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Gadolinium-containing copolymeric chelates—a new potential MR contrast agent

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Abstract

Rationale and objectives: To develop and partially characterize a new class of potential blood pool magnetic resonance (MR) contrast agents. *Methods*: Various copolymeric chelates of gadolinium–diethylenetriamine pentaacetic acid (Gd–DTPA) were prepared with differing molecular weights of polyethylene glycol (PEG) or polypropylene glycol (PPG) as linkers between the monomeric chelate units. Gadolinium content of the polymeric chelates was determined by atomic absorption spectra. Relaxivity of the polymeric chelates was measured at 1.5 Tesla and compared with Gadolinium–DTPA. MR angiography (MRA) was performed in rabbits comparing Gd–DTPA with Gd–copolymers. *Results*: The gadolinium content of the copolymeric chelates ranged from 2.95 to 22.2% on weight basis. The molecular weight of the PEG linkers in the copolymers ranged from about 150 to about 3400. The r_1 (1/T1, $mM^{-1}\ s^{-1}$) for Gd–DTPA = 4.1. The r_1 values for the different Gd-containing polymers ranged from 3.8 to 5.8, with the lowest r_1 for the polymer prepared with the lowest-molecular-weight complex. The higher-molecular-weight complexes resulted in moderately higher relaxivity. MRA with Gd-copolymers, in rabbits, showed markedly greater vascular enhancement relative to an equivalent dose of Gd–DTPA. Vascular enhancement was much more sustained with the copolymeric agent and confined to vascular space; i.e. no appreciable background tissue enhancement—a reflection of distribution into extravascular fluid space—was observed. *Conclusions*: Relative to Gd–DTPA monomers, PEG-containing Gd–DTPA polymeric complexes provided moderate increases in relaxivity but markedly greater efficacy during in vivo MRA. In vitro relaxivity studies of Gd-copolymers showed only an approximately 50% increase in r_1 relaxivity compared with Gd–DTPA. The PEG-containing complex's lack of rigidity may have diminished the effect of spin diffusion on relaxation, thereby accounting for this modest increase. The greater efficacy of Gd-copolymers during in vivo MRA may reflect compartmentalization within the vascular space and possibly enhanced relaxation of the macromolecular copolymers in the blood. Gd-copolymers are promising agents that merit additional study.

Keywords: Gadolinium; Chelates; Polymers; Blood pool; MR contrast agents

1. Introduction

Blood pool magnetic resonance (MR) contrast agents are under development to increase signal intensity from blood during MR angiography as well as for other applications [1–4]. Low-molecular-weight extracellular fluid (ECF) MR contrast agents such as gadolinium–DTPA (Gd–DTPA) equilibrate between the intravascular and extravascular spaces [5–8]. This equilibration decreases the effective concentration of gadolinium within the blood vessels and distributes gadolinium into the interstitial tissues where it may increase background signal noise [9]. Faster scanning sequences for MR angiography (MRA) have decreased the image acquisition time, making it feasible to do MRA with ECF agents such as Gd–DTPA. There are trade-offs between image acquisition time, signal to noise, and field of view, however. For many applications, it would be desirable to have a blood pool MR contrast agent with a prolonged vascular residence time. An ideal agent for MRA would remain sequestered within the vascular space for sufficient time to allow acquisition of high-resolution MR images throughout the desired anatomic region and in many cases, over an extended field of view. One method of creating a blood pool MR con-

