New Gadolinium Complexes as Magnetic Resonance Imaging – Contrast Agents

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We have investigated the physicochemical and paramagnetic properties of the new gadolinium complexes of N-(pyrid-2-yl-methyl)-ethylenediamine-N,N',N'-triacetic acid (Gd-PEDTA) and N-tris(2-aminoethyl)amine-N',N',N'',N'',N''',N'''-hexaacetic acid (Gd-TTAHA). The relaxivities as well as the thermodynamic and conditional stability constants of these complexes with respect to the physiological relevance were determined and discussed in comparison with the commercially available gadolinium(III) diethylenetriaminpentaacetic acid/ gadopentate dimeglumine (Gd-DTPA, Magnevist®). In case of Gd-TTAHA a twofold higher relaxivity and a complex stability similar to Gd-DTPA were determined. It is shown, that lower concentrations of Gd-TTAHA are sufficient for the same signal enhancement in the T_1-weighted MR image compared with Gd-DTPA and, thus, the use of the new contrast agent Gd-TTAHA should diminish risks for health. Therefore, Gd-TTAHA might be used potentially as a new contrast agent for clinical MRI application.

Introduction

In recent years magnetic resonance imaging (MRI) has gained an important role in clinical diagnostics. Signal intensitiy, and therefore the image quality is influenced by the longitudinal and transverse relaxation rates of the water protons. It is common to increase the contrast of the image by increasing the relaxation rates of the water protons by addition of paramagnetic substances (Lauffer, 1987).

Of all elements, gadolinium, a rare earth (lanthanoide) element, has the strongest influence on spin lattice relaxation time (T_1) of water protons. Reasons for that are the high electronic spin (S= 7/2), relatively long electronic relaxation time and a labile hydration sphere. Because of the high toxicity of free gadolinium ions (LD_{50}=0.5 mmol/kg) (Weinmann *et al.*, 1984), its detoxification by complex formation with organic chelating agents is necessary for in vivo application. The clinical use and effectiveness of a paramagnetic contrast agent are mainly determined by its toxicity and relaxivity, respectively. That means that a MRI contrast agent must remain intact under physiological conditions to minimize the concentration of free metal ions and ligand. This is best realized by use of a strongly binding ligand that occupies most of the available coordination sites of the metal. The design of more stable complexes of gadolinium ions is expected to decrease the toxicity, but often the relaxivity is lowered as well.

Four gadolinium(III) complexes of linear and macrocyclic polyamino polycarboxylate are currently in clinical use as extracellular contrast enhancement media (gadopentetate dimeglumine, Magnevist® (Weinmann *et al.*, 1984; Felix *et al.*, 1994), gadodiamide, Omniscan® (Chang, 1993), Gd-DOTA, Dotarem® (Bousquet *et al.*, 1988) and Gd-HP-DO3A , Prohance® (Runge *et al.*, 1990), respectively).

The aim of our study is to present new gadolinium(III) compounds with higher relaxivity and better or similar aqueous complex stability in reference to the commercially available contrast agent especially gadopentate dimeglumine (Gd-DTPA, Magnevist®). An improved relaxivity means that the contrast agent is applicable in lower concentrations resulting in a reduction of biological risks.

This report presents investigations with respect to relaxivity and resulting signal enhancement in T_1-weighted images. Data of the determination of

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the thermodynamic and conditional stability constants with respect to the physiological consequence for a rough estimation of toxicity of two new gadolinium(III) chelates are shown. The results will be discussed in comparison with Gd-DTPA (Magnevist®).

Material and Methods

Chemicals

For all investigations two new gadolinium(III) complexes based on polyamino polycarboxylate were used. These ligands were obtained by the carboxymethylation of the corresponding amine with chloracetate in alkaline solution. The synthesis, purification, and characterization of the ligands N-(pyrid-2-yl-methyl)-ethylenediamine-N,N',N'-triacetic acid (PEDTA) and N-tris(2-aminoethyl)-amine-N',N',N'',N'',N''',N'''-hexaacetic acid (TTAHA) and of the corresponding gadolinium(III) complexes were previously described in detail (Ruloff *et al.*, 1995).

