO3A) showing the lowest osmolality. Ionic compounds will ultimately be limited in their osmotolerance. The osmolality of blood and body fluids is about 0.3 mOsmol/kg. Accidental extravasation of a contrast agent with osmolality of 2000 mOsmol/kg therefore causes pain and tissue damage (Yuh W, unpublished observation). Cohan, et. al. [13] found that tissue necrosis, hemorrhage and edema in rats after $Gd(DTPA)^{2-}$ were similar in graded magnitude to the effects observed using the ionic X-ray contrast agent, di-

Table 1. Structural data governing relaxivity values

Complex	Q[a]	τ_r (ps)[b]	Gd-OH_2 (Å)	$^{20}r_1$ ($mM^{-1}s^{-1}$)
Gd(HP-DO3A)	1.3 ± 0.1	57	2.50[c]	3.7 ± 0.1
Gd(DTPA-BMA)	1.1 ± 0.1	53	2.46[d]	3.8 ± 0.1
$Gd(DTPA)^{2-}$	1.1 ± 0.1	55	2.49[e]	3.8 ± 0.1
Gd(DOTA)$^-$	1.1 ± 0.1	63	2.46[c]	3.5 ± 0.1

[a] Tb(L), refs. [9, 10]
[b] Y(L), C-H = 1.0 Å (Shukla R, personal communication)
[c] Ref. [4]
[d] Dy-O distance. Ref. [33]
[e] Ref. [34]

Table 2. Molar osmolality, and viscosity data at 37 °C

Contrast Agent	Conductivity, 20 °C Sm, mho, $cm^2\ mmol^{-1}$	Osmolality (Osmol/kg)		Viscosity (cP)	
		0.5 M	1.0 M	0.5 M	1.0 M
Gd (HP-DO3A)	1	0.63	1.91	1.3	3.9
Gd (DTPA-BMA)	5.5	0.65	1.90	1.4	3.9
NMG_2 (Gd-DTPA)	117[a]	1.96	5.85	2.9	> 30
NMG (Gd-DOTA)	54[a]	1.35	4.02	2.0	11.3

[a] Sodium salts

atrizoate, and 32, 6, ad 10 times greater, respectively, than ProHance which showed effects similar to those of saline. While the global increase in osmolality after injection of 0.1 mmol/kg of an ionic compound in humans is insignificant, local effects, such as crenation of erythrocytes and other cells, and damage to endothelial surfaces may be significant, especially at a bolus injection site. At elevated doses (> 0.3 mmol/kg) of $Gd(DTPA)^{2-}$ given as rapid boli, dose dependent significant hemodynamic effects (lower peak systolic LV pressure) were demonstrated in animals and attributed to hyperosmolality and possible Ca binding [14]. ProHance was not significantly different from saline. All of these effects are expected to become more serious as power injectors begin to find routine use in MRI and especially in applications which may require higher concentrations.

Interestingly, the charge on the contrast agent has even been shown to affect image signal intensity in special cases. Ibrahim has reported that the enhancement of intervertebral disks in rabbits was 2.6–5.3 times greater with nonionic ProHance than with ionic $Gd(DTPA)^{2-}$ [15]. It appears that cartilage, which contains negatively charged proteoglycans, may hinder the infusion of negatively charged contrast agents by the mechanism of charge repulsion. In this case, the two charge types could yield significantly different results when attempting to differentiate recurrent herniated disk form scar, nonionics producing better enhancement of the cartilage.

The viscosities of 0.5 and 1.0 M solutions of the contrast agents are shown in Table 2. The higher viscosity for the ionic complexes is due in part to the greater number of particles in solution. Moreover, the extra particles are especially problematic in that they are NMG^+ cations (NMG^+ = N-methylglucammonium ion, a sugar amine derivative), which contain multiple hydroxy groups that hydrogen-bond effectively with water molecules. The viscosities of the ionic chelates at 1.0 M concentrations are unacceptably high.

