Synthesis, Characterization, and Relaxivity of Two Linear Gd(DTPA)–Polymer Conjugates

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Two linear polyamide conjugates of Gd(DTPA)2− were synthesized and characterized by high-resolution nuclear magnetic resonance (NMR) spectroscopy and size exclusion chromatography (SEC). DTPA was copolymerized with two different diamines, 1,6-hexanediylene and trans-1,4-cyclohexanediyclic, yielding the polymers DTPA-HMD and DTPA-CHD, with low polydispersity. Their molecular flexibility in solution was studied using 13C spin–lattice relaxation time measurements, indicating that the cyclohexanediyclic linking moiety of the DTPA-HMD polymer is more rigid than that of DTPA-CHD. The influence of the flexibility of the linking functionalities on the relaxivity of the Gd3+–DTPA–polymer conjugates was studied by water nuclear magnetic relaxation dispersion (NMRD). The relaxivity of the Gd(DTPA-CHD) polymer was only slightly higher than that of the Gd(DTPA-HMD) polymer, and only two times higher than the usual values for small Gd-DTPA-like chelates. The low relaxivities obtained for both polymers, much lower than expected from the polymer apparent molecular weights, result from their substantial residual flexibility, and also from a too long, nonoptimal, value of the inner-sphere water exchange rate. These polymeric compounds are also cleared very quickly from the blood of rats, indicating that they are of limited value as blood pool contrast agents for MRA.

INTRODUCTION

Contrast agents (CAs) for magnetic resonance imaging (MRI) are mainly chelates of Gd3+, due to its high magnetic moment and long electronic relaxation time (1−4). A Gd(III)-bound water which exchanges with the bulk water of the body, determines via the enhancement (T1 and T2) of the water protons, the intensity of the water signal and thus the contrast of the images. The relaxation theory predicts that higher relaxation rates are obtained upon increase of the rotational correlation time. These polymeric compounds are also cleared very quickly from the blood of rats, indicating that they are of limited value as blood pool contrast agents for MRA.

Here we report the synthesis, physicochemical characterization and in vivo pharmacokinetics in Wistar rats of two linear polyamide conjugates of Gd(III)-DTPA, Gd-(DTPA-HMD), and Gd(DTPA-CHD) (see their structures in Figure 1), obtained by copolymerization of DTPA with two different diamines, the linear and conformationally flexible 1,6-hexanediyclic and the cyclic and conformationally more rigid trans-1,4-cyclohexanediyclic. The influence of the molecular flexibility of the respective functionalities on the relaxivity of the Gd(III)-DTPA–polymer conjugates is also reported and compared with other systems from the literature. As macromolecular polyamides, they should be biodegradable and biocompatible.
NMR (DMSO-d$_6$ detected, using xylenol-orange as indicator. The samples were dialyzed until no free Gd$^{3+}$ was detected. The mixture was heated at 70 °C under N$_2$ bubbling. Over a period of 3 h a large part of the solvent was slowly distilled under N$_2$ at reduced pressure (70 °C). The metal content (%) in the solutions was adjusted with DCl and CO$_2$-free NaOD to 97% purity, GdCl$_3$$\cdot$6H$_2$O, trans-1,4-cyclohexanediamine (CHD) and 1,6-hexanediamine (HMD) were purchased from Aldrich and used as supplied. Acetic anhydride, pyridine and dimethyl sulfoxide, also from Aldrich, were dried over 3 Å molecular sieves before use.

Synthesis and Characterization of DTPA Copolymers and Gd$^{3+}$ Conjugates. Preparation of DTPA-bis-Anhydride (DTPA-BA). A modification of procedure described in the literature (28, 30) was applied: 0.125 mol of DTPA was mixed with 0.558 mol of dried acetic anhydride and 0.770 mol of dried pyridine. The resulting suspension was stirred for 24 h at 65 °C. Then the solid obtained was filtered, washed with dried diethylether and dried under vacuum, until constant weight at 25 °C. The DTPA-bis-anhydride was characterized by NMR. 1H NMR (DMSO-d$_6$) $\delta$ (ppm) 3.7 (s,8H), 3.29 (s,2H), 2.43 (t,4H), 2.58 (t,4H); 13C NMR (DMSO-d$_6$) $\delta$ (ppm) 171.9, 165.7, 54.5, 52.5, 51.7, 50.7. This product was considered sufficiently pure to be used in the preparation of the polymers.