Gd-DTPA (Magnevist®) was purchased from Schering AG (Germany). The buffer salts trishydroxymethylaminomethane (Tris) and NaCl of analytical grade were purchased from Fluka Feinchemikalien GmbH (Germany) and used without further purification.

For determination of the corresponding relaxivities stock solutions (10 mM) of various gadolinium(III) complexes in trishydroxymethylaminomethane/NaCl-buffer (10 mM/154 mM) were prepared. The pH of the solutions was adjusted to 7.4 with diluted HCl solution on a PM-82 pH meter (Radiometer, Copenhagen (Denmark)) equipped with a combination glass electrode standardized against N. B. S. (National Bureau of Standards) buffer. Standard reference materials were purchased by Sigma (Deisenhofen, Germany).

Seven concentrations (0.25–2.5 mM) of the two new complexes and five Magnevist® concentrations were prepared by dilution of the stock solutions.

Potentiometric measurements

The complex stability constants were determined alcalimetrically using potentiometric titration with an automatic titrator system. Components of the autotitrating system included a pH meter (ABU 80/PHM84), a glass electrode G202C, and a saturated calomel reference electrode K701 (Radiometer, Copenhagen (Denmark)).

The electrodes were calibrated in the thermostated cell with standard acid (HCl) and base (KOH) to read p[H] directly. In each experiment, the temperature was maintained at 25 °C and ionic strength was kept constant at 0.1 M with KCl. Details of the potentiometric methods are described in (Martell *et al.*, 1992). Species distribution was calculated using the programm SPE.EXE (Martell *et al.*, 1992).

NMR-measurements

All NMR-measurements were carried out at 298 K on a Bruker AMX 300 spectrometer operating at 7 T, equipped with a microimaging accessory.

The spin-lattice relaxation times (T_1) were obtained by means of the inversion recovery sequence (16 measured points, repetition time 10s). The spin-spin relaxation times (T_2) were determined using the Carr Purcell Meiboom Gill technique with echo times τ between 200 and 1000 μs. The monoexponential analysis of relaxation data was performed by means of the curve fitting software package Peakfit version 3.0 (Jandel scientific).

T_1-weighted and T_2-weighted MR microscopic images were acquired using spin echo sequences with a repetition time (TR) of 200 and 1000 ms and echo times (TE) of 11 and 80 ms, respectively. The field of view of the images was 20 x 20 mm^2. Pixel resolution and slice thickness were 78 μm and 500 μm, respectively.

Results and Discussion

The ligands used in this study are illustrated in Fig. 1. All three ligands are polyamino polycarboxylate molecules. For application as contrast agents, the binding sites of ligands are very important for chelation of Gd^{3+} to form stable complexes. The coordination number of Gd^{3+} is estimated to be nine or ten (Reuben, 1971). Thus, using DTPA with eight coordination sites as a chelating ligand, only eight of gadolinium's nine or ten possible coordination sites can be filled. This leaves at least one or two sites open for fast-exchanging water protons to approach closely to the paramagnetic center of the complex. Based on luminescence

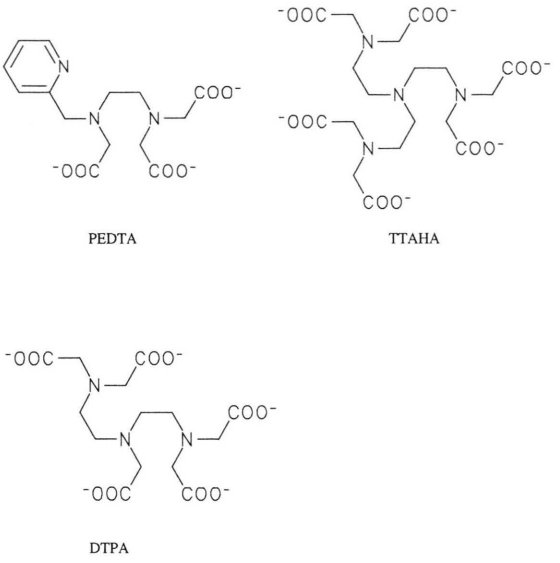

Fig. 1. Schematic structures of the ligands used in this study.
Abbreviations are: PEDTA, N-(pyrid-2-yl-methyl)-ethylenediamine-N,N',N'-triacetate; TTAHA, N-tris(2-aminoethyl)amine-N',N',N'',N'',N''',N'''-hexaacetate; DTPA, diethylentriaminepentaacetate.

