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Erratum: The name of the co-author **M. Schaefer** was misspelled as M. Shaefer in the list of authors and in the address given at the end.

Physico-Chemical Properties of the Macrocyclic Chelate Gadolinium-DOTA

D. Meyer, M. Shaefer, D. Doucet

The in vivo utilization of paramagnetic complexes as non-specific agents for contrast enhanced magnetic resonance imaging (MRI) has been the subject of numerous studies (1). These works have demonstrated the utility of this new class of diagnostic agents and have formed the physico-chemical basis for the appearance of new products, like the macrocyclic chelate Gadolinium-DOTA (2).

The in vivo utilization of paramagnetic complexes is based on two interdependent objectives. The first concerns the improvement of biological compatibility of the paramagnetic metal, and the second is aimed at maintaining sufficient relaxivity of the complexed metal and achieving an optimum dose/effect ratio.

The biological tolerance of paramagnetic complexes can be regarded as the result of two contributory factors. These are:

* an intrinsic factor, characterized by molecular interactions of the complex with the biological systems (access to free metal coordination sites, electrostatic and hydrophobic interaction between the complex and biological macromolecules, and the anionic or cationic nature of the complex);
* the presence of unchelated toxic species (ligand and free metal). The concentration of these species can be directly correlated with the physico-chemical properties of the complex, which control its in vivo dissociation in the presence of competing endogenous species (protons, metals, ligands, and endogenous complexes) at physiological pH.

Among the physico-chemical properties which combine to minimize the dissociation of paramagnetic complexes in vivo, three play a primary role:

* the conditional stability of the complex at physiological pH,
* the specific affinity of the paramagnetic metal for the ligand,
* the dissociation kinetics of the complex.

The latter plays a fundamental part in exchanges between the paramagnetic complex and endogenous metals and ligands. From this point of view, the Gd-DOTA complex demonstrates a remarkable physico-chemical pattern associated with the rigid macrocyclic structure of DOTA (Figure 1).

The macrocyclic structure of the DOTA ligand comprises a preorganized cavity, the size of which is compatible with the spherical symmetry of

gadolinium (4 F^7). X-ray diffraction studies carried out on the Gd-DOTA complex have shown that DOTA encages gadolinium in a very rigid shell (Figure 2).

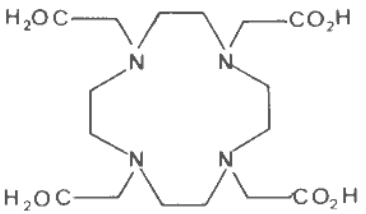

Figure 1:
Structure of DOTA 1,4,7,10 Tetra-aza-cyclododecane N,N',N'',N''' Tetra-acetic acid.

In Na+[Gd-DOTA H_2O]-$4H_2O$, gadolinium is octa-coordinated by four nitrogen atoms of the macrocycle and four oxygen atoms of the carboxylate groups with asymmetry higher than that of Eu-DOTA (3).

An axial coordination site remains available for one water molecule located at 2,459 Å from gadolinium. Distances between gadolinium and the two protons of the bound water molecule are 2.983 Å and 2.909 Å respectively.

The structure of Gd-DOTA is characterized by a marked symmetry (4) and a very rigid conformation, identical in the solid state and in solution (5) with major consequences on the kinetic and thermodynamic properties of the complex.

From the thermodynamic point of view, gadolinium-DOTA is the most stable gadolinium chelate so far identified, with a thermodynamic stability constant ranging from 10^{25} (6) to 10^{28} (7) according to the measuring method used. This outstanding stability of the Gd-DOTA macrocyclic complex can be explained by several structural features:

* the basicity of the DOTA ligand (pKa = 32.3) (8), corresponding to the sum of the protonation constants, is directly correlated with the intensity of the electrostatic interactions between the metal ion and the donor atoms of the ligand (9);

* the formation of the 8 five-membered rings (N-Gd-N, N-Gd-O) resulting from the chelation of gadolinium and donor atoms of the ligand. Five-membered rings are characterized by a low steric strain and have an effect on the entropy of complex stability (10);

* the macrocyclic effect, described in detail in the literature (11) and based on the level of preorganization of the ligand prior to chelation (12). This

effect can be responsible for an increase instability by 4 to 8 orders of magnitude in the case of macrocyclic complexes compared with analogous non cyclic complexes (13).