Polymerization of DTPA-BA with HMD. In a round-bottom flask, 6 mmol of DTPA-BA were dissolved in 30 mL of DMSO and an equimolar amount of HMD was added. The mixture was heated at 70 °C under N$_2$ bubbling. Over a period of 3 h a large part of the solvent was slowly distilled under N$_2$ at reduced pressure (70 °C). The product was precipitated in acetone, filtered, and dissolved in 20 mL of distilled water. The solution was dialyzed for 3 days, using a MWCO 12,000 Membrane purchased from Medicel, London, U.K., and lyophilized to give 0.33 g of polymer (11.6%).

Polymerization of DTPA-BA with CHD. The same procedure as described above was followed except that 5.6 mmol of DTPA-BA and of CHD were used. After lyophilisation, 0.63 g of polymer was obtained (24%).

Preparation of the Gd$^{3+}$ Conjugates. The polymers were dissolved in a small amount of distilled water and equimolar amounts of an aqueous solution of GdCl$_3$ were added dropwise with stirring, for the period of 1 h. The pH was maintained at 5.8 by the addition of 0.1M NaOH. The solutions were dialyzed until no free Gd$^{3+}$ was detected, using xylenol-orange as indicator. The samples were then lyophilized.

Determination of Gd$^{3+}$. The metal content (%) in the polymer conjugates was determined by inductively coupled plasma optical emission spectroscopy (ICP-OES), using a J oeb Yvon-j y 70 Plus spectrometer.

The metal binding to the polymers was also determined by titration of aqueous solutions (D$_2$O) of those polymers with solid aliquots of GdCl$_3$ while maintaining neutral pH by addition of aqueous NaOH. The formation of the complex was monitored by measuring the shift of the $^{17}$O NMR signal of water at 348 K (2, 16). The presence of unbound Gd$^{3+}$ was readily detected by a dramatic increase in the chemical shift and width of the signal. Then, evaluation of the titration data allowed for determination of the percent of DTPA binding moieties of the polymer complexed to Gd$^{3+}$.

NMR Measurements. 1H (499.824 MHz), 13C (125.697 MHz), and $^{17}$O (67.760 MHz) NMR spectra were recorded on a Varian Unity 500 spectrometer (at an external field of 11.8 T) in D$_2$O (99.8% D from Sigma Chem. Co.) solutions. For the 1H NMR spectra, the water signal was used as internal reference, set at δ = 4.75 ppm (at 298 K) and was suppressed by a presaturation pulse. 13C NMR spectra were measured with broad-band proton decoupling, using the methyl resonance of TSP (sodium-3-trimethylsilylpropionate-2,2,3,3-d$_4$) as internal reference, set at δ = 0 ppm. $^{17}$O NMR spin–lattice relaxation times ($T_1$) values were measured using the inversion–recovery technique. Assignments of the 1H and 13C NMR spectra were based on literature data for similar systems (28, 31) and on the results of two-dimensional homonuclear correlation spectra (COSY). The pH of the solutions was adjusted with DCl and CO$_2$-free NaOD (from Sigma Chem. Co.) using a Crison Microph 2002 pH-meter with an Ingold 405-M5 combined electrode. The temperature precision of the experiments was ±0.5 °C.

SEC Measurements. The apparent molecular weights of the polymers were determined by size-exclusion chromatography (SEC) using two series of PL-Aquagel-OH-30 columns (from Polymer Laboratories) coupled to a LC-25 RI detector. The eluent was 0.2 M NaCl. Systematic errors in the molecular weight determinations, leading to apparent values result from the charges of the polymers. The weight-average molecular weight (Mw) and number-average molecular weight (Mn) of the polymers are given by the expressions $M_w = (SN,M^3/3)(SN,M)$ and $M_n = (SN,M)/(SN)$, where N is the number of molecules of molecular weight M, $M_w$ and $M_n$ were calculated using a calibration curve of poly(ethylene glycol) standards obtained using a software program, EzChrom Chromatography V6.6, Scientific Software, Inc. The Mn/Mw ratio, named polydispersity, which is a measure of the width of the molecular weight distribution, was also calculated.

