

NMR RELAXIVITY AND IMAGING OF NEUTRAL MACROMOLECULAR POLYESTER GADOLINIUM (III) COMPLEXES*

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Abstract Five neutral macromolecular polyester gadolinium (III) complexes with pendant hydrophobic alkyl and aromatic functional groups were prepared. The longitudinal relaxation rates of these complexes were measured. One of these Gd (III) complexes was chosen for the acute toxicity test and T_1 -weighted imaging measurement. Preliminary results showed that, compared with Gd-DTPA, the neutral macromolecular gadolinium (III) complexes provide higher T_1 relaxivity enhancement and longer function duration.

Keywords Magnetic resonance imaging (MRI), Neutral contrast agent, Macromolecular gadolinium complexes, Relaxivity, Acute toxicity

INTRODUCTION

Magnetic resonance imaging (MRI) has become an important non-invasive technique in the clinical diagnosis of various diseases, especially those associated with physiological changes, damage or lesions in the organs or tissues. Development of paramagnetic media to enhance the imaging quality and to shorten experiment time has been a field of intense activity^[1-4]. Trivalent gadolinium, for its high paramagnetic property and strong relaxation effects arising from its unpaired $4f^7$ electrons, has received much attention in this area of study^[2-6]. Gd-DTPA (diethylenetriamine pentaacetic acid)^[7, 8] and Gd-DOTA (tetraazacyclododecane- N, N', N'', N''' -tetraacetic acid)^[9], are the two contrast agents routinely used in clinical MRI. Since Gd-DTPA and Gd-DOTA are nonspecific extra-cellular contrast agents, they will be excreted or metabolized rapidly from the body, and this will affect their efficiency in the detection of lesions or physiological changes in the organs^[1-4, 10]. The design and development of novel tissue-specific contrast agents which possess higher proton relaxivity and lower toxicity are one of the most challenging aspects of MRI^[2-7]. A number of efforts have been made to achieve liver-specificity by chemically modifying Gd-DTPA with hydrophobic alkyl^[11] or aromatic groups^[5] to form new amphiphilic Gd (III) contrast agents. A comparative study of electrically neutral Gd-DTPA-BMA (bis-methylamine) and di-anionic Gd-DTPA has shown that the neutral complex has lower

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osmolarity as well as better tolerance *in vivo*^[12]. In addition, macromolecular contrast media has been demonstrated to have prolonged intra-vascular retention and higher proton T_1 relaxivity than small molecular compounds^[6, 13]. Recently, we synthesized and characterized a series of macromolecular Gd (III) complexes containing poly-DTPA-ester ligands with various *N*-substituted aromatic or alkyl groups^[14]. In this work, the longitudinal relaxation rate of these macromolecular Gd (III) complexes was measured. One of these polyester-Gd (III) complexes was subjected to the acute toxicity test and T_1 -weighted imaging measurements.

EXPERIMENTAL

Materials

All the polymeric ligands and their Gd (III) complexes were prepared using a method previously described^[14]. The synthetic scheme and chemical structures of the polymeric Gd (III) complexes (**3a~3e**) having an average degree of polymerization (*n*) of 4~5 are shown in Fig. 1.

