JPP Journal of Pharmacy and Pharmacology

JPP 2005, 57: 351–357
© 2005 The Authors
Received August 17, 2004
Accepted November 17, 2004
DOI 10.1211/0022357055506
ISSN 0022-3573

Synthesis and evaluation of gadolinium complexes based on PAMAM as MRI contrast agents

Guo-Ping Yan, Bin Hu, Mai-Li Liu and Li-Yun Li

Abstract

Diethylenetriaminepentaacetic acid (DTPA) and pyridoxamine (PM) were incorporated into the amine groups on the surface of ammonia-core poly(amidoamine) dendrimers (PAMAM, Generation 2.0–5.0) to obtain dendritic ligands. These dendritic ligands were reacted with gadolinium chloride to yield the corresponding dendritic gadolinium (Gd) complexes. The dendritic ligands and their gadolinium complexes were characterized by ^{1}HNMR, IR, UV and elemental analysis. Relaxivity studies showed that the dendritic gadolinium complexes possessed higher relaxation effectiveness compared with the clinically used Gd-DTPA. After administration of the dendritic gadolinium complexes (0.09 mmol kg^{-1}) to rats, magnetic resonance imaging of the liver indicated that the dendritic gadolinium complexes containing pyridoxamine groups enhanced the contrast of the MR images of the liver, provided prolonged intravascular duration and produced highly contrasted visualization of blood vessels.

School of Materials Science and Engineering, Wuhan Institute of Technology, Wuhan 430073, P. R. China

Guo-Ping Yan

School of Physical and Chemical Sciences, Queensland University of Technology, GPO Box 2434, Brisbane, Q4001, Australia

Guo-Ping Yan

Planar Solutions (Arch/Wacker JV), 6550 South Mountain Road, Queen Creek, AZ 85242, USA

Bin Hu

State Key Laboratory of Magnetic Resonance and Atomic and Molecular Physics, Wuhan Institute of Physics and Mathematics, the Chinese Academy of Sciences, Wuhan 430071, P R China

Mai-Li Liu, Li-Yun Li

Correspondence: G.-P. Yan, School of Physical and Chemical Sciences, Queensland University of Technology, GPO Box 2434, Brisbane, Q4001, Australia.
E-mail: guopyan@hotmail.com

Funding: We thank the Postdoctoral Research Fellowship Scheme of the Queensland University of Technology and the Chinese National Natural Science Foundation (No. 29874028) for their financial support.

Introduction

As a noninvasive and efficient imaging technique, magnetic resonance imaging (MRI) is widely used in the detection and diagnosis of a variety of diseases. MRI is used in conjunction with MRI contrast agents. These contrast agents can enhance various portions of the MR image by changing the relaxation times of the protons in the immediate vicinity to the agent, and make the area of interest much more conspicuous than the surrounding tissue (Lauterbur 1973; Lauffer 1987).

The extracellular fluid agents, for example the clinically used gadolinium diethylenetriaminepentaacetic acid (Gd-DTPA), do not work very well for imaging of the vascular system and for some organs such as the liver, because the majority of the agent is lost to the extracellular fluid space and is rapidly excreted by the body; however, these agents can work well in the brain and spinal cord. The quest to discover novel organ or tissue targeting and macromolecular MRI contrast agents has led to a number of interesting results (Van Beers et al 1997; Caravan et al 1999). Some liver-targeting contrast agents including gadobenate dimeglumine (Gd-BOPTA), gadolinium ethoxybenzyltriamine pentaacetic acid (Gd-EOB-DTPA) and manganese dipyridoxal-diphosphate (Mn-DPDP) have been studied for use in clinical trials (Rocklage et al 1989; Cavagna et al 1991; Schuhmann-Giampieri et al 1993).

On the surface of mammalian hepatocytes there exist a large number of asialoglycoprotein receptors that can selectively recognize and bind to galactose-terminated glycoproteins, and internalize them by a receptor-mediated endocytosis process (Cynthia et al 1996). Zhuo et al (1997) incorporated the galactose moiety into polylysine-(Gd-DTPA) to give liver-specific MRI contrast agents. Their biodistribution in mice showed that the polylysine (Gd-DTDA)-containing galactose moiety could be specially taken up by the liver.

Mn-DPDP is a liver-targeting contrast agent that is taken up specifically by hepatocytes through the pyridoxine transporter at the sinusoidal pole. The ligand consists of two linked pyridoxyl-5′-phosphate groups, the catalytically active form of vitamin B_6 (Rummeny et al 1991). Previously, we incorporated the amine form of vitamin B_6, pyridoxamine (PM), to a series of polyester and polyaspartamide to obtain MRI contrast agents and anticancer conjugates. The experimental data proved that polymers containing pyridoxamine groups

exhibited a liver-targeting property and their uptake by the liver was highly enhanced in mice and rats (Yan et al 2001a, b, 2002a, b).

Macromolecular MRI contrast agents exhibit more effective relaxation compared with the low molecular weight metal complex alone and improve the relaxivity per gadolinium atom because of an increase in rotational correlation time. They show prolonged intravascular retention due to their bulky molecular size, and so they can be used clinically as the blood pool contrast agents (Caravan et al 1999; Yan & Zhuo 2001; Beezer et al 2003; Uzgiris et al 2004). The conjugate of paramagnetic metal chelates with dendrimers is currently being explored as a new type of potential macromolecular MRI contrast agent. This is because dendrimers have some advantages over other polymer carriers including a spherical and highly branched structure, low polydispersity molecule, and large modifiable surface functionality (Balogh et al 1989; Huber & Burchard 1989; Hawker & Frechet 1990; Tomalia et al 1990; Wiener et al 1994, 1996, 1997; Archut & Vögtle 1998; Fischer & Vögtle 1999; Esfand & Tomalia 2001).