Relaxometry Studies. The chelates were dissolved in ultrapure H$_2$O and the water $T_1$ relaxation times of the solutions were measured at 9 MHz and 298 K using an MRS-4 relaxometer (J ožef Stefan Institute, Ljubljana, Slovenia), using an inversion recovery pulse sequence (180°-t-90°) with eight delay times. The relaxivity was determined by calculating the slope of the curve of relaxation rate (1/$T_1$) versus Gd$^{3+}$-concentration (mmol/L).

Proton nuclear magnetic relaxation dispersion (NMRD) profiles were recorded at 310 K on a field cycling relaxometer (Field Cycling Systems, Honesdale, PA) working between 0.02 and 50 MHz. Additional points at 60 and 300 MHz were obtained on a Bruker Minispec mq60 (Bruker, Karlsruhe, Germany) and on a Bruker AMX 300 spectrometer (Bruker, Karlsruhe, Germany). Fitting of the 1H NMRD curves was performed with a previously described software using minimization routines (Minuit, CERN Library) (32, 33).
Pharmacokinetics. The pharmacokinetic studies were carried out according to the following protocol: the Wistar rats were anesthetized by i.p. injection of Nembutal (100 μL/100 g b.w.), the carotid artery was catheterized for rapid blood collection, 200 μL of contrast agent were injected in the blood through the femoral vein; blood samples (300 μL/sample) were collected 1, 2, 5, 10, 15, 30, 45, and 60 min. afterward; the longitudinal relaxation rate $R_1$ was measured at 39 °C on a spin analyzer PC120 (Bruker, Rheinstetten, Germany) on each sample.

RESULTS AND DISCUSSION

The copolymers of DTPA obtained were synthesized by an easy method that leads to polyamides. After purification, these polymers were found to be water soluble. The structure of both polymers is represented in Figure 1. Their $^1H$ NMR spectra, shown in Figure 2, have narrow peaks suggesting that the polymers present are rather flexible. The assignments of the $^1H$ signals were based on their intensities, multiplicities and chemical shifts, and confirmed by $^1H$-COSY spectra. The assignments of the methylene protons of the DTPA moiety were obtained from the previously described proton NMR titration curves of DTPA derivatives (31). In spectrum B, we can observe the polymeric species corresponding to two conformations. The substitution groups in cyclohexane are mainly in diequatorial configuration because of the effect of 1,3-diaxial interactions. However, due to the partial double bond character of the amide N–C bonds, the repeated unit in the polymer can be in three conformations, which are E,Z, E,E, and Z,Z (Figure 3). Only two sets of signals, with intensity ratios 4.9:1, were observed in the $^1H$ NMR spectrum. An inspection of molecular models suggests that the steric strain in the three possible configurations of the disubstituted cyclohexane moieties follows the order E,E > E,Z > Z,Z. Therefore, we assign the two sets of signals to the E,Z and Z,Z configurations, and we assume that the population of the E,E configuration is negligible. In the DTPA-HMD polymer only a single set of broad signals was observed, which can be explained by the fast exchange between the isomers on the NMR time scale.

Figure 2. $^1H$ NMR spectra of the copolymers: (A) DTPA-HMD polymer obtained in D$_2$O + NaOD (57.2 mg/mL) at 25 °C, pH 10.71; (B) DTPA-CHD polymer obtained in D$_2$O + NaOD (41.6 mg/mL) at 25 °C, pH 11.2.
polymer, we see two sets of resonances for carbons a–f. This is in agreement with the $^1$H NMR data.

Table 1 shows the $^{13}$C NMR $T_1$ values, obtained for the two polymers. These values are rather long for the ester and carboxylate carbons, due to the absence of efficient dipolar relaxation from neighboring protons. It is more instructive to compare the $T_1$ values for the CH$_2$ carbons, where the $^{13}$C relaxation rates (1/$T_1$) are proportional to an effective local rotational correlation time, which modulates the dipolar interaction between the $^{13}$C and the directly bound protons, reflecting the local mobility of the molecule (35). This relationship is valid for local rotational correlation times smaller than 10$^{-9}$ s (36) and can be applied to polymers that have fast internal motions. Here we see that, for both polymers, the methylene carbons of the DTPA moieties have the same mobility. This mobility is smaller than that of the linking chain for the DTPA-HMD polymer, but the same for the DTPA-CHD polymer. This surprising result may be a consequence of the slower overall tumbling rate of the second polymer, with its larger molecular weight, rather than of the local mobility of the linking moieties. However, to correlate these data with the relaxivity of the macromolecular complexes we have to take into account the mobility of the DTPA moiety and the correlation time of the water proton.