Together, the low osmolalities and viscosities of the nonionics offer greater formulation and dosing flexibility. For example, the unacceptably high osmolality and viscosity values would prevent formulation of the ionic chelates at 1.0 M, rather than the current 0.5 M concentrations. Higher concentrations will result in smaller injection volumes when larger doses are administered. The 1.0 M formulations should also provide for sharper boli, and greater Gd concentration at the temporal bolus peak. Elevation of the height of the bolus would be an advantage for dynamic first-pass MRI studies when perfusion measurement is the goal.

Stability and dissociation of Gd^{3+} ion from the chelate

Thermodynamic stability governs the ultimate tendency toward dissociation of the Gd^{3+} ion from the chelate, while dissociation rate constants measure the rate of that process. In vitro equilibrium stability for Gd chelate-based MRI agents is defined in terms of three constants. The thermodynamic binding constant, Keq from equation (1), is useful if the energy of the interaction is desired. The strength of this interaction is important in our context as a measure of the likelihood that the Gd chelate will dissociate into free Gd and free chelate, both of which are poorly tolerated. (LD_{50}'s are 30–100 times lower for free Gd^{3+} and for free ligand than for the Gd chelate)[3] A method of comparison more appropriate to physiologic pH is through K', which considers the protonation constants of the ligand, and describes the position of the binding equilibrium at pH 7.4.

Table 3. Equilibrium constants, dissociation kinetics and endogenous ion competition. Data for gadolinium chelates (refs. [16–20])

Complex	log Keq M^{-1}	log K' pH 7.4	$T^1/_2$, dissociation [H +] = 0.01	Reaction with 25 mM	
				Cu/PO_4	Zn/PO_4
Gd (HP-DO3A)	23.8	17.1	1.3 days	< 1 %	< 1 %
Gd $(DOTA)^-$	25.8	18.8	95 days	< 1 %	< 1 %
Gd (DTPA-BMA)	16.9	14.9	< 2 s	35	25
Gd $(DTPA)^{2-}$	22.1	17.7	< 2 s	25 %	21 %

$$Gd + L \underset{k_d}{\overset{k_f}{\leftrightarrows}} Gd(L) \qquad K_{eq} = \frac{k_f}{k_d} \tag{1}$$

Table 3 contains data on the binding constants and the results of dissociation stress tests [16, 17, 18, 19, 20].The equilibrium binding constants are difficult to measure accurately in this range of values, and are estimated to be accurate to ± log, even though they are more precisely determined than that. The binding data demonstrate powerful chelation in all cases. (For comparison, iron binding by transferrin has a log K' = 22 at pH 8.4.) The negatively charged carboxylate oxygens are more powerful donor atoms for Gd than are uncharged oxygen atoms. Thus, when one carboxylate oxygen atom is replaced with a hydroxyl oxygen atom (DOTA to HP-DO3A), or two carboxylate oxygen atoms are replaced with two amide oxygen atoms (DTPA to DTPA-BMA), the K_{eq} and K' decrease. Equilibrium stability data are especially useful in formulation work, where aqueous solutions have years of shelf life to equilibrate. All of the chelates have adequate shelf life.

Dissociation rates [generically, the reverse reaction in equation (1)] are measurable only in acidic media. The studies are complex and reflect differing mechanisms of dissociation for the various chelates, but choosing one pH, a halflife may be calculated from the mechanism to gain some flavor of the difference in the dissociation propensity of the macrocycles vs. the linear chelates. The half-times are reported in Table 3. The kinetic inertia to dissociation imparted by the macrocycle is clearly seen in this stress test. In vivo, the equilibrium state is to the left in equation (1), relative to the position in water at pH 7. The reason is that proteins, surfaces,

