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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^{-1} mM^{-1} 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. ©2000 Elsevier Science Ltd. All rights reserved.

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

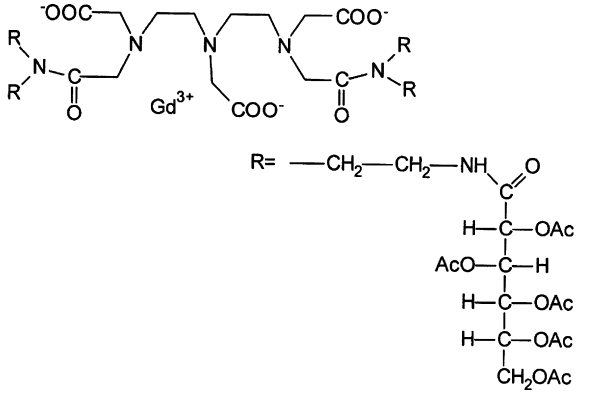

Figure 1. Structure of Gd–D1.

18.55 mM of Gd–D1. The data are presented as the reduced transverse relaxation $(\{T_2^R\}^{-1} = \{T_2^P\}^{-1} \times 55.55/[\text{Gd–D1}]$ where the transverse paramagnetic relaxation rate $-(T_2^P)^{-1}-$ 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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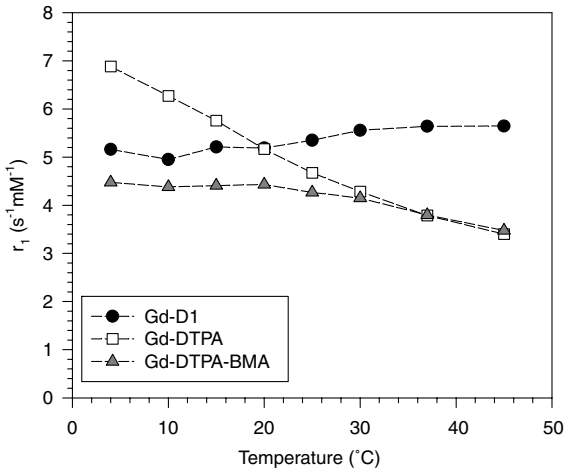

Figure 2. Temperature dependence of the proton relaxivity of the Gd–D1 complex at 20 MHz. The curves of Gd–DTPA and Gd–DTPA–BMA have been added for comparison.

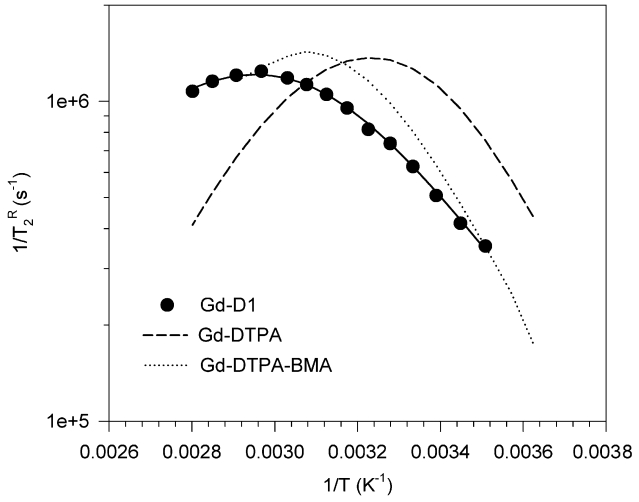

Figure 3. Temperature dependence of the reduced transverse paramagnetic relaxation rate of oxygen-17 of Gd–D1 solution (Bo = 7.5 T). The fitted data of Gd–D1 (plain line) were obtained with the following parameters: $\Delta H^{\#} = 30.8 \pm 0.05$ kJ/mol, $\Delta S^{\#} = -29.9 \pm 0.17$ J/mol K, $B = 2.39 \pm 0.08 \times 10^{20}$ s^{-2}, $\tau_V^{298} = 22.9 \pm 0.8$ ps, $E_v = 17.5 \pm 0.6$ kJ/mol, $A/\hbar = -3.5 \times 10^6$ rad s^{-1}, and $q = 1$. The curves of Gd–DTPA and Gd–DTPA–BMA have been added for comparison.