The SEC chromatograms of our samples of the two polymers and of their Gd$^{3+}$ complexes, obtained using an eluent of high ionic strength, are shown in Figure 5. The results of their analysis (Table 2) show that the DTPA-

Table 1. $^{13}$C $T_1$ Values (s) for the Copolymers DTPA-HMD and DTPA-CHD in D$_2$O at 25 °C, pH 11.0

<table>
<thead>
<tr>
<th>$^{13}$C nucleus (see Figure 1)</th>
<th>DTPA-HMD</th>
<th>DTPA-CHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>e</td>
<td>3.18 ± 0.09</td>
<td>2.76 ± 0.24</td>
</tr>
<tr>
<td>f</td>
<td>1.76 ± 0.08</td>
<td>1.49 ± 0.13</td>
</tr>
<tr>
<td>a</td>
<td>0.23 ± 0.01</td>
<td>0.24 ± 0.03</td>
</tr>
<tr>
<td>b</td>
<td>0.21 ± 0.01</td>
<td>0.20 ± 0.04</td>
</tr>
<tr>
<td>c</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>d</td>
<td>0.21 ± 0.02</td>
<td>0.18 ± 0.02</td>
</tr>
<tr>
<td>d'</td>
<td>0.20 ± 0.01</td>
<td>0.19 ± 0.04</td>
</tr>
<tr>
<td>m</td>
<td>0.27 ± 0.02</td>
<td>0.40 ± 0.03</td>
</tr>
<tr>
<td>n</td>
<td>0.30 ± 0.02</td>
<td>0.19 ± 0.01</td>
</tr>
<tr>
<td>o</td>
<td>0.37 ± 0.03</td>
<td></td>
</tr>
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</table>

* Not determined.

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Figure 3. E,Z, E,E, and Z,Z conformations of the trans-1,4-cyclohexanediamine in the DTPA-CHD polymer.

Figure 4. $^{13}$C NMR spectra of the copolymers: (A) DTPA-HMD polymer obtained in D$_2$O + NaOD at 25 °C, pH 10.7; (B) DTPA-CHD polymer obtained in D$_2$O + NaOD at 25 °C, pH 11.2.
HMD and DTPA-CHD polymers have very similar apparent molecular weights and low polydispersities, indicating that they are quite homogeneous, as we can observe in the SEC chromatograms in Figure 5. A reduction in the apparent molecular weight values of both polymers can be observed after Gd\(^{3+}\) complexation, and their low polydispersity slightly increases. The molecular weights of DTPA-HMD and DTPA-CHD must be lower than those of the corresponding Gd\(^{3+}\) complexes, due to the contribution of the cation. Thus, the observed reduction of the SEC-measured apparent values for the complexed polymers should be a result of the neutralization of charge of the DTPA polymers when bound to Gd\(^{3+}\). In fact, even using an high ionic strenght eluent (0.2 M NaCl), the repulsion forces between the negatively charged polymer carboxylate groups increase the hydrodynamic volumes of the polymeric chains, leading to lower elution volumes and over-evaluated molecular weights. This is confirmed by the observation that using a low ionic strenght eluent (0.01 M NaCl), the apparent molecular weights of the of DTPA-HMD and DTPA-CHD polymers decreased by a factor of 6, while those of their Gd\(^{3+}\) complexes increased only by a factor of 2 (data not shown). Thus, the determination of molecular weights for this type of compounds remains a difficult problem, casting some doubt on the reliability of some of the literature data.

The Gd\(^{3+}\) contents in the two polymers, as measured by ICP, are shown in Table 3. Similar Gd\(^{3+}\) contents were measured by Kellar et al. (29) for the Gd(DTPA-HMD) polymer. The Gd\(^{3+}\) yields obtained show that ca. 20 and 30% of the DTPA units in Gd(DTPA-CHD) and Gd(DTPA-HMD), respectively, are uncomplexed.

The Gd\(^{3+}\) yields, which give the percent of DTPA binding moieties of the polymers complexed to Gd\(^{3+}\), were also determined by measuring the shift of the \(^{17}\)O NMR signal of water at 348 K while titrating D\(_2\)O solutions of those polymers with solid aliquots of GdCl\(_3\) at neutral pH (2). Figure 6 shows the plot resulting from a typical experiment with the DTPA-HMD polymer. The position of the break of the plot, indicating the presence of unbound Gd\(^{3+}\) gave a Gd\(^{3+}\) yield of 87% for the Gd(DTPA-CHD) polymer, a value in reasonably good agreement with that obtained for ICP data.

