Tweedle MF. Work in progress toward nonionic macrocyclic gadolinium (III) complexes. in: Rinck PA (ed). Contrast and contrast agents in magnetic resonance imaging. Trondheim and Mons: The European Workshop on Magnetic Resonance in Medicine (EMRF). 1989. 65-73.

Proceedings of **Contrast and Contrast Agents in Magnetic Resonance Imaging** – A Special Topic Seminar; Trondheim, Norway; 12-13 September 1988.

Work in Progress Toward Nonionic Macrocyclic Gadolinium(III) Complexes

Michael F. Tweedle

1. Introduction

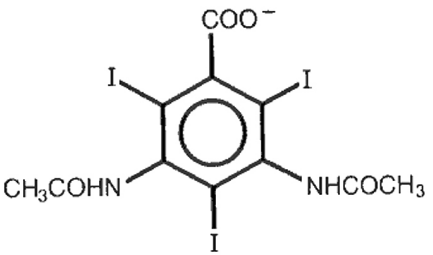
Two hypotheses underlie the work in progress that will be described in this chapter. The first is that nonionic Gd complexes will have potential advantages over ionic Gd complexes, and the second is that complexes based on a certain tetraaza macrocycle will be especially inert to substitutions that result in liberation of the free Gd^{3+} ion. Two caveats must also be stated at the outset. First, the properties of new chemical entities are generally difficult to predict and it is possible that well tolerated ionic, and poorly tolerated nonionic complexes derived from either macrocyclic, macrobicyclic and linear ligand frameworks will be encountered. Second, the results of chemical experiments can be used to support chemical conclusions, but extension of chemistry to biology, and of animal biology to human biology is associated with a high degree of uncertainty.

2. Hypothesis One - Nonionic Gd Complexes

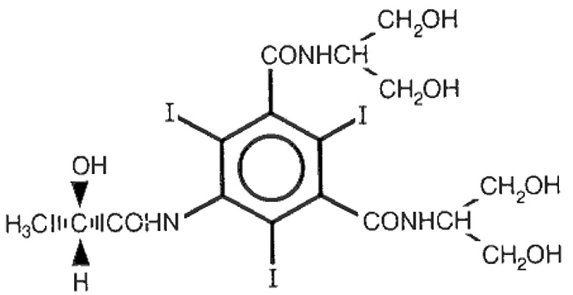
Larger doses of MRI agents than are currently approved may be needed for applications where the problem is one of delivering enough contrast agent to a tissue. Applications in the body, particularly the heart and liver, appear to be the most likely to require higher doses. Current use of $Gd(DTPA)^{2-}$ is primarily in the brain, for detection of disruption of the blood brain barrier. The clinical dose is 0.1 - 0.2 mmol/kg, which is relatively low compared to the 1 - 2 mmol/kg doses used in X-ray imaging. Both types of agents are extracellularly distributed and rapidly excreted renally. 0.1 - 0.2 mmol/kg may be the upper limit for the $Gd(DTPA)^{2-}$ compound if the elevated serum iron and bilirubin levels observed in humans increase as a function of higher dose (1). Limitations on the maximim useful concentration of Gd in a tissue will, of course, be imposed by the T2-dependent loss of signal. However, the signal limiting concentrations in tissue are higher, greater than 2 mM for a SE 30/500 pulse sequence (2, 3), than the concentrations expected in tissues following a 0.1 - 0.5 mmol/kg intravenous injection. For example, less than 0.2 mM was found in a canine brain lesion after 0.25 mmol/kg doses (4). An exception could

occur where the current extracellular agents concentrate, for example in kidneys and urine.

A high degree of water solubility is required of the agents to be discussed. It is well known in the lore of iodinated X-ray contrast agents that water solubility may be imparted by uncharged alkyl-hydroxy side chains (Iopamidol) (5) or the charged carboxylate (Diatrizoate) (6) (See Figure 1 for structures). A large number of molecules has been made and the tolerance in rodents tested. This general indicator of systemic toxicity varies over a wide range, as is shown in Table 1. However, the class of uncharged (nonionic) compounds contains examples which are better tolerated than any of the charged compounds. It cannot be said with certainty what all of the factors are that contribute to the particularly high tolerance of certain molecules such as Iopamidol, but two considerations are worth mentioning.