were determined: τ_V, the correlation time modulating the electronic relaxation of Gd^{3+}; E_v, the activation energy related to τ_V; B, related to the mean-square of the zero field splitting energy Δ ($B = 2.4\Delta^2$); and $\Delta H^{\#}$ and $\Delta S^{\#}$, respectively, the enthalpy and entropy of activation of the water exchange process. The number of coordinated water molecules was set to one and $A/\hbar$, the hyperfine coupling constant between the oxygen nucleus of the bound water molecule and the Gd^{3+} ion, was set to -3.5×10^6 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[5] at 310 K and 331 ± 60 ns[5] and 303 ns[6] 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 τ_M values of 967 ± 36 ns[5] at 310 K and 2130 ± 80 ns[5] and 2220 ns[6] 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 innersphere and outersphere 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 ($d = 0.36$ nm); D, the relative diffusion constant ($D = 2.93 \times 10^{-9}$ m^2/s);[12] r, the distance between the Gd(III) ion and the proton nuclei of water ($r = 0.31$ nm); and τ_M^{310}, the water residence time, was set to the value determined by ^{17}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 τ_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 ($[Gd–D1]_{blood}$) collected before and at different times after injection was determined by relaxometry ($[Gd–D1]_{blood} = R_1^P / r_1^{Gd–D1–blood}$, where R_1^P is the

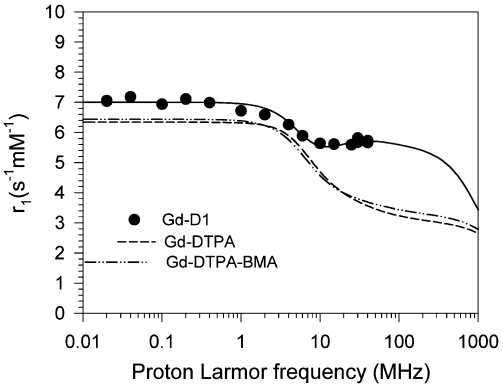

Figure 4. NMRD profile of Gd–D1 complex (at 310 K). The curves of Gd–DTPA and Gd–DTPA–BMA have been added for comparison.

Table 1. Parameters obtained from the theoretical adjustment of the proton NMRD profile

Complexes	τ_M (ns)	τ_R(ps)	τ_{SO} (ps)	τ_V (ps)	B (10^{20} s^{-2})
Gd–D1	889 ± 36[b]	161 ± 2	66 ± 1	20 ± 1	1.52
Gd–DTPA	143 ± 25[a]	54 ± 1.4[a]	87 ± 3[a]	25 ± 3[a]	0.92[a]
	130[c]	44[c]		24[c]	1.1[c]
Gd–DTPA –BMA	967 ± 36[a]	65 ± 2[a]	95 ± 3[a]	18 ± 3[a]	1.17[a]
	1014[c]	47[c]		24[c]	0.98[c]

[a] From Ref. 5.
[b] Fixed to the value obtained by O-17 relaxometry.
[c] Calculated from data of Ref. 6.