The ammonia-core poly(amidoamine) dendrimers (PAMAM) are one kind of dendrimers that have been investigated the most as MRI contrast agents because they are biocompatible, non-immunogenic, water-soluble and possess terminal-modifiable amine functional groups for binding various targeting or guest molecules (Patri et al 2002). Cell-based, acute and subchronic in-vitro and in-vivo toxicity profiles revealed that some kinds of dendrimers displayed low toxicity and so would possibly be good to use as vehicles for drug delivery (Neerman et al 2004).

Dendrimer-based MRI contrast agents have been synthesized, and their pharmacokinetics, whole-body retention, and dynamic MRI evaluated in mice to determine an optimal agent in comparison with Gd-DTPA. The results showed that small dendrimer conjugates were more rapidly excreted from the body than the larger dendrimer conjugates. Thus small dendrimer conjugates might be acceptable for use in further clinical applications (Kobayashi et al 2003). Wiener et al (1994, 1996, 1997) made gadolinium complexes of folate-conjugated PAMAM dendrimers for targeting tumour cells expressing high-affinity folate receptor (hFR). These conjugates showed the increased longitudinal relaxation rate and a 33% increase by contrast enhancement in ovarian tumours compared with a non-specific agent. Other groups have investigated PAMAM dendrimers for drug targeting (Margerum et al 1997; Bryant et al 1999).

In this study, the dendritic gadolinium complexes containing Gd-DTPA and PM groups based on PAMAM (Generation 2.0–5.0) were synthesized, characterized and evaluated as potential MRI contrast agents by relaxivity and magnetic resonance imaging studies (Figure 1).

Materials and Methods

Instruments and reagents

The compounds prepared were characterized using a Spectrum One infrared spectrophotometer, a Lambda Bio40 UV/vis spectrophotometer, a Varian Mercury-Vx300 NMR spectrometer and a Carlo Erba 1106 analyser. The concentration of the paramagnetic species [Gd^{3+}] was

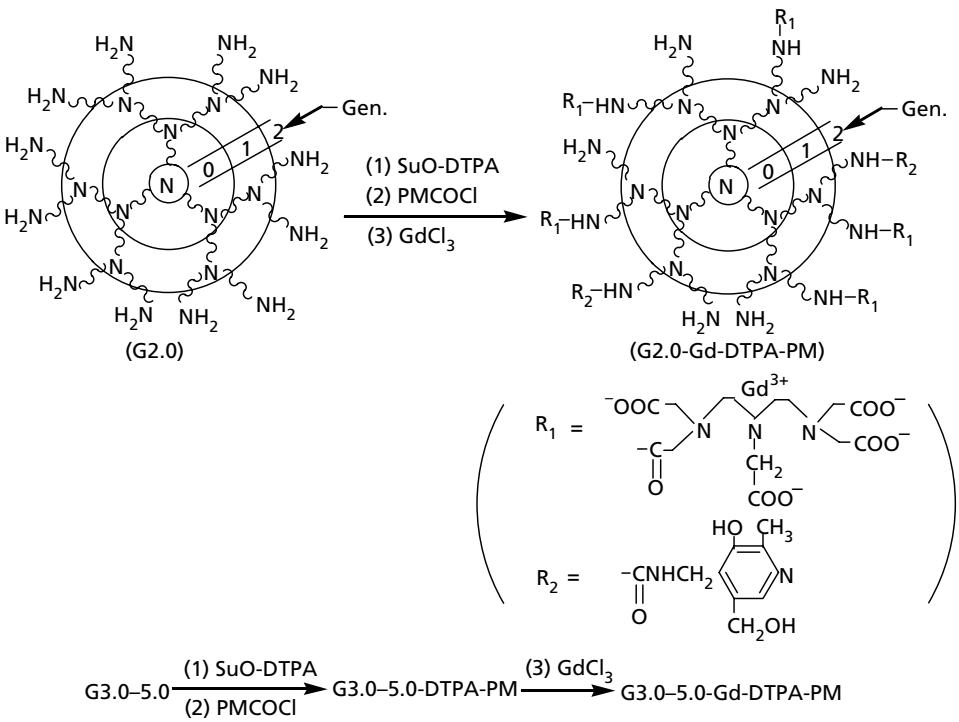

Figure 1 The synthetic route to dendritic MRI contrast agents.

measured by an ICP Atomscan-2000 spectrometer. Longitudinal relaxation time (T_1) for gadolinium complex was determined by a Varian Mercury-Vx300 NMR spectrometer. MR imaging was performed on a 4.7 Tesla BIOSPEC 47/30 MR Scanner (Bruker Medical, Ettlingen, Germany) using Sprague Dawley white rats (150 ± 2 g) as the model.

All chemicals and solvents were of analytical grade. The ammonia-core poly(amidoamine) dendrimers (PAMAM, Generation 2.0–5.0) were obtained from Dendritech Inc. (MI). Chloroformyl-N^4-pyridoxamine (PMCOCl) and DTPA mono(hydroxysuccinimide) ester (SuO-DTPA) were prepared according to Yan et al (2001b).

