A glycosylated complex of gadolinium, a new potential contrast agent for magnetic resonance angiography?

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Abstract—A new low-molecular weight dendrimer-like MRI contrast agent (Gd–D1) has been synthesized and characterized in vitro by proton and oxygen-17 relaxometry. Its pharmacokinetic parameters and biodistribution patterns were evaluated on rats. Its in vitro and in vivo properties, that is, the longitudinal relaxivity (defined as the increase of the water proton longitudinal relaxation rate induced by one millimole per liter of Gd–D1) equal to 5.6 s/mM at 20 MHz and 310 K, the elimination half-time equal to 85 min, and its low accumulation in liver and spleen, underline its potential as a blood-pool MRI contrast agent.

Dendrimer-based MRI contrast agents are designed primarily to enhance the blood-pool signal and the sites of abnormal endothelial permeability.1 They are highly branched polymers with molecular masses larger than 20,000 Da, which allow longer imaging windows without multiple injections. An alternative approach has been explored in the present work by grafting acetylglucose units on Gd-DTPA (Gd–D1 complex, Fig. 1) as described by Takahashi.2

The fact that there is no increase of the relaxivity measured at 20 MHz when temperature decreases from 45–4 °C clearly shows that the relaxivity is limited by the water exchange over the whole range of temperatures investigated (Fig. 2). The water residence time in the first coordination sphere of the complex was obtained from the analysis of the temperature dependence of the transverse paramagnetic relaxation rate of oxygen-17 in a solution containing 18.55 mM of Gd–D1. The data are presented as the reduced transverse relaxation rate $\left(\frac{1}{T_2^w}\right) = \frac{1}{T_2^w} \times 55.55/[Gd–D1]$ where the transverse paramagnetic relaxation rate $\left(\frac{1}{T_2^p}\right)$ is equal to the observed transverse relaxation rate minus the diamagnetic contribution) versus the reciprocal of the temperature and were analyzed as previously described (Fig. 3).3,4 During the theoretical adjustment, the following parameters

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Figure 1. Structure of Gd–D1.
\( B = 2.39 \pm 0.08 \cdot A \) of the bound water molecule and the Gd\(^{3+}\) ion, was set to \( R_s \), the distance of closest approach \( (d = 0.36 \text{ nm}) \); \( D \), the relative diffusion constant \( (D = 2.93 \times 10^{-9} \text{ m}^2/\text{s}) \); \( r \), the distance between the Gd\(^{III}\) ion and the proton nuclei of water \( (r = 0.31 \text{ nm}) \); and \( \tau_{\text{M}} \), the water residence time, was set to the value determined by \(^{17}\text{O} \) NMR. Parameters obtained by the theoretical adjustment of the NMRD profile (Table 1) show that the enhanced relaxivity of Gd–D1 results mainly from an increase of \( \tau_r \) related to the larger molecular weight of the complex.

Plasma pharmacokinetics were assessed on male Wistar rats anesthetized with 60 mg Nembutal/kg bw, ip. The rats were tracheotomized, and the left carotid artery was catheterized for blood collection. The Gd–D1 concentration in blood samples \((\text{Gd–D1}_{\text{blood}})\) collected before and at different times after injection was determined by relaxometry \((\text{Gd–D1}_{\text{blood}} = \frac{R_1}{T_1^*} \text{Gd–D1}_{\text{blood}})\), where \( R_1 \) is the

![Figure 2](image-url)

**Figure 2.** Temperature dependence of the proton relaxivity of the Gd–D1 complex at 20 MHz. The curves of Gd–DTPA and Gd–DTPA–BMA have been added for comparison.

were determined: \( \tau_V \), the correlation time modulating the electronic relaxation of Gd\(^{3+}\); \( E_v \), the activation energy related to \( \tau_V \); \( B \), related to the mean-square of the zero field splitting energy \( \Delta (B = 2.4A^2) \); and \( \Delta H^{\text{iso}} \) and \( \Delta S^{\text{iso}} \), respectively, the enthalpy and entropy of activation of the water exchange process. The number of coordinated water molecules was set to one and \( \Delta h \), the hyperfine coupling constant between the oxygen nucleus of the bound water molecule and the Gd\(^{3+}\) ion, was set to \(-3.5 \times 10^6 \text{ rad s}^{-1}\). Water residence times in the first coordination sphere of the complex equal to 889 ± 36 ns at 310 K and 1497 ± 62 ns at 298 K were obtained; for comparison, values of 143 ± 25 ns\(^2\) at 310 K and 331 ± 60 ns\(^2\) and 303 ns\(^2\) at 298 K were found for Gd–DTPA. The larger water residence time of Gd–D1 agrees with the limitation of the proton relaxivity and with the data reported for bisamide derivatives like the bis methyl amide Gd–DTPA–BMA for which \( \tau_M \) values of 967 ± 36 ns at 310 K and 2130 ± 80 ns at 2220 ns at 298 K were reported.