longitudinal paramagnetic relaxation rate equal to the observed longitudinal relaxation rate minus the diamagnetic contribution of blood and $r_1^{\text{Gd–D1–blood}}$ is the relaxivity of 1 mmol/L of Gd–D1 measured in blood) at 37 °C and 60 MHz, and was converted to plasma concentration by assuming a hematocrit value of 0.53 (blood volume: 58 mL/kg, plasma volume: 31 mL/kg).[13] The Gd–D1 stability in blood and in blood plasma over time was confirmed by measuring r_1 at various time points during 48 h (Δr_1 in blood or blood plasma/r_1 in water = 1.2 ± 0.02). The absence of any interaction with blood plasma proteins was confirmed by measuring the r_1 of Gd–D1 (60 MHz, 37 °C) in 4% human serum albumin, HSA ($r_1^{\text{Gd–D1–HSA}}/r_1^{\text{Gd–D1–H2O}} = 1.05 \pm 0.01$) and in rat serum albumin, RSA ($r_1^{\text{Gd–D1–RSA}}/r_1^{\text{Gd–D1–H2O}} = 1.06 \pm 0.002$). A two-compartment distribution model was used to calculate the pharmacokinetic parameters such as the distribution and elimination half-lives ($T_{d1/2}, T_{e1/2}$), the steady state volume of distribution (VD_{ss}), and the total clearance (Cl_{tot}). The pharmacokinetic parameters calculated from the percentages of the initial blood concentration C_0 after a single bolus injection through the femoral vein at a dose of 0.1 mmol/Kg bw (Fig. 5) reveal a prolonged blood residence of Gd–D1 as compared to Gd–DTPA (Table 2). The VD_{ss} value (0.186 L/kg) reflects a distribution in the interstitial space comparable to that of Gd–DTPA (0.165 L/kg) (Table 2), although its $T_{d1/2}$ is much longer (2.27 min for Gd–D1 and 0.7 min for Gd–DTPA). The prolonged blood residence could possibly be explained by the relatively large molecular weight (2270.64 g); for comparison the dendrimers based on 1,4-diaminobutane core polypropyleneimine (PPI) generation 2 have a molecular weight of 7000 g and an excretion half-life of 3 h.[1] The transmetallation with biological ligands could contribute to the longer elimination half-life as known for other bisamide compounds,[14] but the evaluation of its transmetallation by zinc (II) ions indicated a higher stability than those of Gd–DTPA and Gd–DTPA–BMA.[15,16] According to our previous observations on bisamide compounds,[15] less extensive transmetallation occurs when the substituting groups are bulkier. As shown above, the interaction with blood plasma proteins is excluded as a possible mechanism of $T_{e1/2}$ prolongation.[17]

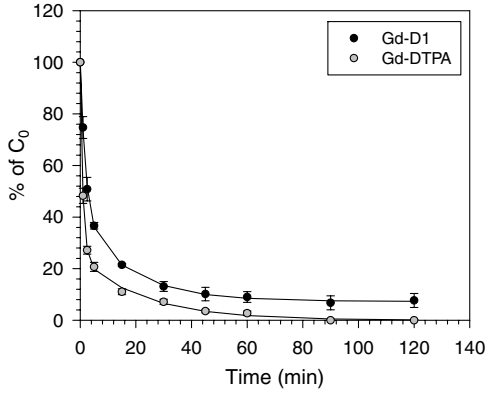

Figure 5. Plasma pharmacokinetic profile of Gd–D1 versus Gd–DTPA in rats. The data are represented as percentages of C_0. The solid line represents the fit of data to a biexponential profile.

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

Pharmacokinetic parameters	Gd–DTPA	Gd–D1
$T_{d1/2}$ (min)	0.70 ± 0.039	2.27 ± 0.65**
$T_{e1/2}$ (min)	14.94 ± 1.25	85.04 ± 12.6*
Cl_{tot} (mL/kg/min)	8.66 ± 1.18	7.13 ± 0.74
VD_{ss} (L/kg)	0.165 ± 0.019	0.186 ± 0.007

*$p < 0.01$, **$p < 0.05$ versus Gd–DTPA.

The biodistribution was determined in rats, 2 h after a 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-atomic 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 transporters[13] or with asialoglycoprotein receptors[18] 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 $T_{e1/2}$. 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-

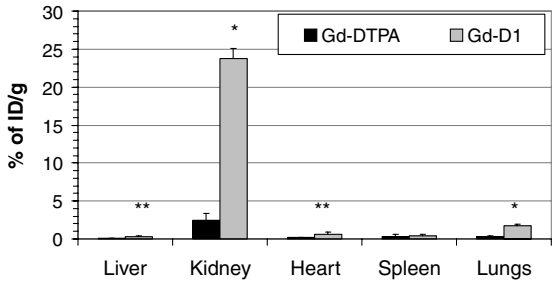

Figure 6. The biodistribution of Gd–D1 in Wistar rats 2 h after single iv administration of 0.1 mmol Gd/kg. The results are represented as averages ± SEM; the Student t test was calculated for Gd–D1 versus Gd–DTPA: *$p < 0.01$, **$p < 0.05$.

layed excretion and increased retention in liver, spleen, and kidneys represent the major limitation for their clinical use. The present small-molecular dendrimer-like compound has advantages as a blood-pool contrast agent not only from the relaxometric point of view (its relaxivity is 68% larger than that of Gd–DTPA at 60 MHz and 310 K), but also from the biological one, that is, convenient $T_{e1/2}$ = 85 min and significantly lower accumulation in liver and spleen as compared to other dendrimer compounds.

