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Synthesis, in vitro and in vivo studies of Gd-DTPA-XDA-D1-Glc(OH) complex as a new potential MRI contrast agent

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ABSTRACT

A new type of dendritic molecules Gd-DTPA-XDA-D1-Glc(OH), which work as a functionalized ligand coordinating gadolinium(III) ion at the center of their frameworks with two glucose moieties on the molecular surfaces, were readily synthesized with high yield. The structures were established by IR, ^{1}H, ^{13}C NMR, and mass spectral studies. Its bio-distribution patterns were evaluated on rats.

Over the past two decades Magnetic Resonance Image (MRI) has become a very powerful tool of diagnostic medicine.[1,2] Paramagnetic materials have been investigated as MRI contrast agents (CAs). These materials enhance the contrast of the image, indirectly by remarkably shortening the magnetic relaxation time of water protons coordinated, by comparison with protons of the surrounding tissues.[3] The most frequently used CAs are stable gadolinium(III) complexes with hydrophilic poly(aminocarboxylate) ligands resulting in rapid extracellular distribution and renal elimination. Gd(III) is preferred because of its favorable magnetic properties. Gd-complexes with amphiphilic properties have previously been prepared and evaluated as blood-pool and liver imaging agents. Long chain amides and esters of Gd-DTPA (diethylenetriaminepentaacetic acid) complex are the most common.[4]

The MRI diagnosis is an excellent method to draw molecular and living body imagings, especially the strong point of the method is to be able to draw clearest 3D images among the available diagnosis. The most commonly clinically used MRI contrast agent is Gd-DTPA (Magnevist), which is one of the safest drugs. However, because of the blood vessel penetrating character of Gd-DTPA, magnetic resonance angiography by the CAs is often accompanied with narrow window, and then sometimes double or triple dose is required. To improve the week point of Gd-DTPA, we are developing chemical modification of Gd-DTPA by combining some functional residues at the outer face of the molecule.[5]

In the continuation of our work on MRI contrast agents[6] we designed a molecule (Fig. 1) to have more solubility in water. The glycoside groups have a specific target and combine with asialoglycoprotein receptor (ASGPR) on the surface of hepatocyte. Also, the glycoside groups, which were introduced into DTPA, can

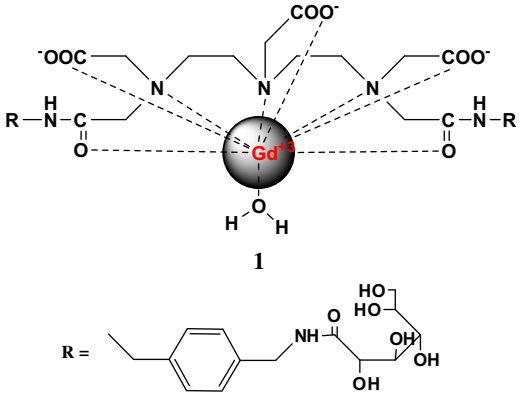

Figure 1. The structure of Gd-DTPA-XDA-D1-Glc(OH) (**1**).

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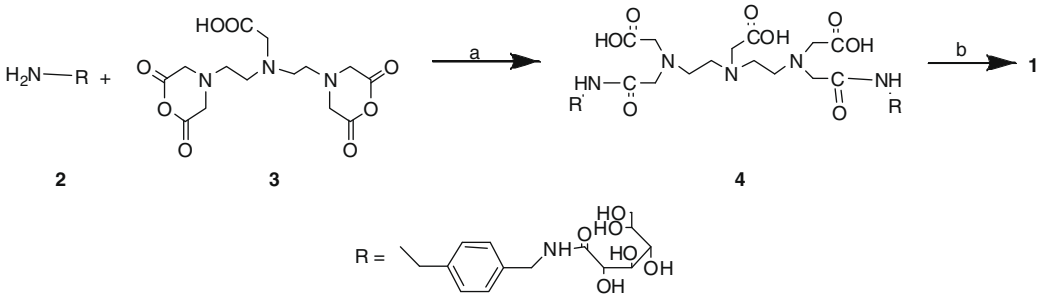

Scheme 1. Reagents and conditions: (a) DMF, 60 °C, 24 h; (b) $GdCl_3$, Pyridine, H_2O, 60 °C, 24 h.

improve the water-solubility of contrast agent. So in this work, DTPA was used as a core of the ligand and glycoside was used as a biofunctional group to prepare a series of dendritic Gd-complexes for novel MRI contrast agents.