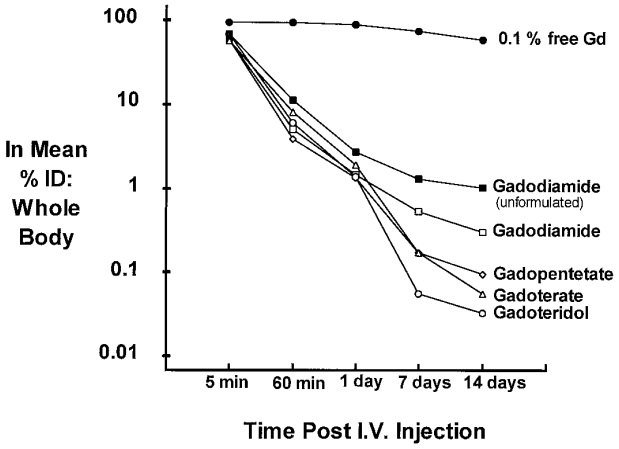

Fig. 2. Residual ^{153}Gd in mice (whole animal) as percent injected dose (% ID) after intravenous administration of Gd chelates. $Gd(DTPA)^{2-}$ (gadopentetate), $Gd(DOTA)^{-}$ (gadoterate) Gd(HP-DO3A) (gadoteridol), and Gd(DTPA-BMA) (gadodiamide) were studied as formulated drug substances unless otherwise indicated

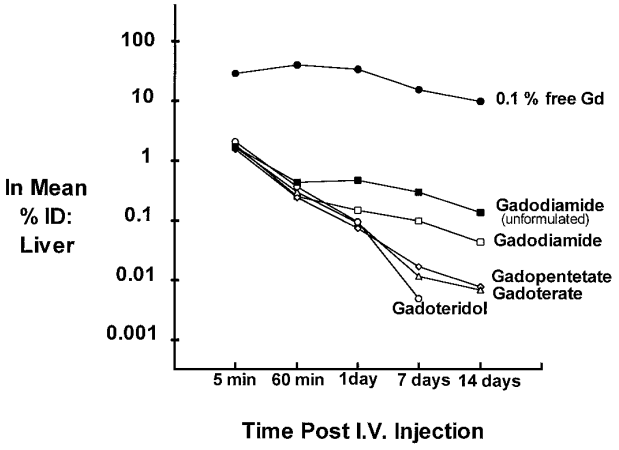

Fig. 3. Residual ^{153}Gd in mice livers as percent injected dose (% ID) after intravenous administration of Gd chelates. $Gd(DTPA)^{2-}$ (gadopentetate), $Gd(DOTA)^{-}$, (gadoterate) Gd(HP-DO3A) (gadoteridol) and Gd(DTPA-BMA)$^{-}$ (gadodiamide) were as formulated drug substances unless otherwise indicated

and a host of other cations (e.g., Fe^{3+}, Cu^{2+}, Zn^{2+}, Ca^{2+}) compete with the Gd^{3+} ion for the ligand and a number of other anions (e.g., PO_4^{3-}, CO_3^{2-}, OH^-) compete with the ligand for the Gd^{3+}. The copper and zinc ion stress test data in Table 3 measure the free Gd formed in 15 min after mixing Gd chelate with copper or zinc cations in a phosphate medium. The combined stress of phosphate and a metal ion is enough to dechelate the linear agents in 15 min, but not the macrocycles. Both thermodynamics and kinetics may play roles in this experiment, but the results lead to an obvious conclusion. Overall, the most important chemical feature governing relative stability and dissociation inertia is the presence or absence of the macrocycle.

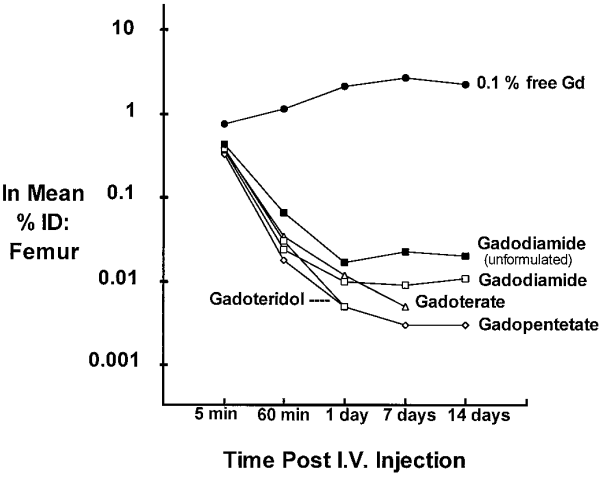

Fig. 4. Residual ^{153}Gd in mice femurs as percent injected dose (% ID).$Gd(DTPA)^{2-}$ (gadopentetate) $Gd(DOTA)^{-}$, (gadoterate) Gd(HP-DO3A) (gadoteridol) and Gd(DTPA-BMA)$^{-}$ (gadodiamide) were studied as formulated drug substances unless otherwise indicated

In vivo stability

A more biologically relevant stress test is for the residual free Gd remaining in animals fourteen days after intravenous administration of radiolabeled Gd chelates [21, 22]. The effect of in vivo competition may be appreciated because the excretion rate of free Gd^{3+} is on the order of 1 % per day in mice, compared to > 95 % per day for the Gd chelates. Any free Gd^{3+} that is formed from Gd chelate dissociation should be detectable after 14 days. Figure 2 shows the results plotted as log-percentage injected dose (% ID) versus time.