studies several groups have estimated 1 and 1.2 water molecules per analogous terbium and europium complexes, respectively (Chang *et al.*, 1990; Bryden *et al.*, 1982). Compared with the well-known ligand DTPA with 8 binding sites, TTAHA and PEDTA possess 10 and 6 binding sites, respectively.

The determination of the longitudinal and transverse relaxivity served as a general characterization of paramagnetic compounds. Using these data the influence of the contrast agent with respect to signal enhancement can be estimated under *in vivo* concentrations.

As predicted by relaxation theory (Lauffer, 1987), relaxation rates show a linear response to increasing complex concentration in buffer solution (Fig. 2). In comparison with Gd-DTPA (Magnevist®) a significant difference between the longitudinal (T_1) and transverse (T_2) relaxivities of Gd-TTAHA and Gd-PEDTA were observed. The relaxivities of these two new compounds are roughly twice as high than as those of Magnevist® (Table I).

The relaxation times of water in the presence of a paramagnetic complex is influenced by the inner

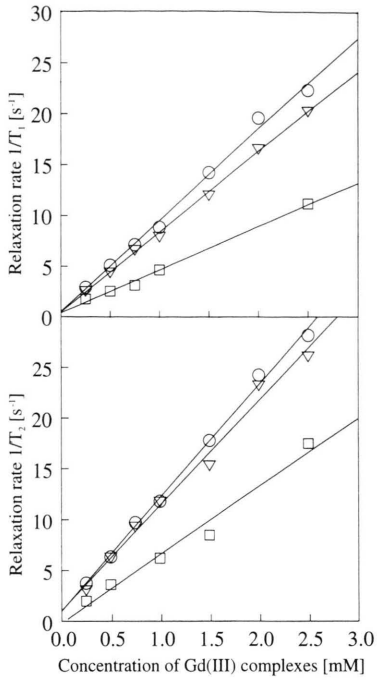

Fig. 2. Dependence of the water T_1 (spin-lattice) and T_2 (spin-spin) relaxation times on the concentration of the gadolinium(III) complexes – Gd-TTAHA (○), Gd-PEDTA (▽), Gd-DTPA (□) measured at 300 MHz and 298 K (pH 7.4).

Table I. Relaxivities (300 MHz, 298 K) and aqueous solubilities of various gadolinium(III) complexes.

Complex	Relaxivity [$s^{-1}mM^{-1}$]		Water solubility [mol/l]
	T_1	T_2	
Gd-TTAHA	9.5	10.5	0.5
Gd-PEDTA	8.8	9.6	0.1
Gd-DTPA	4.2	6.7	1
Gd-EDTA*	6.9	8.2	1

* As determined at 40 MHz by Weinmann *et al.* (1984).

sphere water coordination number (q) and correlation time (τ_c), each of them can vary significantly (Lauffer, 1987; Chang *et al.*, 1990). For small gadolinium(III) chelates at high frequencies, the rotation correlation time (τ_r) dominates τ_c, and a higher relaxation rate can be obtained by increasing τ_r (Lauffer, 1987). Assuming that the molecular weight and molecule size of these new complexes are nearly the same like Gd-DTPA an increased τ_r as reason for the high relaxivity must be excluded.

Therefore we think, that the enhanced relaxivitiy of the new gadolinium(III) complexes might be caused by the binding of more than one water molecule in the first coordination sphere of the central atom. A further possible explanation for the enhanced water relaxivity might be also based on an increased exchange rate of the coordinated water molecules $1/\tau_m$ (Ruloff, 1997).

PEDTA with six donor atoms principally has three or four binding sites for water molecules, which should confere a high relaxivity. Due to the hydrophobic pyridine ring, the addition of water molecules to the paramagnetic metal ion is hindered. This could explain the relatively low water solubility and relaxivity (Table I).