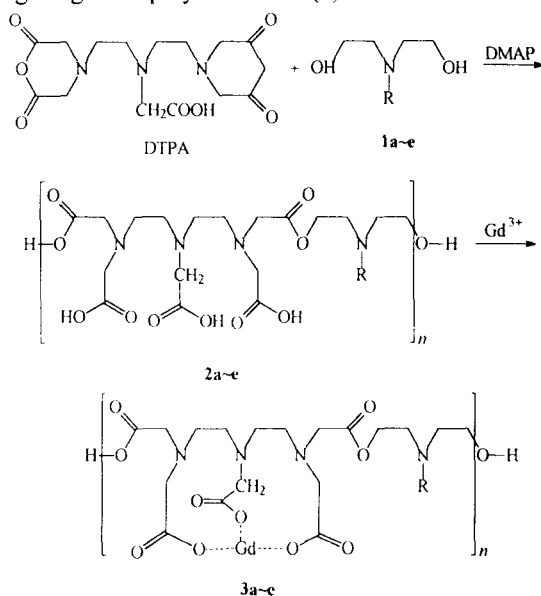


Fig. 1 Molecular structure and synthetic scheme of the complexes **3a~3e**

R: a) $-\text{C}_6\text{H}_5$, b) $-\text{CH}_2\text{C}_6\text{H}_5$, c) $-\text{C}_6\text{H}_4-\text{CH}_3$ (*p*), d) $-\text{C}_8\text{H}_{17}$ (*n*), e) $-\text{C}_4\text{H}_9$ (*n*)

Relaxation Measurement

The longitudinal relaxation times (T_1) of the solvent water protons in the presence of polymeric Gd (III) complexes (**3a~3e**) were measured on a Varian-XL 200 (4.7 T) NMR spectrometer using the standard inverse recovery method at 21 °C. The concentrations of these complexes were in the range of millimole per liter (mmol/L). Twelve relaxation delays were used for each of the measurements with spectral width of 3000 Hz.

Acute Toxicity

Complex **3b** was chosen for the acute toxicity test and for the comparison of MR imaging with Gd-DTPA. The acute toxicity test was done by single intravenous (tail vein) injection of different volumes of a 0.2 mmol/L solution of **3b** at a rate of 2 mL/min into rats (18~22 g, male and female). The animals were under intense care and observation for 7 days after the injection. The results show that complex **3b** has no obvious toxicity under the experimental conditions.

MR Imaging Experiments

MR imagings of the Wistar rats (190 ± 20 g) were performed on a Bruker Biospec 47/30 MRI machine (4.7 T). After the rat was anesthetized with pentobarbital sodium solution i.p, axial images

on 2 mm thick slices were acquired using the spin-echo pulse sequence (Fig. 2) with spin-echo time (TE) of 13 ms, repeat time (TR) of 500 ms and number of averages (NA) of 2. After the rat received the injection of **3b** or Gd-DTPA (dose: 0.11 mmol/kg) intravenously, the imaging on the same slice was continued every 5 min for over 1 h.

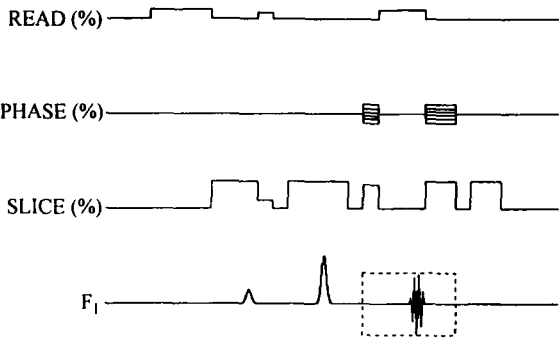


Fig. 2 Spin-echo pulse sequence used for the imaging experiments

RESULTS AND DISCUSSION

Relaxivity

The longitudinal relaxation times (T_1) of the water protons in the aqueous solutions containing these five macromolecular Gd (III) complexes (**3a**~**3e**) were given in Table 1. In the paramagnetic aqueous solution and without the solute-solute interaction, the water proton relaxation rates ($1/T_1$) are linearly dependent on the concentration of the paramagnetic species ($[M]$) as described in Eq. (1)

$$(1/T_1)_{\text{obsd}} = (1/T_1)_d + R_1 [M]$$

(1)

where $(1/T_1)_{\text{obsd}}$ and $(1/T_1)_d$ are water proton relaxation rates in the presence and absence of paramagnetic media, respectively. R_1 is the relaxivity defined as the slope of this dependence with the units of l/mmol/s and reflects the ability of the relaxation enhancement of a paramagnetic contrast agent. The R_1 for each of the five Gd (III) complexes (**3a**~**3e**) in distilled water at 21°C was calculated using Eq. (1) and listed in Table 1. It can be seen from the Table that all of these neutral functional macromolecular Gd (III) complexes provide higher relaxivity than Gd-DTPA. Compared with Gd-DTPA, the relative relaxivity for **3c** is 1.3, and those for the other 4 complexes are over 1.5. **3b** is the most efficient agent as a relaxation medium and thus it is chosen for the imaging experiments. Naturally, the increment in the R_1 is the result of the enlargement of the molecular size of the neutral Gd (III) complex relative to Gd-DTPA.