Synthesis of the dendritic ligands

PAMAM (Generation 2.0, G2.0, 0.52 g, 0.5 mmol) and 5 mL triethylamine were dissolved in 60 mL distilled water. The solution of chloroformyl-N^4-pyridoxamine (PMCOCl, 0.3 g, 1.2 mmol) in DMF (20 mL) was added slowly at -15 to -20°C. The reaction was stirred for 4 h at -5°C and then for 20 h at room temperature. After filtration, the filtrate was collected and cooled to -5°C.

The solution of DTPA mono(hydroxysuccinimidyl) ester (SuO-DTPA, 1.1 g, 2.4 mmol) in DMF (20 mL) was added dropwise to the mixture at -5°C. The reaction was stirred for 4 h at 0°C and then for 48 h at room temperature. The product was filtered and precipitated with ethanol and diethyl ether. The solid was reprecipitated from distilled water using ethanol and diethyl ether, filtered and dried under vacuum to yield a yellow dendritic ligand containing pyridoxamine group G2.0-DTPA-PM (2.3 g, 81%). ^{1}H NMR (D_2O, δ): 7.9–7.7(C_5NH), 5.8($HOCH_2$), 4.2($NHCH_2$), 3.7 ($CH_2C{=}O$), 3.4∼3.2(NCH_2CH_2N), 3.0(CH_3), 2.9–2.8(NCH_2CH_2N), 2.6∼2.3 ($NHCH_2CH_2NH$). IR(KBr, cm^{-1}): 3421(NH), 3013(C-H), 1724, 1639(COO, CONH), 1396, 1228 (C-N), 1089 (C-O); UV(H_2O, nm): 324.

The dendritic ligands with differing dendrimers (Generation: G3.0–5.0) were synthesized by the same method to give the following: G3.0-DTPA-PM (77%), G4.0-DTPA-PM (M_1, 73%; M_2, 75%; M_3, 70%) and G5.0-DTPA-PM (72%).

G3.0-Gd-DTPA-PM: ^{1}H NMR (D_2O, δ): 7.9–7.7(C_5NH), 5.8($HOCH_2$), 4.2($NHCH_2$), 3.7 ($CH_2C{=}O$), 3.4∼3.2(NCH_2CH_2N), 3.0(CH_3), 2.9–2.7(NCH_2CH_2N), 2.6∼2.3 ($NHCH_2CH_2NH$). IR(KBr, cm^{-1}): 3433(NH), 3013(C-H), 1722, 1637(COO, CONH), 1397, 1232(C-N), 1090(C-O); UV(H_2O, nm): 329.

G4.0-Gd-DTPA-PM (M_1): ^{1}H NMR (D_2O, δ): 7.9–7.6 (C_5NH), 5.7($HOCH_2$), 4.1($NHCH_2$), 3.7 ($CH_2C{=}O$), 3.5∼3.2(NCH_2CH_2N), 3.0(CH_3), 2.9–2.8(NCH_2CH_2N), 2.7∼2.3 ($NHCH_2CH_2NH$). IR(KBr, cm^{-1}): 3427 (NH), 3018 (C-H), 1722, 1638(COO, CONH), 1397, 1233(C-N), 1094(C-O); UV(H_2O, nm): 328.

G4.0-Gd-DTPA-PM (M_2): ^{1}H NMR (D_2O, δ): 7.9–7.6 (C_5NH), 5.7($HOCH_2$), 4.1($NHCH_2$), 3.7 ($CH_2C{=}O$), 3.5∼3.2(NCH_2CH_2N), 3.0(CH_3), 2.9–2.8(NCH_2CH_2N), 2.7∼2.3 ($NHCH_2CH_2NH$). IR(KBr, cm^{-1}): 3432(NH), 3074 (C-H), 1724, 1639(COO, CONH), 1397, 1237(C-N), 1127(C-O); UV(H_2O, nm): 331.

G4.0-Gd-DTPA-PM (M_3): ^{1}H NMR (D_2O, δ): 7.9–7.6 (C_5NH), 5.7($HOCH_2$), 4.1($NHCH_2$), 3.7 ($CH_2C{=}O$), 3.5∼3.2(NCH_2CH_2N), 3.0(CH_3), 2.9–2.8(NCH_2CH_2N), 2.7∼2.3 ($NHCH_2CH_2NH$). IR(KBr, cm^{-1}): 3431(NH), 3014(C-H), 1726, 1638(COO, CONH), 1396, 1227(C-N), 1126(C-O); UV(H_2O, nm): 328.

G5.0-Gd-DTPA-PM: ^{1}H NMR (D_2O, δ): 8.0–7.8(C_5NH), 5.7($HOCH_2$), 4.2($NHCH_2$), 3.8 ($CH_2C{=}O$), 3.5∼3.2(NCH_2CH_2N), 3.0(CH_3), 2.9–2.8(NCH_2CH_2N), 2.7∼2.3 ($NHCH_2CH_2NH$). IR(KBr, cm^{-1}): 3425(NH), 3018(C-H), 1722, 1639(COO, CONH), 1397, 1233(C-N), 1177(C-O); UV(H_2O, nm): 328.