The relaxivity data of Gd-TTAHA show a significant and interesting dependence on the pH-value (Fig. 3). At very low pH values (pH<3), the

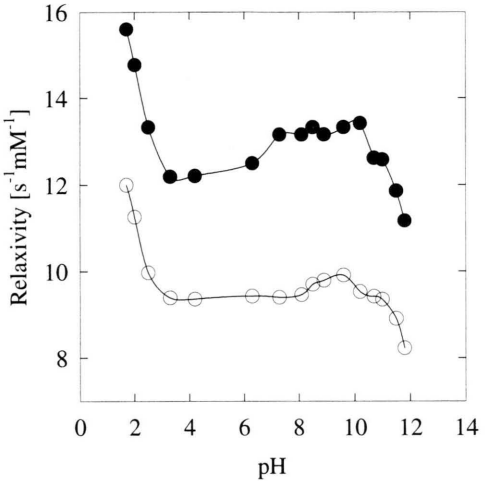

Fig. 3. pH dependence of the Gd-TTAHA relaxivities (T_1 (○) ,T_2 (●), at 300 MHz and 298 K.

carboxylate group and the nitrogen atom are successively protonated and are no longer coordinated to the central atom. Upon the dissociation of functional groups an increase in the number of water molecules coordinated to the metal ion is observed. These species exhibit higher relaxivity values due to their enhanced hydration state. Between pH values of 3 and 8, a relatively constant value is achieved. However, the relaxivity of Gd-TTAHA decreases when pH 9 exceeds. This can be caused by two effects. First, at high pH values the nitrogen of the free iminodiacetate group is deprotonated and a complexation of the free arm of the ligand is possible. On the other hand, a partial substitution of coordinated water molecules with OH^- ligands cannot be excluded. In both cases the number of coordinated water molecules in the first coordination sphere is reduced. This explains the decreased relaxivity at high pH values. Potentiometric titration evidence strongly indicates that the complex exists as disodium hydrogenated compound at pH 5.8. At pH 9 the protonated form turns into the deprotonated chelate complex (Fig. 5). These data support the first hypothesis.

The water solubility of Gd-TTAHA and Gd-PEDTA are 0.5 M and 0.1 M, respectively. These values are low compared with Magnevist®, but nevertheless high enough for their use in MR imaging.

In contrast to the relaxation rates, which generally increase with the low concentration of the paramagnetics, the signal intensity, however, does not show a linear dependence on the concentration of the contrast agent. To examine the influence of Gd-TTAHA and Gd-DTPA on the T_1- and T_2-weighted MR images, respectively, we used a phantom probe containing varying concentrations of the gadolinium(III) complexes. The results shown in Fig. 4 indicate, that Gd-TTAHA as well as Gd-DTPA increases the signal intensity in aqueous solution in a concentration range from 0.05 to 2 mM by means of T_1-weighted spin-echo images (Fig. 4a,b). Using T_2-weighted spin-echo sequences (Fig. 4c,d) high concentration of Gd-TTAHA and Gd-DTPA (1 and 2 mM), respectively, results in a decreasing of signal intensity, because of the shortening of the T_2 water relaxation time. The T_1-weighted images (Fig. 4 a,b) show, that due to the high relaxivity of the new contrast agent Gd-TTAHA higher signal enhancement factors in reference to Gd-DTPA can be obtained under identical conditions. This indicates, that a lower concentration of Gd-TTAHA is required to obtain the same signal enhancement in reference to Gd-DTPA. The application of lower amounts of the contrast agent, therefore, should result in a reduction of toxicity.

In addition to relaxivity, complex stabilities and toxicities are of great importance for the possible application of gadolinium(III) complexes as contrast agents.