Diatrizoate



Iopamidol

Figure 1:
Chemical structures of X-ray imaging agents. Water solubility is imparted to the ionic agent, diatrizoate, by means of the charged carboxylate. In the nonionic agent, iopamidol, the water solubility is imparted by alkyl-hydroxy substituents.

Intravenous Acute Tolerance in Mice for Monomeric 2,4,6-triiodinated Benzene Derivatives

Compound Type	Number	LD 50 g-I/kg [a] Range	Mean	S.D.
Ionic	(53)	0.5 - 14	7.4	2.6
Nonionic	(46)	0.5 - 22	9.4	5.3
Diatrizoate (NMG)		5.4		
Iopamidol		21.8		

(a) Collected from the extensive tables in reference 6.

Table 1

First, elementary chemistry tells us that charged compounds interact electrostatically with oppositely charged compounds, while nonionic compounds do not. It might be expected, for example, that anionic (negatively charged) compounds might bind endogenous Ca^{2+} with greater affinity than do nonionic compounds. This appears to be so for anionic versus nonionic X-ray agents (7). Electrostatic interactions may also contribute to protein binding or enzyme inhibition, both of which are reported to be greater for ionic than for nonionic agents (8, 9).

Second, the colligative properties of the agents in their formulations should match blood plasma and extracellular fluid as closely as possible. Hyperosmolality relative to plasma results when the formulation has an osmolality greater than 0.3 Osmol/kg-water. Hyperosmolality of an injectate has a number of potential adverse physiological effects, for example the crenation of blood cells, that it is preferable to avoid. It has been reported that patients feel pain or a burning sensation when the injectate has an osmolality above 0.7 ± 0.05 Osmol/kg-water (10). The osmolality of nonionics is generally much lower than the equivalent concentration of ionic agents.

2. Hypothesis Two - Macrocyclic Gd Complexes

The second hypothesis is that nonionic complexes may be made from a twelve-membered tetraazamacrocycle that will retain water solubility and

remain inert to substitution despite having only three charged carboxylate donor atoms. By substitution, we mean any process which leads to dissociation of the Gd^{3+} ion from the complexing ligand. Substitution of the Gd^{3+} ion is a potential problem because the uncomplexed Gd^{3+} ion is poorly tolerated and has a very long biological residence time following intravenous administration (11). The LD 50 in mice og $GdCl_3$ is less or equal to 0.3 mmol/kg, and the LD 50 of strong chelating agents like the ones to be discussed is as low or lower (2). This strategy is illustrated in Figure 2, along with a second strategy using the linear amine from the DTPA ligand.

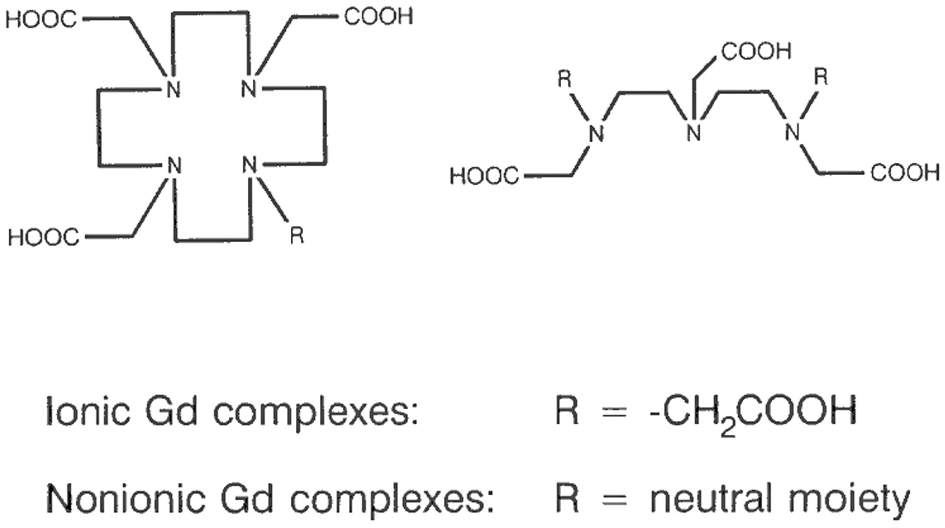


Figure 2:
Two strategies for making nonionic Gd complexes. Only three deprotonated carboxylates may be used in the complexing ligand to neutralize the Gd^{3+} ion.