The proton NMRD profile of Gd–D1 (Fig. 4) was acquired at 310 K. As compared to Gd–DTPA, the relaxivity at high field (10–60 MHz) is significantly higher, that is, 5.6 s\(^{-1}\) mM\(^{-1}\) at 20 MHz and 5.7 s\(^{-1}\) mM\(^{-1}\) at 60 MHz. The fitting of the NMRD curve was performed according to the classical inner sphere and outer sphere theories.\(^{5,7–11}\) Some parameters were fixed during the fitting procedure: \( q \), the number of coordinated water molecules \((q = 1)\); \( d \), the distance of closest approach \((d = 0.36 \text{ nm}) \); \( D \), the relative diffusion constant \((D = 2.93 \times 10^{-9} \text{ m}^2/\text{s}) \); \( r \), the distance between the Gd\(^{III}\) ion and the proton nuclei of water \((r = 0.31 \text{ nm}) \); and \( \tau_{\text{M}} \), the water residence time, was set to the value determined by \(^{17}\text{O} \) NMR. Parameters obtained by the theoretical adjustment of the NMRD profile (Table 1) show that the enhanced relaxivity of Gd–D1 results mainly from an increase of \( \tau_r \) related to the larger molecular weight of the complex.

![Figure 3](image-url)

**Figure 3.** Temperature dependence of the reduced transverse paramagnetic relaxation rate of oxygen-17 of Gd–D1 solution \( (B_0 = 7.5 \text{ T}) \). The fitted data of Gd–D1 (plain line) were obtained with the following parameters: \( \Delta H^{\text{iso}} = 30.8 \pm 0.05 \text{ kJ/mol} \), \( \Delta S^{\text{iso}} = -29.9 \pm 0.17 \text{ J/mol K} \), \( B = 2.39 \pm 0.08 \times 10^{20} \text{ s}^{-2} \), \( \tau_{\text{M}} = 22.9 \pm 0.8 \text{ ps} \), \( E_v = 17.5 \pm 0.6 \text{ kJ/mol} \), \( 
\frac{\Delta h}{\text{D}} = -3.5 \times 10^6 \text{ rad s}^{-1} \), and \( q = 1 \). The curves of Gd–DTPA and Gd–DTPA–BMA have been added for comparison.

![Figure 4](image-url)

**Figure 4.** NMRD profile of Gd–D1 complex (at 310 K). The curves of Gd–DTPA and Gd–DTPA–BMA have been added for comparison.

<table>
<thead>
<tr>
<th>Complexes</th>
<th>( \tau_M ) (ns)</th>
<th>( \tau_B ) (ps)</th>
<th>( \tau_S ) (ps)</th>
<th>( \tau_V ) (ps)</th>
<th>( B ) (10^20 s^-2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gd–D1</td>
<td>889 ± 36</td>
<td>161 ± 2</td>
<td>66 ± 1</td>
<td>20 ± 1</td>
<td>1.52</td>
</tr>
<tr>
<td>Gd–DTPA</td>
<td>143 ± 25</td>
<td>54 ± 14</td>
<td>87 ± 3</td>
<td>25 ± 3</td>
<td>0.92</td>
</tr>
<tr>
<td>130</td>
<td>44</td>
<td>24</td>
<td>1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gd–DTPA–BMA</td>
<td>967 ± 36</td>
<td>65 ± 2</td>
<td>95 ± 3</td>
<td>18 ± 3</td>
<td>1.17</td>
</tr>
<tr>
<td>1014</td>
<td>47</td>
<td>24</td>
<td>0.98</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\) From Ref. 5.

\(^{b}\) Fixed to the value obtained by O-17 relaxometry.