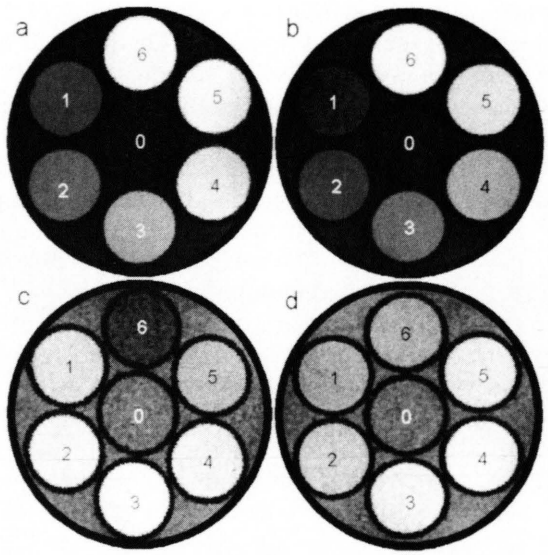

Fig. 4. Effect of Gd-TTAHA (a, c) and Gd-DTPA (b, d) concentrations on spin-echo signal intensity by means of T_1-weighted (a, b) and T_2-weighted (c, d) images, respectively. Seven tubes containing increasing concentrations of the gadolinium(III) complexes. The following concentrations were used: 0: 0 mM, 1: 0.05 mM, 2: 0.1 mM, 3: 0.25 mM, 4: 0.5 mM, 5: 1 mM, 6: 2 mM. All solutions included 154 mM NaCl.

Table II shows the potentiometrically determined protonation constants of TTAHA and PEDTA compared with values reported earlier for DTPA (Smith *et al.*, 1974). The completely protonated acid TTAHA possesses 10 different pK_a values. Four dissociation equilibriums have pK_a values below 2 and therefore cannot be determined potentiometrically. Direct potentiometric titration was also used to determine the stability constants of the complexes formed by TTAHA and the metal ions Gd(III), Ca(II), and Zn(II). The experiments were run in 1:1 metal-ligand systems, and the data are shown in Table II.

Table II. Protonation constants of the ligands TTAHA, PEDTA, and DTPA and the stability constants of the corresponding Gd^{3+}, Ca^{2+}, and Zn^{2+} complexes (μ = 0.1 M (KCl), 25 °C).

	log*K*		
Equilibrium	TTAHA	PEDTA	DTPA[b]
[HL]/[L][H]	10.66 ± 0.02	9.83 ± 0.01	10.49
$[H_2L]/[HL][H]$	8.56 ± 0.02	5.95 ± 0.01	8.60
$[H_3L]/[H_2L][H]$	8.38 ± 0.02	3.61 ± 0.01	4.28
$[H_4L]/[H_3L][H]$	2.92 ± 0.02	2.00 ± 0.01	2.64
$[H_5L]/[H_4L][H]$	2.39 ± 0.05		2.00
$[H_6L]/[H_5L][H]$	2.00 ± 0.10		1.60
[GdL]/[Gd][L]	19.00 ± 0.10	15.56 ± 0.02	22.46
[GdHL]/[GdL][H]	8.30 ± 0.02		2.39
[CaL]/[Ca][L]	9.73 ± 0.03	9.56[a]	10.75
[CaHL]/[CaL][H]	8.64 ± 0.02		6.11
[ZnL]/[Zn][L]	18.91 ± 0.05	14.87[c]	18.70
[ZnHL]/[ZnL][H]	8.01 ± 0.02		5.60
$[ZnH_2L]/[ZnHL][H]$	3.68 ± 0.02		
$[ZnH_3L]/[ZnH_2L][H]]$	2.33 ± 0.05		

The values were taken from a) Hoyer *et al.* (1967); b) Smith *et al.* (1974); c) Beyer (1964).
Abbreviations are: μ: ionic strength, L: ligand, *K*: stability constant.

The stability constant of the 1:1 gadolinium(III) complex of TTAHA is log*K* = 19.0. The relatively low complex stability of Gd-PEDTA (log*K*=15.4) is probably caused by the lower coordination number. This correlates well with the stability of Gd-EDTA (log*K* = 17.3, coordination number 6) (Cacheris *et al.*, 1987).