To neutralize the tripositive charge of Gd^{3+}, the complexing ligand should have only three negatively charged donor atoms, rather than the four or five of DOTA and DTPA, respectively. It was not certain at the beginning of the work that such a complex, DO3A in Figure 2, would be inert in the presence of other ions that are available to it in vivo. It was also a possibility that water solubility would have to be coaxed into the molecule by means of alkyl substitutions at the secondary amine (11).

3. Results and Discussion

The parent ligand of the R-DO3A series is shown in Figure 2 (R = H). R-DO3A ligands, and their Gd complexes are prepared by published methods (12). Neutral metal complexes are often less soluble than complex salts in polar solvents like water. It was, therefore, surprising that the parent molecule of the series, Gd(DO3A), was water soluble at room temperature to better than 2 M. The parent ligand, DO3A, is a highly versatile synthetic intermediate, and has provided a wide range of alkyl derivatives such as those shown in Figure 3. Some alkyl substituted R-DO3A derivatives are very water soluble with values commonly on the order of 1 M. However, water solubility has been the most difficult physical property to predict.

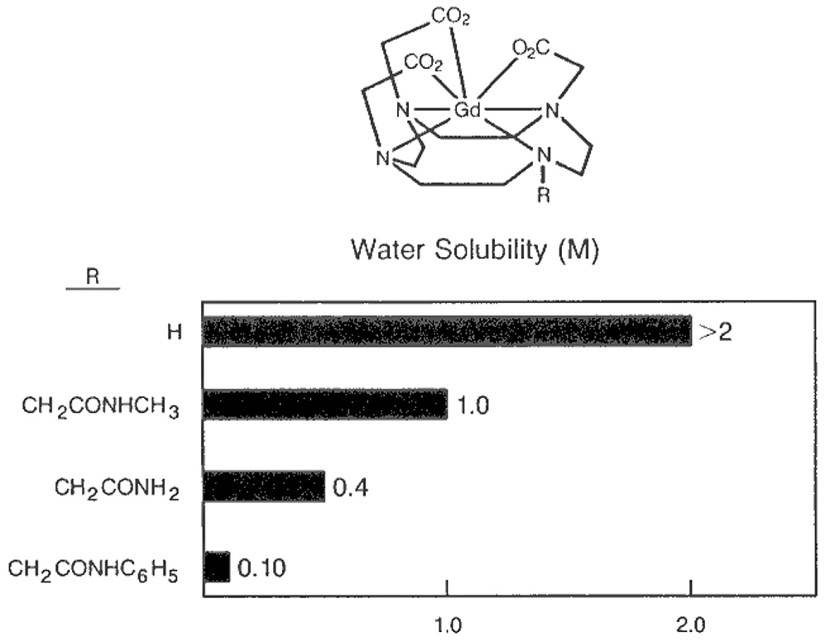


Figure 3:
The DO3A ligand forms water soluble nonionic Gd complexes. DO3A is a versatile intermediate, and many of its alkyl derivatives are water soluble. However, water solubility is difficult to predict.

The osmolality of a 0.5 M solution of Gd(DO3A) was measured to confirm that the complex was electrically neutral (i.e. nonionic). These data are collected in Table 2, along with viscosity and relaxivity data.

Physico Chemical Properties of Compounds and their 0.5 M Aqueous Solutions

Compound	Osmolality[a]	Viscosity[a]	Relaxivity[b]
NMG2[Gd(DTPA)]	1.9	2.9	4
Gd(DO3A)	0.5	1.1	5
Diatrizoate	0.9	2	-
Iopamidol	0.4	2	-

(a) 0.5 M solutions at 37°C. Data for Diatrizoate and Iopamidol are interpolated from product data at higher and lower concentrations (13); estimated uncertainty is 10%. **(b)** 20 MHz, 30°C, 0.1 to 1 mM range. Data are highly dependent on frequency, temperature, and viscosity. Reproducibility is within ~10%.