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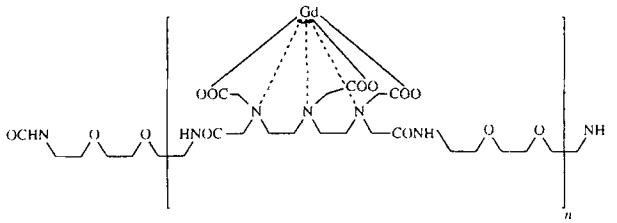
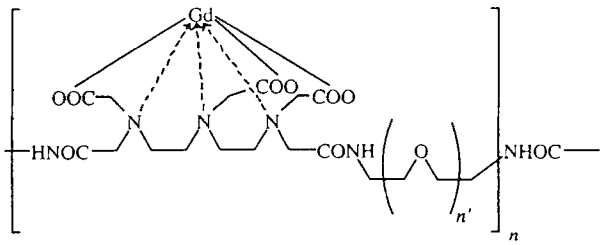
trast agent is to increase the molecular weight of the compound so that the size of the molecule does not readily pass through the fenestrations of the capillaries. As an additional advantage, higher-molecular-weight contrast agents may increase the efficiency of the relaxation mechanism between the paramagnetic Gd^{3+} and the water protons for which the relaxivity is measured.



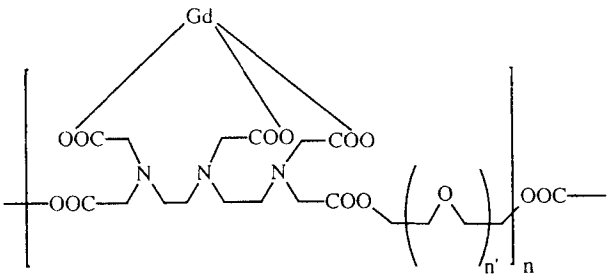
Gd-poly-DTPA-EOEA

Fig. 1. Structure of Gd–poly-DTPA–EOEA.



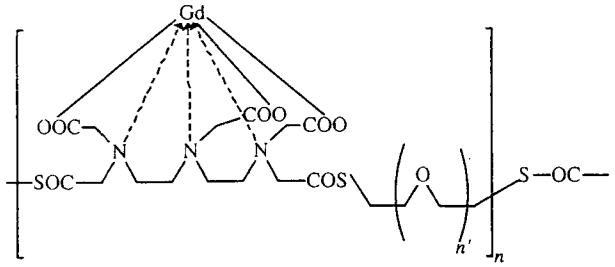
Gd-poly-DTPA-NH-PEG

Fig. 2. Structure of Gd–poly-DTPA–NH-PEG.



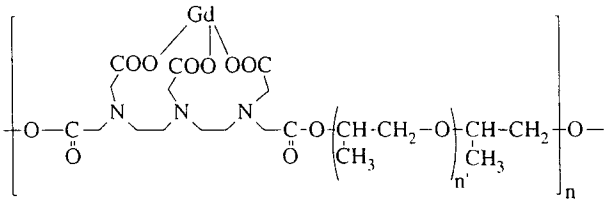
Gd-poly-DTPA-PEG

Fig. 3. Structure of Gd–poly-DTPA–PEG.



Gd-poly-DTPA-S-PEG

Fig. 4. Structure of Gd–poly-DTPA–S-PEG.



Gd-poly-DTPA-PPG

Fig. 5. Structure of Gd–poly-DTPA–PPG.

Copolymers may offer the advantage of slower tumbling times, thereby effectively increasing the dipolar interactions, directly or indirectly, between a paramagnetic species and water protons, thus leading to higher relaxivities. Copolymers composed of chelates with a linker monomer incorporate the chelate into the polymeric backbone to create a closely defined stoichiometric ratio of linker, chelate, and metal ion. Copolymeric chelates may make it easier to develop a well-defined and regular spacing of chelate in the macromolecule than with frame-graft polymers such as Gd–DTPA grafted onto albumin or dextran.