The coupling of amino glycoside branch **4**[7] and DTPA anhydride (**5**)[8] results the ligand **6**[9] which on further reaction with $GdCl_3{\cdot}6H_2O$ forms compound **1**[10] (Scheme 1).

These ligands are composed of DTPA[8] and glucose units, which may immobilize gadolinium ion at the focal points by eight coordination sites, allowing one water molecule to chelate and encapsulates the metal ions inside the glycoside clusters. Along with the 'glycoside cluster effect'[11] the carbohydrate aggregation may offer a potential advantage for site-specific delivery of the contrast agents at a molecular level since carbohydrates play significant roles in recognition processes on cell surface.[11–13] Like previous works on Gd(III)-DTPA complexs,[14,15] our new Gd-complex has showed good solubility in aqueous media, although the acetylated glycosides might reinforce their own hydrophobic features.

We developed the synthesis of new dendritic molecules and their utilization as functionalized ligands. Along with our synthetic strategy, a multi gram synthesis responsible for practical use as radiopharmaceuticals can be administered. The chelates with higher-molecular weight compounds are indispensable for prevention of their diffusion from the intravascular space during MRI examinations.[16] Accordingly; these gadolinium(III) chelates may fulfill many criteria and for superior contrast agents after creation of structural modifications. Following intensive investigations on a wide variety of carbohydrate-modified ligands, the feasibility of their metal complexes as new potentional candidates for MRI contrast media are now in progress.

In vitro evaluation. r_1 relaxivity that divided T_1 relaxation time by gadolinium concentration is used as a guide to contrast intensification of MRI contrast agent because the relaxation time depends on the gadolinium concentration of MRI contrast agent. Because gadolinium ion that did not form complexes have influence on measurements of relaxation time, they were adjusted to pH 7.0 in water, added Chelex®100 Resin, stirred for 6 h, and removed free gadolinium ion. The removal of free gadolinium ion was confirmed with color test by Xylenol Orange. Gadolinium concentration was measured with ICP-AES because relaxation time depended on gadolinium concentration of contrast agents. T_1 was measured by TD-NMR of 0.47 T at 37 °C. T_1 was measured not only in water but also in serum albumin which was most existing protein in blood. r_1 was calculated by the following expression, the results of the r_1 value and the standard deviation calculated are shown in Table 1.

Table 1
Comparison of r_1 relaxivity

Gd complexes	r_1 [s^{-1} mM^{-1}]		Standard deviation (mean ± S.D.)	
	In H_2O	In albumin	In H_2O	In albumin
Gd-DTPA	3.5	3.5	—	—
1	10.5	19.75	0.0612	0.0070

$$r_1 = \frac{\frac{1}{T_1} \times 1000 - r_1^{H_2O}}{Gd^{3+}}$$

r_1; relaxivity [s^{-1} mM^{-1}]$_T$, T_1; relaxation time [ms], r_1; relaxivity of H_2O [s^{-1} mM^{-1}], [Gd^{3+}]; gadolinium concentration [mM].

In vivo evaluation. We imaged the MR image of the rats with MRI machine at 3.0 T. Concentration of MRI contrast agent were adjusted by normal saline solution, Gd-DTPA was 0.1 mmol/kg, Gd-DTPA-XDA-D1-2Glc(OH) (**1**) were 0.05 mmol/kg. In Figures 2 and 3, MR image in the left figure is rat before administration, and the right figures are rat after 1 min administration, 5 min after administration, 20 min after administration, respectively.