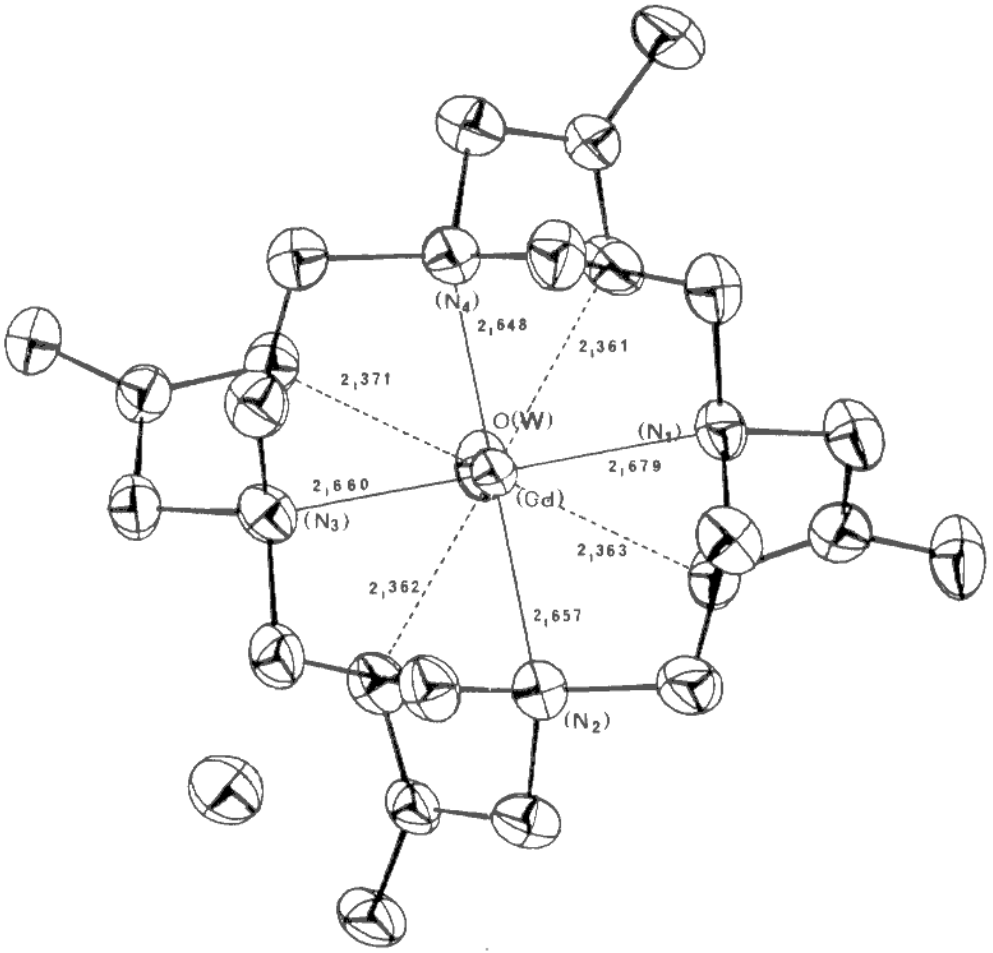

Figure 2:
Structure of $Na^+[Gd\ DOTA\ H_2O]^-,4H_2O$. The interatomic distances are in Angstroems.

At physiological pH, the DOTA ligand is diprotonated. These protonated species prevent the formation of stable complexes (14) and the metal must eject these protons before chelation.

The conditional stability constant is the result of this competition between metal and proton and can be easily calculated from the protonation constants of the ligand and thermodynamic stability constant of the complex with an appropiate computation program (15).

The curve (Figure 3) clearly demonstrates that the stability of gadolinium DOTA decreases with the pH value. At pH=7, gadolinium DOTA remains more stable than its non-cyclic analogues, about 4 to 5 orders of magnitude (16).

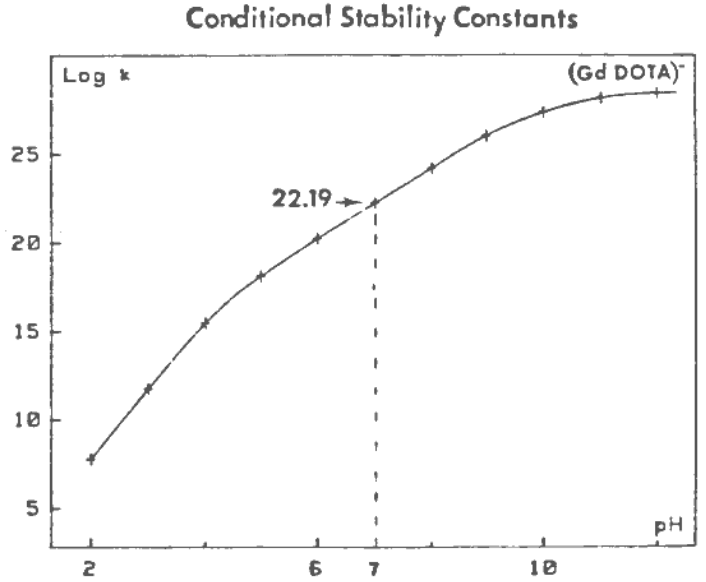

Figure 3:
Conditional stability constants are determined from the thermodynamic (5) and protonation constants (8) with Titfit program (15).