Complexation

The dendritic gadolinium complexes with differing dendrimers (Generation: G2.0–5.0) were synthesized according to the following standard procedure. G2.0-DTPA-PM (1.15 g, 0.4 mmol) was dissolved in 20 mL distilled water and gadolinium chloride ($GdCl_3$, 0.53 g, 2 mmol) was added. This solution was stirred for 1 h and the pH was adjusted to pH 5 with 2 M NaOH solution. The solution was then stirred for a further 12 h at room temperature. After dialysis, the dialysed solution was evaporated and the solid residue was dried under vacuum to yield the dendritic gadolinium complex G2.0-Gd-DTPA-PM (1.0 g, 72%). G3.0-Gd-DTPA-PM (73%), G4.0-Gd-DTPA-PM (M_1, 65%; M_2, 70%; M_3, 68%) and G5.0-Gd-DTPA-PM (70%) were similarly synthesized.

G2.0-Gd-DTPA-PM: IR (KBr, cm^{-1}): 3428(NH), 3011(C-H), 1595, 1443, 1408(COO, CONH), 1326 (C-N), 1093(C-O). Anal. (%) found (calc.): Gd 14.1(14.3), C 41.5(42.2) N 16.5(16.8), H 6.25(6.2); UV(H_2O, nm): 327.

G3.0-Gd-DTPA-PM: IR (KBr, cm^{-1}): 3432(NH), 3010(C-H), 1595, 1442, 1408(COO, CONH), 1325(C-N), 1093(C-O). Anal. (%) found (calc.): Gd 14.2(14.4), C 41.7(42.5) N 16.3(16.6), H 6.2(6.1); UV(H_2O, nm): 329.

G4.0-Gd-DTPA-PM (M_1): IR (KBr, cm^{-1}): 3426 (NH), 3014 (C-H), 1595, 1441, 1408 (COO, CONH), 1326(C-N), 1094(C-O). Anal. (%) found (calc.): Gd 11.5 (11.6), C 43.8(44.4), N 17.8(18.0), H 6.7(6.6); UV(H_2O, nm): 328.

G4.0-Gd-DTPA-PM (M_2): IR (KBr, cm^{-1}): 3433(NH), 3076 (C-H), 1595, 1439, 1407 (COO, CONH), 1325(C-N), 1093(C-O). Anal. (%) found (calc.): Gd 9.5(9.6), C 45.3(45.7), N 18.9(19.1), H 7.0(6.9); UV(H_2O, nm): 331.

G4.0-Gd-DTPA-PM (M_3): IR (KBr, cm^{-1}): 3433(NH), 3011(C-H), 1595, 1439, 1407(COO, CONH), 1325(C-N), 1093(C-O). Anal. (%) found (calc.): Gd 10.8(10.9), C 44.4(44.9), N 18.1(18.3), H 6.8(6.7); UV(H_2O, nm): 328.

G5.0-Gd-DTPA-PM: IR (KBr, cm^{-1}): 3400(NH), 3014(C-H), 1595, 1442, 1408(COO, CONH), 1326(C-N), 1094(C-O). Anal. (%) found (calc.): Gd 12.3(12.5), C 43.2(43.8) N 17.2(17.5), H 6.5(6.4); UV(H_2O, nm): 328.

Relaxivity

The solvent longitudinal relaxation time (T_1) for the gadolinium complexes in distilled water (pH = 7) was determined using a Varian Mercury-Vx300 NMR spectrometer.

MR imaging

MR imaging of the liver was carried out on Sprague Dawley rats (150 ± 2 g) using a 4.7 Tesla BIOSPEC 47/30 MR Scanner. The rats were anaesthetized with urethane (10%, 10 mL kg^{-1}), positioned supine and fixed to a polystyrene cradle with adhesive tape to minimize respiratory motion. After performing non-enhanced MR imaging, a solution of Gd-DTPA (0.1 mmol kg^{-1}, Magnevist), G2.0-Gd-DTPA-PM (0.09 mmol kg^{-1}), G4.0-Gd-DTPA-PM (M_1, 0.09 mmol kg^{-1}), G4.0-Gd-DTPA-PM (M_2, 0.09 mmol kg^{-1}) or G4.0-Gd-DTPA-PM (M_3, 0.09 mmol kg^{-1}) in 0.9% sodium chloride was injected via the tail. Coronal images of the liver were obtained with a T_1-weighted spin-echo sequence. (Repetition time (TR) 500 ms, echo time (TE) 15 ms, the field of view was 40 mm, with an image matrix of 128×128. Six slices were taken and slice thickness was 2 mm, with a l-mm interslice gap.)

Statistical analysis

Six T_1-weighted image slices were taken for the liver of each rat. The top and bottom slices were not included in the data analysis to prevent confounding partial volume effects at the edges of the liver. The average relative signal intensities (pre-injection and post-injection at different times) within the region of interest (indicated areas 1 and 2) were normalized to the water label background ($RI_{background}$) at each of the imaging conditions. The percentage of contrast enhancement in the signal from the liver was calculated according to the following equation:

$$\text{Enhancement } (\%) = 100\% \ (RI_{post} - RI_{pre})/(RI_{pre} - RI_{background}) \quad (1)$$

The effect of the various factors on the results derived from image analysis were examined using the Kruskal–Wallis and Wilcoxon signed rank tests, where appropriate. The effect of the concentration and the observed solvent relaxation time (T_{1obsd}) of gadolinium complex on the relaxivity (R_1) was performed using the Kruskal–Wallis test.