\(^{c}\) Calculated from data of Ref. 6.
Biodistribution of Gd–D1 in Wistar rats 2 h after single iv injection of 0.1 mmol Gd/kg bw. The organs were weighted, dried overnight at 60 °C, and subsequently digested in acidic conditions by microwaves. The gadolinium content was determined by inductively coupled plasma-atmospheric emission spectroscopy. The biodistribution data (Fig. 6) show significantly higher concentrations of Gd–D1 chelate as compared to Gd–DTPA in different organs, particularly in kidneys (24% of ID/g), liver (0.36% of ID/g), heart (0.64% of ID/g), and lungs (1.7% of ID/g). The in vivo transmetallation or hydrolysis of Gd–D1 could contribute to the release of Gd ions and their concentration in tissues known to sequester free Gd (e.g., liver and bones), but this process is expected to occur only at extreme pH values. The interaction with glucose transporters13 or with asialoglycoprotein receptors14 is not possible because the acetylated glucose units in Gd–D1 cannot be recognized anymore by such cell membrane receptors. We presume that the relatively high Gd concentration found in various tissues may be related to blood contamination as a result of the prolonged T1/2. The high Gd concentration found in kidneys 2 h after administration seems to be related to the delayed blood clearance as compared to Gd–DTPA. On the other hand, this result could suggest that the Gd–D1 chelate has a renal elimination. Of course, such a route of excretion can only be confirmed by urine measurement of the Gd–D1 concentration, but molecules with this molecular size and no functional groups that allow their retention in kidney are known to be freely excreted through the fenestrated capillaries of the kidney.19

Large macromolecular contrast agents are useful for magnetic resonance angiography (MRA), but their de-

### Table 2. The pharmacokinetic parameters of Gd–DTPA and of Gd–D1 determined in Wistar rats

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Gd–DTPA</th>
<th>Gd–D1</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1/2 (min)</td>
<td>0.70 ± 0.039</td>
<td>2.27 ± 0.65**</td>
</tr>
<tr>
<td>T1/2 (min)</td>
<td>14.94 ± 1.25</td>
<td>85.04 ± 12.6*</td>
</tr>
<tr>
<td>Cltot (mL/kg/min)</td>
<td>8.66 ± 1.18</td>
<td>7.13 ± 0.74</td>
</tr>
<tr>
<td>VDss (L/kg)</td>
<td>0.165 ± 0.019</td>
<td>0.186 ± 0.007</td>
</tr>
</tbody>
</table>

*p < 0.01, **p < 0.05 versus Gd–DTPA.

Figure 5. Plasma pharmacokinetic profile of Gd–D1 versus Gd–DTPA in rats. The data are represented as percentages of C0. The solid line represents the fit of data to a biexponential profile.

Figure 6. The biodistribution of Gd–D1 in Wistar rats 2 h after single iv administration of 0.1 mmol Gd/kg. The results are represented as averages ± SEM; the Student t test was calculated for Gd–D1 versus Gd–DTPA. *p < 0.01, **p < 0.05.
the biological one, that is, convenient $T_{1/2} = 8.5$ min and significantly lower accumulation in liver and spleen as compared to other dendrimer compounds.

Acknowledgments

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References and notes

2. Takahashi, M.; Hara, Y.; Aoshima, K.; Kurihara, H.; Oshikawa, T.; Yamashita, M. Tetrahedron Lett. 2000, 41, 8485. (A solution of d-(+)-Glucono-1,5-lactone (7.05 g) in 45 mL of DMF was added to diethylenetriamine (2.04 g). The mixture was stirred for 5 h at room temperature and then was left in the refrigerator overnight. (Boc)2O (2.01 g) was added to the solution (4.23 g) at 0 °C and stirred overnight at room temperature. The mixture was added to Et3N (10.0 g) and Ac2O (9.40 g) at 0 °C, and then stirred for 2 days at room temperature. After the completion of the reaction, work-up gave a brown crystal. Trifluoroacetic acid (6.40 g) was added to the solution of the crystal (8.42 g) in CH2Cl2 (5 mL) and the mixture was allowed to react for 2 h at room temperature. After the completion of the reaction, work-up and the product isolation by recycle GPC gave diethylenetriamine bisgluconate (yield 74%, 6.0 g). DTPA anhydride (0.441 g) was added to the solution of the gluconate (2.17 g) in 20 mL of CH3CN and the mixture was refluxed for 2 h. After the completion of the reaction, the evaporation of the solvent gave DTPA derivative of bis(diethylenetriamine bisgluconate) (D1).

D1 was dissolved in 3.5 mL Gd2O3(0.0123 g) solution in H2O/MeOH (2/1). The mixture was refluxed for 45 min. Work-up and removal of free Gd(III) ion gave Gd–D1 (0.15 g) in 98% yield. Uncomplexed Gd3+ ions were removed by treatment with Chelex. The absence of free Gd ions was checked by use of xylene orange indicator.


16. The evaluation of the long time index $\Delta$ is equal to 0.88 as compared to 0.49 and 0.10 for Gd–DTPA and Gd–DTPA–BMA, respectively.