Table 2

The values are fully consistent with the proposed formulations. The lower osmolality and viscosity, together with similar effectiveness in terms of relaxivity per mole of agent could be used to formulate the nonionic agents with more flexibility. For example, if a high dose were required to be delivered as a rapid bolus in a small volume, 0.25 mmol/kg of the nonionic could be delivered at ~0.6 Osmol/kg in 35 mL to a 70 kg patient. The same patient given $Gd(DTPA)^{2-}$ at 0.6 Osmol/kg would need to receive about 115 mL (NMG2Gd(DTPA) is not currently approved for use at 0.25 mmol/kg). The alternative would be to inject smaller volumes at much greater hyperosmolality, and accept the ususal physiologic consequences.

The acute systemic tolerance of the nonionic compounds in vivo in rodents appears to be high. LD 50 values in mice and rats of 7 - 14 mmol/kg have been found for the Gd(R-DO3A) family. NMG_2[Gd(DTPA)] as Magnevist resulted in an LD 50 of 6 mmol/kg in both mice and rats (14). Some preliminary data on DTPA-derived complexes (Figure 2 with R = $CH_2CONHCH_3$ (15) and $CH_2CONHCH_2CHOHCH_2OH$ (16)) indicate that greater acute tolerance in rodents may be expected for some nonionic derivatives in this class as well. Although the number of examples of Gd complexes is yet extremely small

compared to the number of triiodinated benzene compounds, the trends in acute tolerance in rodents are, so far, the same.

In their proposed application, we seek to minimize chemical reactions of the Gd complexes in vivo, especially those that involve dissociation of Gd^{3+} ion. Ideally, we would like new compounds to have high thermodynamic and conditional stability (no ability to react) and great kinetic inertia (slow reactions when reactions are possible). It may be useful if the chelating ligands are as specific as possible for Gd(III) over other entities to be encountered in vivo. To explore this, an ITLC-SG method was developed to allow us to screen new molecules for their reactivity in high concentrations of endogenous ions that might react with the Gd complexes (17). The data in Figure 4 indicate that the DO3A ligand is highly specific for Gd(III) over other, endogenously available ions. Whether the lack of reactivity is controlled by thermodynamic preference of DO3A for Gd^{3+} or kinetic inertia toward substitution of Gd(DO3A) is under investigation.

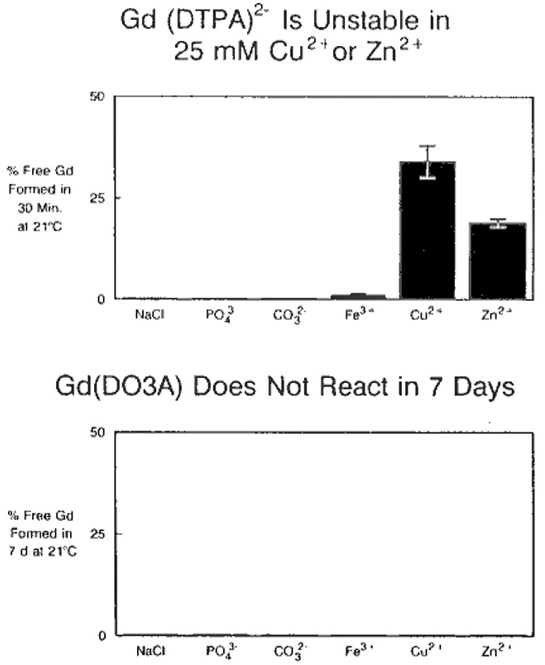


Figure 4:
Gd(DO3A) is inert to substitution by endogenously available ions; ≤ 1% reaction was detected in 7 days. $Gd(DTPA)^{2-}$ reacts in 30 minutes with Cu^{2+} and Zn^{2+} ions, producing Gd(III) ion.

4. Summary

Following two hypotheis, that nonionic Gd complexes would provide a pool of candidate compounds from which would emerge some unusually well tolerated examples, and that Gd complexes based on a twelve-membered macrocycle would provide substitutionally inert Gd complexes, a series of new nonionic Gd complexes has been synthesized and studied.

Water solubility has proven to be unexpectedly high, yet unpredictable. Metal ion selectivity and lability, colligative properties, and acute tolerance are generally very favorable, making some examples of this new series candidates for clinical testing.

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Acknowledgements

The author would like to thank his colleagues in the Squibb Institute for Medical Research for their collaborations.

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