The TTAHA ligand has in principle ten donor atoms for the coordination with a gadolinium(III) atom. The potentiometric measurements (Table II) and determination of the complex formation constant of Gd-TTAHA, yield that the complex exists as a disodium hydrogenated compound at pH 5–8 (Fig. 5). The distribution curves of the 1:1 TTAHA-Gd(III) system shown in Fig. 5 indicate that the species is present as a function of p[H]. The curves clearly show that the metal ion is about 80% complexed at p[H] = 2. The protonated form, GdHL, is converted to the deprotonated chelate GdL at p[H] > 9. Therefore at p[H] = 7, the protonated form GdHL, is the predominant species at approximately 95–100%. This means that one nitrogen atom is protonated. Thus, a co-

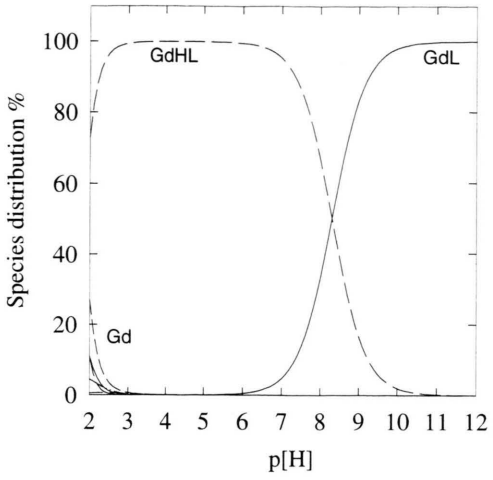

Fig. 5. Species distribution plot of the TTAHA/Gd(III) system as a function of p[H] (total ligand(TTAHA): 100 mM; total Gd^{3+}: 100 mM).
GdHL means the protonated form of the gadolinium (III) complex and GdL the deprotonated form of the complex.

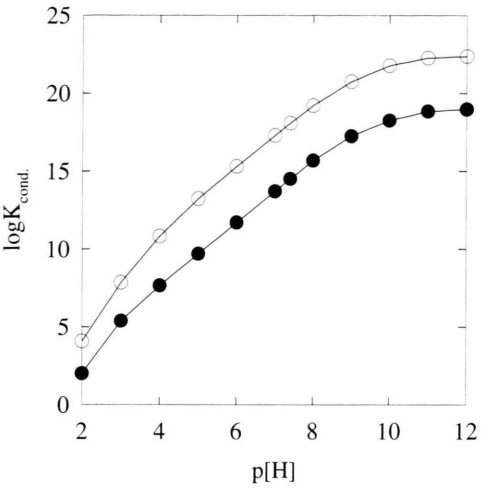

Fig. 6. p[H] dependence on conditional stability constant for Gd-TTAHA (●) and Gd-DTPA (○).

ordination of one iminoacetate group at the gadolinium(III) is not possible. Due to the seven coordination sites of TTAHA, two or three binding sites may be occupied by water molecules in the first coordination sphere. This explains the roughly twice as high relaxivity of Gd-TTAHA in comparison to Gd-DTPA at pH 7 where not a single water molecules is coordinated. Additionally, luminescence studies of the corresponding europium(III) complex yielded that about two water molecules are bound to the central atom (data not shown).

For medical application the conditional stability constant (K_{cond}) is more significant than the thermodynamic stability constant. It specifies the degree of metal chelation at a given pH and is therefore a more realistic measure of the stability of the metal complex at physiological pH.

The stability of a complex under physiological conditions can be readily determined if the pK_a values are known. The pK_a values of the ligand along with the thermodynamic stability constant can then be used to determine a conditional stability constant (Cacheris *et al.*, 1990).

Fig. 6 shows the variation of the conditional stability constants of Gd-TTAHA and Gd-DTPA with p[H]. At p[H] 7.4 Gd-DTPA is more stable than Gd-TTAHA by a factor of $10^{3.8}$ but Gd-TTAHA has a comparable stability like Gd-DTPA-BMA, the bis(methylamide) derivate of DTPA ($\lg K_{cond}$ = 14.9, pH 7.4) (Chang *et al.*, 1990; Chang, 1993). At a pH high enough to ensure complete deprotonation of the ligands (pH>11), the conditional stability constants are equal to their thermodynamic values.