The relaxivities of the two Gd\(^{3+}\) polymers at 9 MHz and 298 K are also shown in Table 3. The relaxivities for the polymers were less than twice the value of 5.6 s\(^{-1}\) mM\(^{-1}\), observed for the Gd(DTPA)\(^2+\) chelate itself in the same conditions. The relaxivity of Gd(DTPA-HMD) is quite similar to the value reported in the literature (29). As CHD has a more rigid structure than HMD, allowing only chain-chain conformational interconversions but no single C–C bond rotational motions, it should hinder the rotational motion of the DTPA-linking moieties in the polymeric chain. As can be seen in Table 3, the relaxivity of the Gd(DTPA-CHD) polymer was not as high as expected, being only slightly higher than that of the Gd(DTPA-HMD) polymer. This could result from the substantial residual flexibility of that polymer, and also from a nonoptimal value of the inner-sphere water exchange rate.

To be able to better understand the factors determining the relaxivity of these polymers, we undertook a full proton nuclear magnetic relaxation dispersion (NMRD) study of these systems. The proton NMRD profiles recorded at 310 K (see Figure 7) show a hump between 10 and 100 MHz characteristic of slowly tumbling paramagnetic complexes. The analysis of the proton relaxivity takes into account the outersphere (37) and the innersphere magnetic interactions (38, 39). For Gd(DTPA-HMD), the values evaluated from the NMRD profiles analysis at 310 K for the rotational correlation time \(\tau_r\), for the electronic parameters \(\tau_s\), the electronic relaxation time at low field, and \(\tau_v\), the correlation time modulating the electronic relaxation) and the residence time of the inner-sphere water molecule \(\tau_w\) (see Table...
Chain, which dominate the fast local motions in the extended polymeric residence time of their innersphere water molecules.

The clearance from the blood. With a blood half-life shorter than 10 min, they show the same pharmacokinetic behavior as Gd(DTPA)²⁻, the extravascular/extracellular parent molecule.

CONCLUSIONS

We have obtained two main chain DTPA polymers by copolymerization of DTPA with two diamines of different structures and internal flexibilities. Despite the derivatization of two terminal carboxylate groups of each DTPA residue, with a somewhat reduced Gd³⁺ binding ability of the resulting amide moieties, the stability of the polymeric complexes should still be high enough to be used in vivo. This is supported by the observation that the reduced thermodynamic stability of small Gd³⁺-chelates of DTPA-bis amides relative to Gd(DTPA)²⁻ is compensated by their comparable or even favorable conditional stability constants and selectivity factors for Gd³⁺ relative to other biologically relevant cations such as Zn²⁺, leading to nonincreased in vivo dissociation of Gd³⁺ (31, 41, 42).

The Gd³⁺ complexes of the two polymers were obtained with Gd³⁺ yields of about 80 and 70% for Gd(DTPA-CHD) and Gd(DTPA-HMD), respectively, as measured by ICP. Solvent water ¹⁷O NMR shift monitoring of titration experiments of the polymers with solid GdCl₃ gave comparable results. The incomplete loading of the polymers with Gd³⁺, resulting in the presence of ca. 20–30% of uncomplexed, negatively charged DTPA units, while increasing their solubility and SEC-determined apparent molecular weights due to the negative charges present, will also contribute to an increased internal mobility and reduced relaxivities.

The Gd³⁺ micromolecular complexes show somewhat higher relaxivities than the small parent Gd³⁺ chelate, Gd(DTPA)²⁻. The rigidity of the cyclic diamide linking moiety did improve the relaxivities of the complexes in water, but not as much as expected. This may result from the short length of the linking groups, which prevents the hydrophobic interaction between polymeric chains and the formation of a compact polymer, as happens with similar polymers with longer chains in the linking groups (29). A substantial residual flexibility of those polymers may still be present. Alternatively, the local motions located at the linking groups in the polymer chains might be less important than those of the DTPA moieties in the short length of the linking groups, which prevents the hydrophobic interaction between polymeric chains and the formation of a compact polymer, as happens with similar polymers with longer chains in the linking groups (29). A substantial residual flexibility of those polymers may still be present. Alternatively, the local motions located at the linking groups in the polymer chains might be less important than those of the DTPA moieties in