Several mechanisms have been proposed that could lead to more efficient water proton relaxation rates. For example, Wagner and Jackels have determined that water proton relaxation beyond the first coordination sphere of metal complexes are predominantly controlled by fluctuations in intermolecular dipolar interactions [10]. Burton et al., among others, have found that the relaxation efficiency of water protons from paramagnetic metal complexes often increases significantly when the rotational correlation time, τ, is increased by attachment of the metal complex by either (1) a slowly rotating macromolecule or (2) by addition of a viscous solute [11]. The first objective, designing a macromolecule, can be achieved by synthesizing a large-molecular-weight copolymer. The second objective, increasing viscosity, can be achieved by designing a molecule with suitable microviscosity properties. Polyethylene glycols (PEGs) are commonly used, biocompatible, pharmaceutical excipients that increase the viscosity of an aqueous milieu [12]. We are currently addressing this issue by synthesizing gadolinium copolymeric chelates that (1) are of sufficient molecular weight to increase the complex correlation time and (2) contain PEG within the copolymer in an effort to increase the microviscosity around the gadolinium complex. The objective of these analogs is to generate complementary effects that increase relaxivity of these molecules.

Table 1
Characteristics of Gd-copolymers

Compound	Molecular weight[a]	Gd content calculated	Percentage of Gd found
Gd-DTPA	548	28.67	NA
Gd-poly-DTPA-EOEA	662[a]	23.72	22.16
Gd-poly-DTPA-NH-PEG	4314[a]	3.64	2.95
Gd-poly-DTPA-PEG	2400[a]	6.54	6.78
Gd-poly-DTPA-S-PEG	4320[a]	3.64	3.56
Gd-poly-DTPA-PPG	1700[a]	9.14	8.02

[a] Per polymeric unit

In prior reports, we and other groups have described manganese-based copolymeric chelates as MR contrast agents [13–18]. These prior compounds were composed of ethylenediaminetetraacetic acid (EDTA) monomers and linker monomers of diaminoethylene glycol. In this report, we describe synthesis and preliminary characterization of gadolinium-based copolymers composed of DTPA and varying-molecular-weight PEG and polypropylene glycol (PPG) linker monomers.

2. Materials and methods

2.1. Synthesis, in vitro, and in vivo characterization of Gd-copolymers

2.1.1. General method for synthesis of copolymers

Diethylenetriamine pentaacetic acid (DTPA) dianhydride (Aldrich, Milwaukee, WI) was added in small portions into a stirring solution of ω,ω′-difunctionalized PEG (Aldrich) of varying molecular weights or PPG in dried methanol. Stirring was continued until the initially turbid solution became clear. The mixture was heated to 45°C and stirred for one additional hour. The solution was filtered and concentrated in vacuo to yield a white powder. The copolymer was dissolved in water, and one molar equivalent of gadolinium carbonate (Aldrich) was added. The solution was stirred for 2 days at room temperature followed by additional stirring at 50°C for 3 h. The solution was filtered and dialyzed in an 8000-mw, cutoff dialysis membrane against a water dialyzate. The dialyzed solution was frozen and lyophilized to obtain the Gd-containing polymer residue.

Five Gd-copolymers were synthesized:

1. Gadolinium poly-2,2′-ethylenedioxydiethylamine-co-diethylenetriamine pentaacetic acid (Gd–poly-DTPA–EOEA)
2. Gadolinium poly-ω,ω′-diamino-polyethylene glycol-co-diethylenetriamine pentaacetic acid (Gd–poly-DTPA–NH–PEG)
3. Gadolinium poly-polyethylene glycol-co-diethylenetriamine pentaacetic acid (Gd–poly-DTPA–PEG)
4. Gadolinium poly-ω,ω′-thiol-polyethylene glycol-co-diethylenetriamine pentaacetic acid (Gd–poly-DTPA-S-PEG)
5. Gadolinium poly-polypropylene glycol-co-diethylenetriamine pentaacetic acid (Gd–poly-DTPA–PPG)

A specific synthetic example is noted below.

2.1.2. Synthesis of poly-DTPA–EOEA

In a 50-ml, round-bottomed flask was dissolved 0.74 g (5 mmol) of Bis-2,2′-ethylenedioxydiethylamine (EOEA) in anhydrous methanol (50 ml). To this solution was added in small portions with stirring, 1.79 g (5 mmol) of DTPA. The turbid reaction mixture was stirred until clear, followed by heating at 45°C for 1 h. The solution was then filtered and dried in vacuo to obtain the copolymer.