The toxicity is very important for clinical use as contrast agents. Free gadolinium ions have an ionic radius similar to that of calcium and have thus been shown to disturb calcium-dependent functions such as muscular contraction and neurotransmission (Wolf *et al.*, 1984). This leads to a high degree of acute toxicity of Gd(III) ions. The toxicity of the metal chelate correlates with its *in vivo* stability which is a function of many factors such as solution thermodynamics, solubility, selectivity, and kinetics (Cacheris *et al.*, 1990). Some researchers have used the *in vitro* thermodynamic stability constant of the complex as an indicator of the in vivo affinity of the metal ion and ligand. It was reported that the *in vitro* thermodynamic stability constants of Gd-EDTA, Gd-DTPA and Gd-DOTA, correlate well with their acute toxicity (Bousquet *et al.*, 1988). Gd-TTAHA ($\lg K$=19) is less stable in aqueous solution in comparison with Magnevist® ($\lg K$=22.4) (Tse *et al.*, 1985), but more stable than the new commercially available contrast agent Gd-DTPA-BMA (Omniscan®, $\lg K$ = 16.9 (Chang *et al.*, 1990; Chang, 1993)), which shows a surprisingly low toxicity (LD_{50} = 34 mmol/kg).

On the other hand it was reported that a direct dependence of toxicity upon thermodynamic stability does not exist (Cacheris *et al.*, 1990; Mann, 1993). The thermodynamic stability constant of gadolinium(III) complexes is not the only stability constant relevant for considerations of the toxicity of Gd^{3+} complexes. Based on the assumption that most of the toxicity of such complexes arises from the release of the highly toxic Gd^{3+} ion *in vivo*, it is important to know the stability of the ligand with endogenous metal ions such as Zn^{2+}, Cu^{2+}, Fe^{2+}, and Ca^{2+} since these metals can be replaced by Gd^{3+}. A better correlation to the *in vivo* gadolinium(III) chelate stability will be obtained by the selectivity constants (K_{sel}). K_{sel} is a potent indicator of the affinity of the chelating agent for gadolinium in comparison to the other ions present e.g. Zn^{2+}, Ca^{2+}, and Cu^{2+} (Cacheris *et al.*, 1990; Mann, 1993). Gd-DTPA-BMA has a high selectivity for Gd^{3+} over Zn^{2+} and Ca^{2+} (Cacheris *et al.*, 1990). That means, that the greater selectivity especially for Gd^{3+}over Zn^{2+} is related positively to the LD50 value. Zn^{2+} plays an important role in organisms because it builds very stable complexes with nitrogen ligands (Carvalho *et al.*, 1992). Based on this aspect we have determined the complex stability of Gd^{3+}, Ca^{2+}, and Zn^{2+} with TTAHA (Table II). Accordingly, the corresponding stability constants with PEDTA and DTPA are also given in Table II (Smith *et al.*, 1974; Hoyer *et al.*, 1967; Beyer, 1964). Ca^{2+} is not expected to displace Gd^{3+} from Gd-TTAHA due to its relatively low stability constant ($\lg K_{M/ML}$= 9,67). However, Zn^{2+} has a moderately high stability constant ($\lg K_{M/ML}$=18.91). This indicates, that the Gd^{3+} and Zn^{2+} complex stability with TTAHA are in the same order of magnitude. A higher selectivity of TTAHA to Gd^{3+} over Zn^{2+} cannot be expected. This could have physiological consequences because Zn^{2+} is an important metal for many enzymes.

Conclusion

Two new gadolinium(III) complexes of polyamino polycarboxylate molecules were physicochemically characterized by means of relaxivity measurements and determination of the complex stability constants under physiological conditions These properties suggest that one of these new compounds (Gd-TTAHA) is potentially useful as contrast agent for MR imaging. Compared with Magnevist®, Gd-TTAHA is only slightly less stable in water, but shows a higher relaxivity. That means, that a lower concentration is required for the same signal enhancement in the MR images. For that reason the new gadolinium(III) complex Gd-TTAHA may be used potenially for application to MRI with less risk of toxicity.

Acknowledgments

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