After in vivo administration of gadolinium chelates, a number of exchanges can occur in theory as a result of competition with endogenous metals, although this phenomenon is limited by the fast elimination kinetics of these chelates. To prevent this situation, chelates must have a high specific affinity for gadolinium and must be kinetically inert.

The specific affinity of the ligand for the paramagnetic metal makes it possible to predict the thermodynamic extent of the exchanges. This notion can be expressed as the ratio of the stability constants of gadolinium-DOTA to

those of complexes formed by DOTA with other endogenous metal ions (Table 1).

SPECIFIC AFFINITY OF DOTA FOR Gd^{3+}

Metal	$\frac{K\ (Gd\text{-}DOTA)^-}{Log\ K\ (M\text{-}DOTA)^{n-}(14)}$
Ca^{2+}	11.3
Zn^{2+}	7.4
Cu^{2+}	6.3

Table 1

These ratios demonstrate that DOTA is very specific for gadolinium as compared with some other endogenous metals. This characteristic can be explained by the inability of DOTA to octa-coordinate with transition metals such as copper (17). In this respect, DOTA is very different from its non-cyclic analogues which are universal ligands.

Kinetic inertia now appears as the paramount factor in the limitation of the in vivo dissociation process (18). Several studies have demonstrated the impact of the macrocyclic structure in terms of kinetic inertia and dissociation in human serum (19).

In the case of gadolinium DOTA, several studies (20) confirm its very slow kinetics of dissociation in the presence of H^+ ions

$$[Gd\text{-}DOTA]^- + H^+ \quad kH \quad Gd^{3+} + [DOTA\ H]^{3-}.$$

The comparison of the half-life of dissociation between gadolinium CyDTA (trans-1,2 diaminocyclohexane-N,N,N',N' tetraacetate) - a rigid non-cyclic chelate (21) - and gadolinium- DOTA is particularly relevant (Table 2). This kinetic inertia can be explained by the inability of the ligand to accomodate simultaneously one proton and metal inside the cavity in an intermediate state. During the dissociation process, the very rigid conformation of DOTA probably imposes the simultaneous release of all coordination sites of metal occupied by nitrogen atoms; in other words, no progressive dissociation process like that described in the case of linear complexes (22) can occur here.

Complexes	k^{ML} S^{-1}	k^{ML}_{H} $M^{-1}.S^{-1}$	t 1/2 pH = 7
Gd-CyDTA (21)	10^{-5}	1.3	19 hours
Gd-DOTA (20)	0	1.10^{-5}	$2\ 10^4$ years

$$(kobs = k^{ML} + k^{ML}_{H}\ [H+] + ...)$$

Table 2

All these kinetic and thermodynamic data demonstrate the inability of gadolinium-DOTA to achieve an in vivo dissociation. This characteristic is fundamental to prevent the free species (free ligand and metal) from expressing their toxicity.

The theory of relaxation T1 and T2 induced by paramagnetic agents has been extensively studied (23) and enables us to make an overall assessment of the parameters governing this phenomenon.

In the case of [Gd-DOTA H_2O]$^-$,Mg1, which is characterized by one water molecule directly coordinated to the metal and very marked symmetry, the induced longitudinal relaxivity R_1 and transverse relaxivity R_2 in an aqueous medium are 3.4 $mM^{-1}s^{-1}$ and 4.3 $mM^{-1}s^{-1}$ respectively, at a temperature of 37°C and a frequency of 20 MHz. Relaxometry studies (24) carried out on Gd-DOTA and Gd-DTPA (Figure 4) demonstrate their equivalent paramagnetic efficacy at high field strength.

For Gd-DOTA, the dispersion occurring at 7 MHz is characterized by a correlation time r_C which is equivalent to 3.10^{-11} s. The relaxivity ratio between the two plateaus at high and low field strength reaches about 10/3, which indicates that the relaxivity of Gd-DOTA is dominated by a rotational correlation time r_R independent of the frequency. On the contrary, the relative flattening of the Gd-DTPA dispersion curve at low field strength can probably be interpreted by a progressive domination of r_C by electronic relaxation time r_S which is frequency dependent (25).