2.1.3. Synthesis of Gd–Poly-DTPA–EOEA.

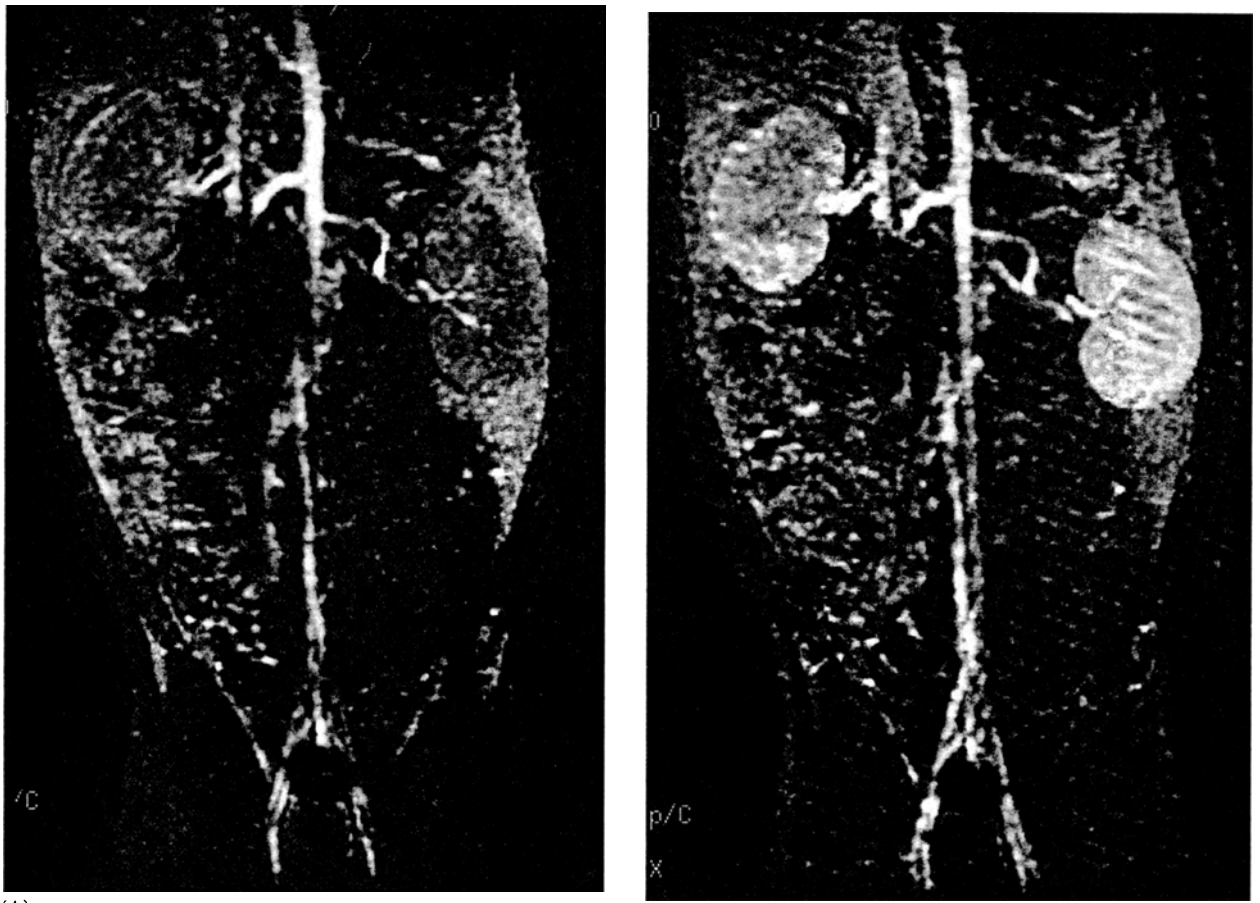
Gadolinium carbonate, 1.1 g, was suspended in 20 ml of water followed by addition of a solution of 1.5 g of