Free Gd is very slowly excreted, as seen in the top curve of Fig. 2. The chelates are all rapidly excreted (into urine), with > 95 % excreted by 1 day. At 7 and 14 days, it is seen that the macrocyclic chelates have the lowest residual free Gd in whole mice. This finding is consistent with their lower dissociation rates. $Gd(DTPA)^{2-}$ shows residual Gd three times greater than that of Gd(HP-DO3A), and Gd(DTPA-BMA) shows considerably greater residual Gd at 7 and 14 days. If Gd chelate dissociation were the mechanism involved in leaving residual Gd in the animals, adding excess ligand would be expected to diminish the problem by driving equation (1) to the right. The high residual Gd found for the weakest chelate, Gd(DTPA-BMA), is somewhat reduced in the commercial formulation by adding 5 % excess chelating agent, but it is still tenfold greater than the values obtained for Gd(HP-DO3A).

Free Gd, when injected as the chloride or acetate deposits in liver and bone [23]. Figures 3 and 4 show data from these individual organs (mice). The same patterns are observed as in the whole animal. The macrocycles yield extremely low residual Gd, followed by $Gd(DTPA)^{2-}$. Gd(DTPA-BMA), whether formulated or unformulated, resulted in much greater residual Gd. Moreover, the shape of the curves are the same for free Gd and Gd(DTPA-BMA), further suggesting that the

residual Gd detected in the whole animal for Gd(DTPA-BMA) was dechelated Gd^{3+}. For the case of $Gd(DTPA)^{2-}$, experimental evidence confirms the dechelation conclusion: Kosokat and Urich injected double labeled $^{153}Gd(^{14}C\text{-}DTPA)^{2-}$ into rats in a similar biodistribution experiment and detected ^{153}Gd: ^{14}C ratios in liver and bone of 6 and 9, respectively, concluding that most of the ^{153}Gd detected was from dechelated $^{153}Gd(DTPA)^{2-}$ [24].

Aside from the general concern one must have for chronic heavy metal retention, there are some observations of an acute nature related to dechelation of Gd chelates. As mentioned above the LD_{50} values of free Gd^{3+} and free ligand are very low, due in some part to rapid precipitation of Gd^{3+}, and Ca^{2+} binding by the ligand. In addition, free, unchelated Gd^{3+} ion is known to block neuromuscular transmission by blocking Ca^{2+} channels in tissue preparations [25, 26]. Some indirect evidence of Gd release also exists in humans. For example, acute deterioration of myasthenia gravis was observed following $Gd(DTPA)^{2-}$administration [27]. Administration of $Gd(DTPA)^{2-}$4 h prior to administration of ^{67}gallium citrate resulted in an unexpected skeletal biodistribution that suggested to the authors that $Gd(DTPA)^{2-}$ had dechelated [28]. This explanation was disputed in favor of free chelating ligand already in the formulation [29], but more thorough exploration via computer simulation strongly supported a dechelation mechanism [30]. The free chelating agent that results from dechelation may also have acute effects. In a study of 31 patients, serum Zn dropped by 10 % and urinary Zn increased by 26 μmol in 3 h after the linear Gd(DTPA-BMA) administration, but no significant changes were found using Gd(HP-DO3A) [31]. In an in vitro experiment, ACE (angiotension converting enzyme) activity was inhibited by the linear chelates $Gd(DTPA)^{2-}$ (–81 %) and Gd(DTPA-BMA) (–92 %). An excess of zinc partially or totally suppressed the effect. The macrocyclic agent, $Gd(DOTA)^{-}$ showed no significant effect on ACE activity [32].

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