Results and Discussion

Synthesis and characterization

Poly(amidoamine) dendrimers (PAMAM, Generation 2.0–5.0) are water-soluble molecules with high numbers of amine groups on their surface and in this instance they have been used as the carriers for MRI contrast agents. Experimental data including ^{1}H NMR, IR, UV and elemental analysis indicated that DTPA and PM were covalently bound to the dendrimers and the subsequent gadolinium complexes formed. ^{1}H NMR spectra showed that the dendritic ligands had the characteristic peaks of PM groups at 8.0–7.6 ppm. The dendritic ligands in aqueous solution after dialysis showed UV absorption at 327–331 nm; however, dendrimers show no characteristic absorption at the above-mentioned wavelengths. IR spectra of the dendritic ligands showed characteristic absorption peaks of carboxyl at 1639∼1635 cm^{-1}, while the IR spectra of their gadolinium complexes had lost those peaks but strong absorption peaks were present at 1596∼1594 cm^{-1}.

Relaxivity of metal complexes

In the absence of solute–solute interactions, the solvent relaxation rates were linearly dependent on the concentration of the paramagnetic species ([M]); relaxivity, R_1, was defined as the slope of this dependence (Lauffer 1987; Nicolle et al 2002):

$$(1/T_1)_{obsd} = (1/T_1)_d + R_1[M] \quad (2)$$

Where $(1/T_1)_{obsd}$ was the observed solvent relaxation rate in the presence of a paramagnetic species, $(1/T_1)_d$ was the solvent relaxation rate in the absence of a paramagnetic species. In this experiment, the concentrations of the paramagnetic species [Gd^{3+}] were measured by an ICP Atomscan-2000 spectrometer. Thus R_1 for gadolinium complexes in distilled water could be calculated (Table 1).

The water exchange rate and rotational correlation times are the critical factors to limit proton relaxivity for macromolecular gadolinium chelates as potential MRI contrast agents. Thus the dendritic gadolinium complexes have higher proton relaxivities than the low molecular weight chelates on the basis of the higher rigidity, faster water exchange rate and slower rotation (Nicolle et al 2002). Table 1 illustrates that the dendritic gadolinium complexes containing pyridoxamine groups exhibited higher relaxation effectiveness compared with Gd-DTPA. Moreover, their relaxivities were enhanced accordingly when the content of Gd^{3+} increased; for example, the relaxivity of G4.0-Gd-DTPA-PM (M_3) was higher compared with G4.0-Gd-DTPA-PM (M_2) and less compared with G4.0-Gd-DTPA-PM (M_1).

MR imaging

The T_1-enhanced positive MR images of the liver were obtained after injection with a low dose of MRI contrast agent. Contrast agents can enhance the image contrast of tissues in which the agent accumulates, and then indicate the status of organ function or blood flow.

Table 1 Relaxivity of gadolinium complex

Gadolinium complex	[Gd^{3+}] (mM)	T_1obsd (s)	R_1 ((mM·s)$^{-1}$)
Gd-DTPA	1.22 ± 0.049	0.105 ± 0.011	7.3 ± 1.3
G2.0-Gd-DTPA-PM	1.32 ± 0.053	0.044 ± 0.002	16.7 ± 1.6
G3.0-Gd-DTPA-PM	1.13 ± 0.045	0.0479 ± 0.003	17.9 ± 2.1
G4.0-Gd-DTPA-PM (M_1)	1.16 ± 0.046	0.0436 ± 0.002	19.2 ± 1.8
G4.0-Gd-DTPA-PM (M_2)	1.06 ± 0.042	0.0697 ± 0.006	12.9 ± 1.9
G4.0-Gd-DTPA-PM (M_3)	1.00 ± 0.04	0.0586 ± 0.004	16.5 ± 2.0
G5.0-Gd-DTPA-PM	1.18 ± 0.047	0.0521 ± 0.004	15.7 ± 1.5

Temperature: 17°C. NMR frequency: 300 MHz. $T_{1d} = 1.60 \pm 0.08$ s.

Compared with the signal intensities (SI) of the liver in the rat injected with Gd-DTPA (0.1 mmol kg^{-1}) (Yan et al 2001a), the signal intensities of the liver in the rat injected with G4.0-Gd-DTPA-PM (M_2) (0.09 mmol kg^{-1}) or G4.0-Gd-DTPA-PM (M_3) (0.09 mmol kg^{-1}) were enhanced, the irradiated portion of the liver was brighter and the demarcation became clearer at the same time intervals during the detection time. In Figures 2 and 3 it can be seen that G4.0-Gd-DTPA-PM greatly enhanced the contrast of the MR images of the liver after its injection, and provided prolonged intravascular duration and exhibited highly resolved visualization of the blood vessels.