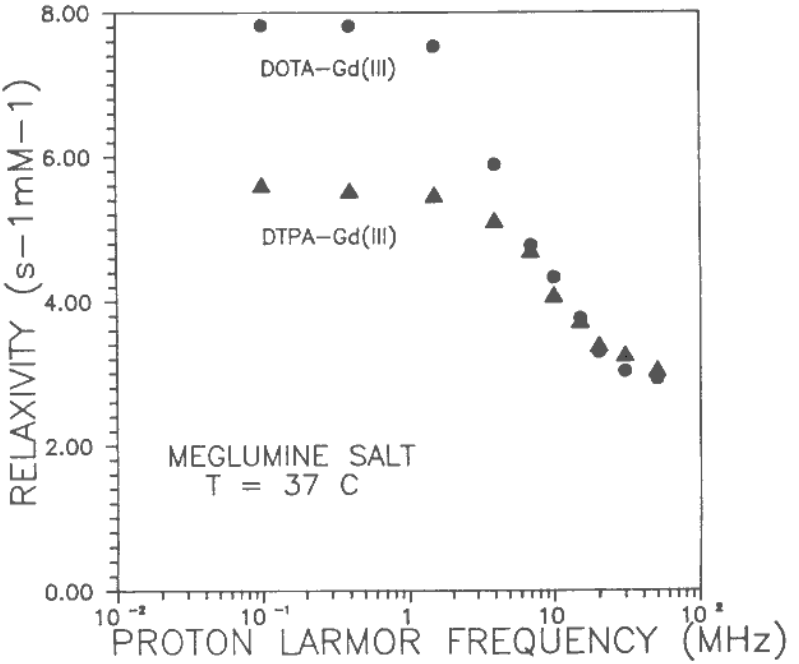

Figure 4:
Relaxivity of Gadolinium complexes. Relaxivities are determined on a field cycling instrument (I.B.M. Research, Mons University, Belgium) with an absolute uncertainty of ±1%.

Due to its greater symmetry, Gd-DOTA exhibits a higher in vitro efficacy at low field strength than Gd-DTPA.

It would seem that the symmetry of gadolinium complexes is a major contributing factor in maintaining an electron relaxation time long enough to prevent interference with r_C.

The macrocyclic chelate Gd-DOTA is currently being tested in humans. These first clinical trials, which have involved over two thousand patients, have confirmed the good tolerance of this complex and its diagnostic efficacy in vivo. The median lethal dose (LD 50) of Gd-DOTA was determined in Swiss mice and Sprague Dawley rats (Table 3) at a rate of 2 ml/min by intravenous injection of its N-methyl glucamine salt.

At different concentrations and osmolalities, these results confirm a very low toxicity of Gd-DOTA. For example, the LD 50 value in mice is 110 times higher than the effective imaging dose (0.1 mmol/kg). This safety ratio appears to be

higher than that of currently used uroangiographic iodinated contrast agents. No dependence between the toxicity and the osmolality of an aqueous solution is observed. Considering these results and the particularly favorable physico-chemical properties, the LD 50 of the macrocyclic chelate Gd-DOTA can essentially be attributed to a very low intrinsic toxicity of the complex with no contribution of highly toxic dissociated species such as free gadolinium.

PHARMACOLOGICAL DATA OF MACROCYCLIC CHELATE Gd-DOTA

Animals	Concentration mol / 1	Osmolality mosm / kg	LD 50 mmol / kg
Mice	0.505	1 400	11.3
	0.627	1 800	11.2
Rats	0.505	1 400	>12.6 (a)
	0.914	3 100	19.8

Table 3:
LD 50 after intravenous injection in mice (Swiss) and rats (Sprague Dawley). Injection rate was 2 ml/min.
(a) At this concentration and with administration of a volume of 25 ml/kg (equal to the plasma volume of rat), LD 50 cannot be reached.

After injection of Gd-DOTA in humans, no significant variation in biological parameters has been observed in the tests carried out in two hundred patients. In particular, no significant variation in plasma iron level was detected 1, 2, 4, 9 and 24 hours after injection of Gd-DOTA at 0.1 mmol/kg body weight.

CONCLUSION

The use of gadolinium-DOTA as a paramagnetic agent for in vivo contrast enhanced MRI studies appears to be quite promising due to its physico-chemical characteristics. The very rigid macrocyclic structure of DOTA has a positive impact on the specific affinity and kinetic inertia of the complex. Thus,

it contributes to minimize the in vivo dissociation process and avoids potential biological disturbances produced by the presence of free species.

It is now quite clear that this new class of macrocyclic chelates will form the basis of future design and innovations in the field of MRI contrast media.

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