Bio-distribution of gadolinium in vivo was examined using rats with liver tumor. Gd-DTPA-XDA-D1-Glc(OH) (0.05 mmol/kg,

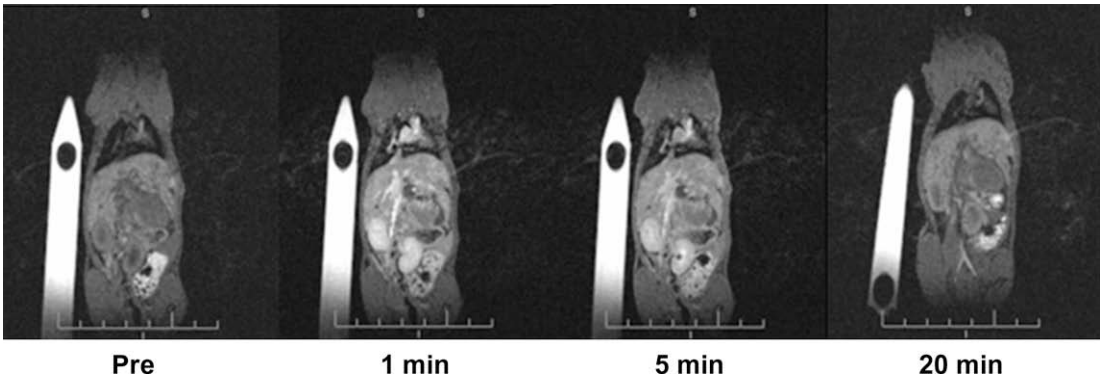

Figure 2. Rat's MRI when Gd-DTPA (0.1 mmol/kg) was administered. The time in min indicates the time after the administration of the contrast agent. The time (Pre, 1 min, 5 min, and 20 min) passes from left to right.

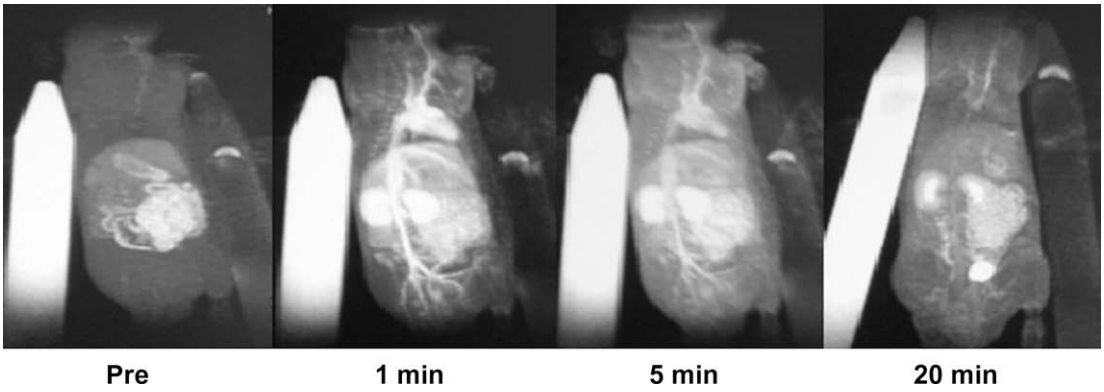

Figure 3. Rat's MRI (at Pre, 1 min, 5 min, and 20 min) when Gd-DTPA-XDA-D1-2Glc(OH) (**1**; 0.05 mmol/kg) was administered.

0.3 mL) was injected intravenously into rats (see Fig. 3). At 1 min, 5 min, and 20 min after injection, the liver, kidney, muscle, blood, pancreas, and spleen were excised. The results are shown in Figure 3, from the figure, we can find Gd-DTPA-XDA-D1-Glc(OH) displays good selectivity to liver, kidney, blood vessel, and spleen.

Figure 3 shows the bio-distribution of the Gd-DTPA complex. The gadolinium concentration of Gd-DTPA in the muscle was the same level as that of the Gd-DTPA-XDA-D1-Glc(OH). Although, the gadolinium concentration of Gd-DTPA in the kidney was higher than that of Gd-DTPA-XDA-D1-Glc(OH), the values of Gd-DTPA in the liver and blood were much lower than those of Gd-DTPA-XDA-D1-Glc(OH) and gadolinium concentration was not observed in the pancreas and spleen.