Thirty minutes after injection of G4.0-Gd-DTPA-PM (M_2, 0.09 mmol kg^{-1}), the signal enhancement of the liver was 46–49% (Figures 4 and 5). This signal enhancement was better than Gd-DTPA (0.1 mmol kg^{-1}, 19%) but was not as good as G2.0-Gd-DTPA-PM (0.09 mmol kg^{-1}, 58–60%), G4.0-Gd-DTPA-PM (M_3, 0.09 mmol kg^{-1}, 72–74%) or G4.0-Gd-DTPA-PM (M_1, 0.09 mmol kg^{-1}, 95–98%). Thus, G4.0-Gd-DTPA-PM (M_2), with the lower average percentage of Gd-DTPA attached to the amine of PAMAM, could enhance the contrast of the MR images of the liver better than Gd-DTPA. As the signal enhancement of the liver increased, the relaxivity (R_1) of the dendritic gadolinium complex became higher. In addition, the dendritic gadolinium complexes showed a prolonged intravascular duration time of approximately 2 h. The results indicated that the dendritic gadolinium complexes containing pyridoxamine groups could be used as MRI contrast agents for the liver.

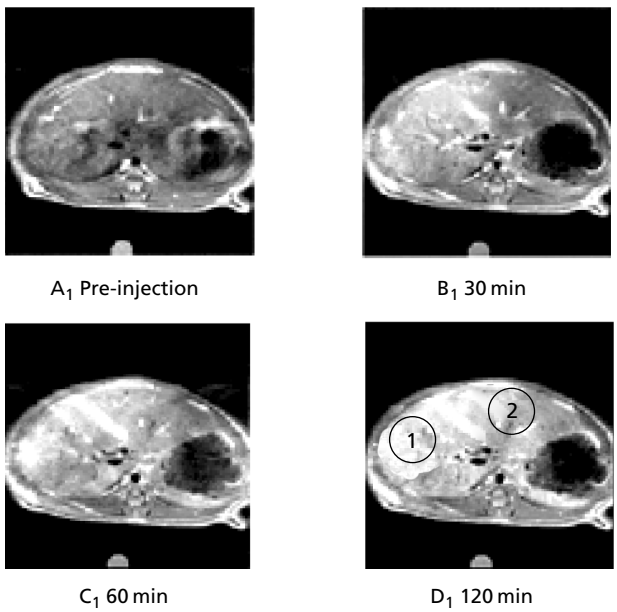

Figure 2 A_1 shows the T_1-weighted image of the liver of a rat not administered any MRI contrast agent; B_1, C_1 and D_1 are the T_1-weighted images of the liver from a rat injected with dendritic G4.0-Gd-DTPA-PM (M_2, 0.09 mmol kg^{-1}) after 30, 60 and 120 min, respectively. Indicated areas 1 and 2 were used to calculate contrast enhancements listed in Figures 4 and 5.

Conclusion

Dendritic gadolinium complexes containing pyridoxamine groups possessed higher relaxation effectiveness compared with Gd-DTPA. MR imaging of rat liver after the animals were administered the lower dose of G4.0-Gd-DTPA-PM or G2.0-Gd-DTPA-PM showed enhanced signal intensity (SI). G4.0-Gd-DTPA-PM and G2.0-Gd-DTPA-PM

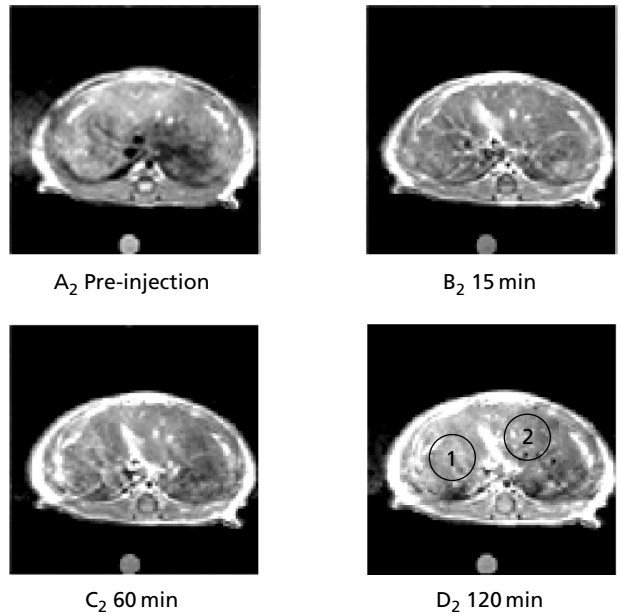

Figure 3 A_2 shows the T_1-weighted image of the liver of a rat not administered any MRI contrast agent; B_2, C_2 and D_2 are the T_1-weighted images of the liver of a rat injected with dendritic G4.0-Gd-DTPA-PM (M_3, 0.09 mmol kg^{-1}) after 15, 60 and 120 min. Indicated areas 1 and 2 were used to calculate contrast enhancements listed in Figures 4 and 5.

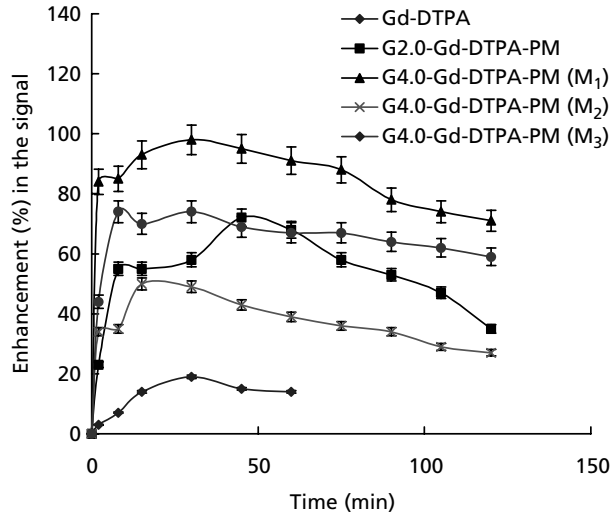

Figure 4 Enhancement (%) in the signal from the liver (indicated area 1) at different times after injection of various MRI contrast agents.