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References and notes

1. Wang, S. J.; Brechbiel, M.; Wiener, E. C. *Invest. Radiol.* **2003**, *38*(10), 662.
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 Et_3N (10.0 g) and Ac_2O (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 CH_2Cl_2 (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 CH_3CN 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 Gd_2O_3(0.0123 g) solution in H_2O/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 95% yield). Uncomplexed Gd^{3+} ions were removed by treatment with Chelex. The absence of free Gd ions was checked by use of xylenol orange indicator.[20]
3. Vander Elst, L.; Maton, F.; Laurent, S.; Seghi, F.; Chapelle, F.; Muller, R. N. *Magn. Reson. Med.* **1997**, *38*, 604.
4. Botteman, F.; Nicolle, G.; Vander Elst, L.; Laurent, S.; Merbach, A. E.; Muller, R. N. *Eur. J. Inorg. Chem.* **2002**, 2686.
5. Laurent, S.; Vander Elst, L.; Muller, R. N. Contrast Med. *Mol. Imaging* **2006**, *1*, 128.
6. Powell, D. H.; Ni Dhubhghaill, O. M.; Pubanz, D.; Helm, L.; Lebedev, Y. S.; Schlaepfer, W.; Merbach, A. E. *J. Am. Chem. Soc.* **1996**, *118*, 9333.
7. Muller, R.N.; Declercq, D.; Vallet, P; Giberto, F.; Daminet, B.; Fischer, H.W.; Maton, F.; Van Haverbeke, Y. Proc. ESMRMB, 7th Annual Congress, Strasbourg, 1990; p. 394.
8. Vallet, P. Ph. D. Thesis, University of Mons-Hainaut in Belgium, 1992.
9. Solomon, I. *Phys. Rev.* **1955**, *99*, 559.
10. Bloembergen, N. J. *Chem. Phys.* **1957**, *27*, 572.
11. Freed, J. H. *J. Chem. Phys.* **1978**, *68*, 4034.
12. Vander Elst, L.; Sessoye, A.; Laurent, S.; Muller, R. N. *Helv. Chim. Acta* **2005**, *88*, 574.
13. Burtea, C.; Laurent, S.; Colet, J-M.; Vander Elst, L.; Muller, RN. *Invest. Radiol.* **2003**, *38*, 320.
14. Cabella, C.; Geninatti Crich, S.; Corpillo, D.; Barge, A.; Ghirelli, C.; Bruno, E.; Lorusso, V.; Uggen, F.; Aime, S. *Contrast Med. Mol. Imaging* **2006**, *1*, 23.
15. Laurent, S.; Vander Elst, L.; Copoix, F.; Muller, R. N. *Invest. Radiol.* **2001**, *36*, 115.
16. The evaluation of the long time index[5] is equal to 0.88 as compared to 0.49 and 0.10 for Gd–DTPA and Gd–DTPA–BMA, respectively.
17. McMurry, T. J.; Parmelee, D. J.; Sajiki, H.; Scott, D. M.; Outellet, H. S.; Walovitch, RC.; Tyeklàr, Z.; Dumas, S.; Bernard, P.; Nadler, S.; Midelfort, K.; Greenfield, M.; Troughton, J.; Lauffer, R. B. *J. Med. Chem.* **2002**, *45*, 3465.
18. Prata, M. I. M.; Santos, A. C.; Torres, S.; André, J. P.; Martins, J. A.; Neves, M.; Garcìa-Martín, M. L.; Rodrigues, T. B.; López-Larrubia, P.; Cerdán, S.; Geraldes, C. F. G. C. *Contrast Med. Mol. Imaging* **2006**, *1*, 246.
19. Okuhata, Y. *Adv. Drug Deliv. Rev.* **1999**, *37*, 121.
20. Barge, A.; Cravotto, G.; Gianolio, E.; Fedeli, F. *Contrast Med. Mol. Imaging* **2006**, *1*, 184.