Table 4. Parameters Obtained from the Fitting of the Proton NMRD Data Obtained at 310 K

<table>
<thead>
<tr>
<th></th>
<th>τ_S₀(ps)</th>
<th>τ_V(ps)</th>
<th>τ_R(ps)</th>
<th>τ_M(ps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gd(DTPA-HMD)</td>
<td>120</td>
<td>21</td>
<td>580</td>
<td>1.33</td>
</tr>
<tr>
<td>Gd(DTPA-CHD)</td>
<td>140</td>
<td>32</td>
<td>997</td>
<td>1.45</td>
</tr>
</tbody>
</table>

The following parameters were fixed: the distance between Gd³⁺ and the inner-sphere water proton (r = 0.31 nm), the distance of closest approach (d = 0.36 nm), the relative diffusion constant (D = 3.5 × 10⁻⁹ m² s⁻¹) and the number of inner-sphere water molecules (q = 1). τ_S₀ = electronic relaxation time at low field. τ_V = correlation time modulating the electronic relaxation. τ_R = rotational correlation time. τ_M = residence time of the innersphere water molecule.

4) are comparable to those reported by Toth et al. (27) (from this publication, a τ_M value of 1.03 ms can be calculated at 310 K for the compound with n = 6). The observed constancy of τ_R is in line with the general observation that derivatization has hardly any effect on the water exchange rate of bisamides, which limit the relaxivity values. The main difference between both compounds is the rotational correlation time, which is longer for Gd(DTPA-CHD) than for Gd(DPTA-HMD). The τ_R values obtained by the fitting of the proton NMRD curves are shorter than those expected for such polymers and are indicative of internal motion. Therefore, the unexpectedly low relaxivity of these polymers can be explained by their relatively short τ_R and the long residence time of their innersphere water molecules.

The present results show that introducing short, conformationally rigid DTPA-linking moieties does not prevent the fast local motions in the extended polymeric chain, which dominate τ_R and keeps the relaxivity low. Other studies with this type of DTPA copolymers, containing as linking units long hydrophilic poly(ethylene-glycol) (PEG) (40), or hydrophobic alkyl chains with a varying number (n) of methylenes (29), showed that higher relaxivities were obtained only for long hydrophobic chains with n > 10. For these polymers, hydrophobic interactions between different chains lead to intramolecular aggregates and the formation of more rigid compact, globular structures, with slower local motions and global rotation, leading to higher relaxivity (27, 29). Studies with other polymeric carriers (15, 16) have also shown that higher relaxivities are obtained for more rigid compact, globular structures, for example by increasing the degree of substitution of the carrier.

The results of pharmacokinetic studies of the two polymeric compounds Gd(DTPA-HMD) and Gd(DTPA-CHD) injected in Wistar rats are shown in Figure 8. This shows that both compounds exhibit a fast exponential clearance from the blood. With a blood half-life shorter...
determining the correlation time of the water molecule bound to the Gd$^{3+}$ ion.

The rotational correlation time is longer for Gd(DTPA-CHD) than for Gd(DTPA-HMD), indicating a substantial decrease of the residual flexibility of the first polymer. However, the rotational correlation times obtained for these polymers are shorter than expected, indicating that internal motion is present. The low relaxivity of these polymers can thus be explained by their relatively short $r_{2\alpha}$ and the long residence time of the inner-sphere water molecule.

With a blood half-life shorter than 10 min., both compounds exhibit the same pharmacokinetic behavior as Gd(DTPA)$^{2-}$, the extravascular/extracellular parent molecule. Molecules with molecular weights lower than about 15 kDa pass freely through glomerular filtration in the kidneys. Large size polymers are usually cleared slowly from the blood because of a restricted glomerular filtration. However, the clearance has to be related not only to the molecular weight of the product but also to its spatial conformation. In the present case, the polymers are made of linear and flexible chains and are characterized by a molecular weight not large enough to prevent the glomerular filtration. Thus, although the polymers are stable in vivo for the short time they stay in circulation, the fast excretion properties severely restrict the applicability of this type of Gd$^{3+}$ polymeric complexes as MRA contrast agents.

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LITERATURE CITED