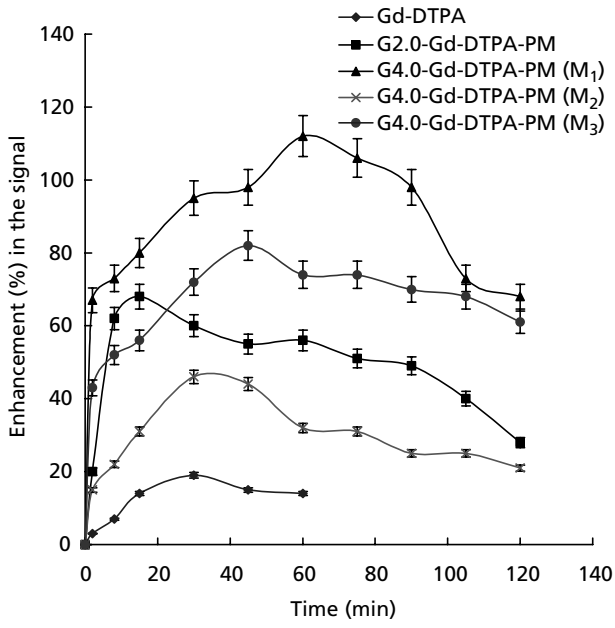

Figure 5 Enhancement (%) in the signal from the liver (indicated area 2) at different times after injection of various MRI contrast agents.

greatly enhanced the contrast of the MR image of the liver and provided prolonged intravascular duration and possessed highly contrasted visualization of the blood vessels. The results indicated that the dendritic gadolinium complexes containing pyridoxamine groups could have potential as MRI contrast agents for the liver.

References

Archut, A., Vögtle, F. (1998) Functional cascade molecules. *Chem. Soc. Rev.* **27**: 233–240

Balogh, L., de Leuze-Jallouli, A., Dvornic, P., Kunugi, Y., Blumstein, A., Tomalia, D. A. (1999) Architectural copolymers of PAMAM dendrimers and ionic polyacetylenes. *Macromolecules* **32**: 1036–1042

Beezer, A. E., King, A. S. H., Martin, I. K., Mitchel, J. C., Twyman, L. J., Wain, C. F. (2003) Dendrimers as potential drug carriers; encapsulation of acidic hydrophobes, within water soluble PAMAM derivatives. *Tetrahedron* **59**: 3873–3880

Bryant, L. H., Brechbiel, M. W., Wu, C. C., Bulte, J. W. M., Herynek, V., Frank, J. A. (1999) Synthesis and relaxometry of high-generation (G = 5, 7, 9, and 10) PAMAM dendrimer-DOTA-gadolinium chelates. *J. Magn. Reson. Imag.* **9**: 348–352

Caravan, P., Ellison, J. J., McMurry, T. J., Lauffer, R. B. (1999) Gadolinium (III) chelates as MRI contrast agents: structure, dynamics, and applications. *Chem. Rev.* **99**: 2293–2352

Cavagna, F., Dapri, M., Maggioni, F., de Hahn, C., Felder, E. (1991) Gd-BOPTA/Dimeg: experimental disease imaging. *Magn. Reson. Med.* **22**: 329–333

Cynthia, R., Rogers, J., Arias, I. M. (1996) Use of an asialoglycoprotein receptor-targeted magnetic resonance contrast agent to study changes in receptor biology during liver regeneration endotoxemia in rats. *Hepatology* **23**: 1631–1638

Esfand, R., Tomalia, D. A. (2001) Poly(amidoamine) (PAMAM) dendrimers: from biomimicry to drug delivery and biomedical applications. *Drug Discovery Today* **6**: 427–436

Fischer, M., Vögtle, F. (1999) Dendrimers: from design to application—a progress report. *Angew. Chem. Int. Ed.* **38**: 884–905

Hawker, C. J., Frechet, J. M. J. (1990) Preparation of polymers with controlled molecular architecture: a new convergent approach to dendritic macromolecules. *J. Am. Chem. Soc.* **112**: 7638–7647

Huber, K., Burchard, W. (1989) Scattering behavior of wormlike star macromolecules. *Macromolecules* **22**: 3332–3336

Kobayshi, H., Kawamoto, S., Jo, S. K., Bryant, H. L., Brechbiel, M. W., Star, R. A. (2003) Macromolecular MRI contrast agents with small dendrimers: pharmacokinetic differences between sizes and cores. *Bioconjugate Chem.* **14**: 388–394

Konda, S. D., Aref, M., Wang, S., Brechbiel, M., Wiener, E. C. (2001) Specific targeting of folate-dendrimer MRI contrast agents to the high affinity folate receptor expressed in ovarian tumor xenografts. *Magn. Reson. Mat. Phys. Biol. Med.* **12**: 104–113

Lauffer, R. B. (1987) Paramagnetic metal complexes as water proton relaxation agents for NMR imaging: theory and design. *Chem. Rev.* **87**: 901–927

Lauterbur, P. C. (1973) Image formation by induced local interactions: examples employing nuclear magnetic resonance. *Nature* **242**: 190–191