The high gadolinium concentration in liver and blood indicates the Gd-DTPA-XDA-D1-Glc(OH) has good selectivity to organs. However to compare with the values of gadolinium concentration at 2 h after injection, the values of gadolinium concentration are not changed at 24 h after injection, which indicate the Gd-DTPA-XDA-D1-Glc(OH) cannot be excreted from body timely.

The MR imaging of rats with liver tumors was shown in Figure 3. The liver tumors have been found at 2 h after injection of Gd-DTPA-XDA-D1-Glc(OH). However, the liver tumors cannot be found after injection of Gd-DTPA. The results indicate Gd-DTPA-XDA-D1-Glc(OH) possesses higher tumor-selectivity than Gd-DTPA.

We have succeeded in the synthesis of a new gadolinium complex, Gd-DTPA-D1-Glc(OH) as a MRI CAs. The higher accumulation in the blood vessel and higher tumor-selectivity of Gd-DTPA-D1-Glc(OH) than Gd-DTPA indicates that the Gd-complex has a potential as MRI angiography and early stage findings and medical treatments for tumors.

Acknowledgements

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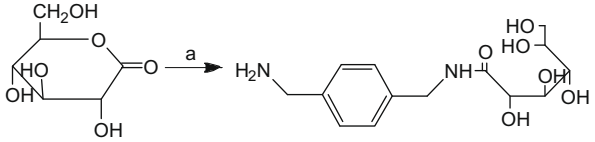
7. To a solution of D-(+)-glucono-1,5-lactone (**A**,1 g, 7.3 mmol) in dry DMF(20 mL) was added *p*-xylenediamine (1.3 g, 7.3 mmol) then stirred for 24 h at rt. After completion of the reaction, the solution was purified, and the solvent was evaporated to dryness under reduced pressure to get **B** as yellow crystals with 90%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.10–8.05, 7.21–7.19, 4.50–4.05, 3.95–3.32, 2.88, 2.73, 2.50. ^{13}C NMR (75 MHz, $CDCl_3$); δ 44.0, 45.2, 64.6, 69.5, 71.8, 72.0, 72.6, 128.4, 135.2, 141.6 ppm. MALDI-TOF MS: *m*/*z* (%) = 316 (100) M^+.



A **B**

Reagents and conditions: (a) *p*-Xylenediamine, rt, 24 h.

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9. The synthesis of dendritic ligand **4** employed a convergent method to couple core and glycoside branch. To the solution of terminal **2** (1.4 g, 4.4 mmol) in DMF (25 mL) was added DTPA dianhydride (**5**, 0.8 g, 2.2 mmol) and stirred for 24 h at 60 °C. After the completion of the reaction and evaporation of the solvent gave a series of ligand with two sugars **6** with 90%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.29–8.02, 7.95–7.02, 4.28–4.06, 3.96–3.3.15, 2.89–2.50. ^{13}C NMR (75 MHz, $CDCl_3$): δ 43.6, 44.0, 52.6, 54.8, 59.6, 59,8, 60.6, 64.6, 69.5, 71.8, 71.9, 72.4, 72.6, 128.4, 135.2, 171.4, 172.6, 173.2. MALDI-TOF MS: *m*/*z* (%) = 985 (100) M^+.
10. To a solution of ligand **4** (1.7 g, 1.7 mmol) in water was added pyridine (1.4 mL, 17.75 mmol) and the reaction mixture was stirred thoroughly for 10 min at rt. To this $GdCl_3{\cdot}6H_2O$ (0.80 g, 1.7 mmol) was added slowly and the reaction was kept at 60 °C and stirred for 24 h. After completion of the reaction water was removed under vacuum and the crude product was dissolved in water and the excess of Gd was removed by using Chelex® resign and checked by use of xylenol orange indicator.[17] After removal of excess Gd the resin was filtered off and after the completion of the reaction, the solvent was removed by rotary-evaporator under reduced pressure then dried to yield **1** with 90%. MALDI-TOF MS: *m*/*z* (%) = 1140 (100) $M^+ - H_2O$.
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