Table 1 Relaxivity measurement of macromolecular polyester Gd (III) complexes at 21°C ($T_d = 2.64$ s)

Complexes	T_1 (s)	R_1 (mmol/s)	Relative relaxivity
3a	123.9	7.5	1.5
3b	87.6	8.7	1.7
3c	156.2	6.4	1.3
3d	113.2	8.2	1.6
3f	129.4	8.3	1.6
Gd-DTPA*	151.2	5.0	1.0

*Gd-DTPA was used as reference and its relaxivity was set to 1.0.

MR Imaging

In the imaging experiments, the water ¹H signal intensity of rat liver (parenchyma) increased after the intravenous injection of **3b** and Gd-DTPA (dose: 0.1 mmol/kg), respectively. The images before and after injection of **3b** and Gd-DTPA were shown in Fig. 3 and Fig. 4, respectively. The

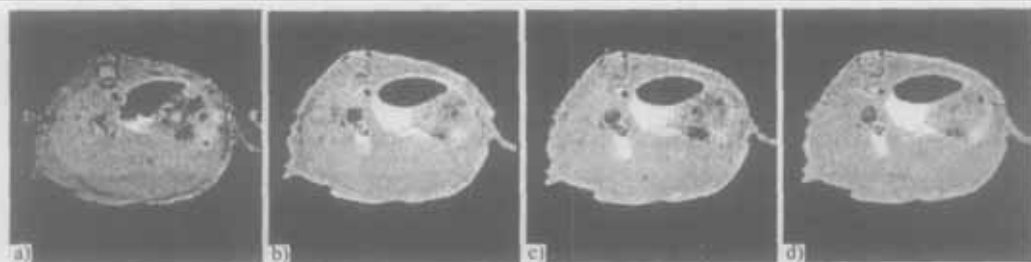


Fig. 3 Comparison of T_1 -weighted portraits of the same axial slice of a Wistar rat (190g) Without contrast agent a) and 5 min b), 25 min c) and 57 min d) after injection of **3b** as contrast agent (dose: 0.11 mmol/kg)



Fig. 4 Comparison of T_1 -weighted portraits of the same axial slice of a Wistar rat (230g) Without contrast agent a) and 5 min b), 26 min c) and 57 min d) after injection of **3b** as contrast agent (dose: 0.11 mmol/kg)

signal intensities taken from these images were used to evaluate the enhancing effects of these two relaxation agents, and the signal intensity before injection of the contrast agents (Fig. 3a and Fig. 4a) was used as reference. It can be seen from the image experiments that the signal intensity increased after the intravenous injection of **3b** (Fig. 3b) and Gd-DTPA (Fig. 4b). The enhancements reached 10% 5 min after each injection, and continuously increased. The highest enhancements were observed at 25 to 40 min: 35~45% for **3b** (Fig. 3c) and 25~40% for Gd-DTPA (Fig. 4c), then decreased afterward. 57 min after the injections, the intensity enhancements were 23% for **3b** (Fig. 3d) and 14% for Gd-DTPA (Fig. 4d), respectively. These results indicate that the complex **3b** produces stronger and longer relaxation enhancement in the rat liver tissue than Gd-DTPA. This is probably due to the bonding of macromolecular complexes to the plasma protein. The benzyl group in **3b** molecule possesses a amphiphilic property, which causes more of the polyester Gd (III) complex to be taken by the liver cell and to undergo hepatobiliary excretion^[3].

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