Margerum, L. D., Campion, B. K., Koo, M., Shargill, N., Lai, J. J., Marumoto, A., Sontum, P. C. (1997) Gadolinium (III) DO3A macrocycles and polyethylene glycol coupled to dendrimers effect of molecular weight on physical and biological properties of macromolecular magnetic resonance imaging contrast agents. *J. Alloys Compounds* **249**: 185–190

Neerman, M. F., Zhang, W., Parrish, A. R., Simanek, E. E. (2004) In vitro and in vivo evaluation of a melamine dendrimer as a vehicle for drug delivery. *Int. J. Pharmaceut.* **281**: 129–132

Nicolle, G. M., Toth, E., Schmitt-Willich, H., Raduchel, B., Merbach, A. E. (2002) The impact of rigidity and water exchange on the relaxivity of a dendritic MRI contrast agent. *Chem. Eur. J.* **8**: 1040–1048

Patri, A. K., Majoros, I. J., Baker, J. R. (2002) Dendritic polymer macromolecular carriers for drug delivery. *Curr. Opin. Chem. Biol.* **6**: 466–471

Rocklage, S. M., Cacheris, W. P., Quay, S. C., Hahn, F. E., Raymond, K. N. (1989) Magnanese (II) N, N′–dipyridoxyl ethylenediamine-N, N′-diactate-5, 5′-bis(phosphate): synthesis and characterization of a paramagnetic chelate for magnetic resonance imaging enhancement. *Inorg. Chem.* **28**: 477–485

Rummeny, E., Ehrenheim, C., Gehl, H. B., Hamm, B., Laniado, M., Lodemann, K. P., Schmiedel, E., Steudel, A., Vogl, T. G. (1991) Manganese-DPDP as a hepatobiliary contrast agent in the magnetic resonance imaging of liver tumors: results of clinical phase II trials in Germany including 141 patients. *Invest. Radiol.* **26**: S142–145

Schuhmann-Giampieri, G., Schmitt-Willich, H., Press, W. R., Negishi, C., Weinmann, H. J., Speck, U. (1993) Preclinical evaluation of Gd-EOB-DTPA as a contrast agent in MR imaging of the hepatobiliary system. *Radiology* **183**: 59–64

Tomalia, D. A., Naylor, A. M., Goddard, W. A. (1990) Starburst dendrimers: molecular-level control of size, shape, surface chemistry, topology, and flexibility from atoms to macroscopic matter. *Angew. Chem. Int. Ed. Engl.* **29**: 138–175

Uzgiris, E. E., Cline, H., Moasser, B., Grimmond, B., Amaratunga, M., Smith, J. F., Goddard, G. (2004) Conformation and structure of polymeric contrast agents for medical imaging. *Biomacromolecules* **5**: 54–61

Van Beers, B. E., Gallez, B., Pringot, J. (1997) Contrast-enhanced MR imaging of the liver. *Radiology* **203**: 297–306

Wiener, E. C., Brechbiel, M. W., Brothers, H., Magin, R. L., Gansow, O. A., Tomalia, D. A., Lauterbur, P. C. (1994) Dendrimer-based metal chelates: a new class of magnetic resonance imaging contrast agents. *Magn. Reson. Med.* **31**: 1–8

Wiener, E. C., Auteri, F. P., Chen, J. W., Brechbiel, M. W., Gansow, O.A., Schneider, D. S., Belford, R. L., Clarkson, R. B., Lauterbur, P. C. (1996) Molecular dynamics of ion-chelate complexes attached to dendrimers. *J. Am. Chem. Soc.* **118**: 7774–7782

Wiener, E. C., Konda, S., Shadron, A., Brechbiel, M., Gansow, O. (1997) Targeting dendrimer-chelates to tumours and tumour cells expressing the high-affinity folate receptor. *Invest. Radiol.* **32**: 748–754

Yan, G. P., Zhuo, R. X. (2001) Research progress of magnetic resonance imaging contrast agents. *Chin. Scien. Bull.* **46**: 1233–1237

Yan, G. P., Zhuo, R. X., Xu, M. Y., Li, L. Y., Tang, Y. F. (2001a) Liver-targeting macromolecular MRI contrast agents. *Sci. China*. Ser. B, **44**: 344–352

Yan, G. P., Zhuo, R. X., Zheng, C. Y. (2001b) Study on the anticancer drug 5-fluorouracil-conjugated polyaspartamides containing hepatocyte-targeting group. *J. Bioact. Compatible Polym.* **16**: 277–293

Yan, G. P., Zhuo, R. X., Xu, M. Y., Zhang, X., Li, L. Y., Liu, M. L., Ye, C. H. (2002a) Hepatic targeting macromolecular MRI contrast agents. *Polym. Int.* **51**: 892–898

Yan, G. P., Zhuo, R. X., Yang, Y. H., Li, L. Y., Liu, M. L., Ye, C. H. (2002b) Tumor-selective macromolecular MRI contrast agents. *J. Bioact. Compatible Polym.* **17**: 139–151

Zhuo, R. X., Fu, Y. J., Liao, J. (1997) Synthesis, relaxivity and biodistribution of novel magnetic resonance imaging (MRI) contrast agents: polylysine (Gd-DTPA/DOTA) with pendent galactose moieties as hepatocyte-targeting groups. *Chin. Chem. Lett.* **8**: 157–160