Table 2
Relaxivity of Gd-copolymers (in PBS)

Compound	r_1 ($mM^{-1}\ s^{-1}$)	r_2 ($mM^{-1}\ s^{-1}$)
Gd-DTPA	4.1	5.2
Gd-poly-DTPA-EOEA	3.8	4.5
Gd-poly-DTPA-NH-PEG	5.1	6.6
Gd-poly-DTPA-PEG	5.8	7.9
Gd-poly-DTPA-S-PEG	5.5	7.4
Gd-poly-DTPA-PPG	5.2	7.0

Table 3
Relaxivity of Gd-copolymers (in serum)

Compound	r_1 ($mmol^{-1}\ s^{-1}$)	r_2 ($mmol^{-1}\ s^{-1}$)
Gd-DTPA	4.1	6.2
Gd-poly-DTPA-EOEA	3.7	4.7
Gd-poly-DTPA -NH-PEG	–	–
Gd-poly-DTPA-PEG	5.6	8.2
Gd-poly-DTPA-S-PEG	6.4	9.4
Gd-poly-DTPA-PPG	4.8	7.5



(A) (B)

Fig. 6. MRA with 0.1 mmol per kg Gd–DTPA: (A) precontrast; (B) postcontrast at $T = 0$ min; (C) $T = 5$ min postcontrast; and (D) composite (left to right): precontrast, $T = 0$, 3 and 5 min. Note contrast in bladder at $T = 5$ min.

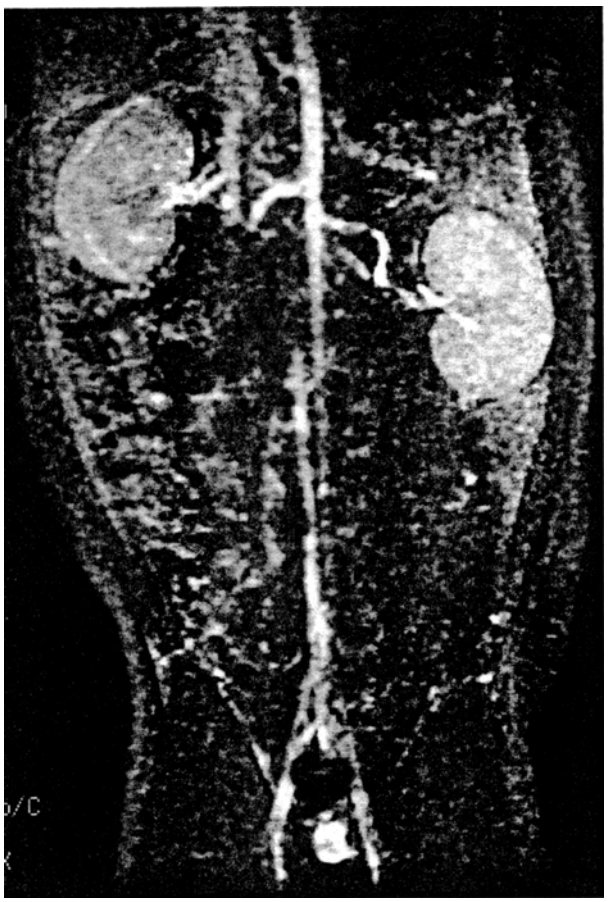
poly-DTPA–EOEA in 30 ml of water as synthesized previously. The mixture was stirred for 2 days at room temperature, followed by heating to 50°C and stirring for an additional 3 h. The reaction mixture was filtered and dialyzed in an 8,000-mw, cutoff dialysis membrane against water for 24 h. The dialyzed solution was then concentrated in vacuo, followed by drying to yield the white, powdery complex. Gadolinium content was determined by atomic absorption spectra analysis (Desert Analytics, Tucson, AZ).

2.1.4. Preparation of NMR Samples

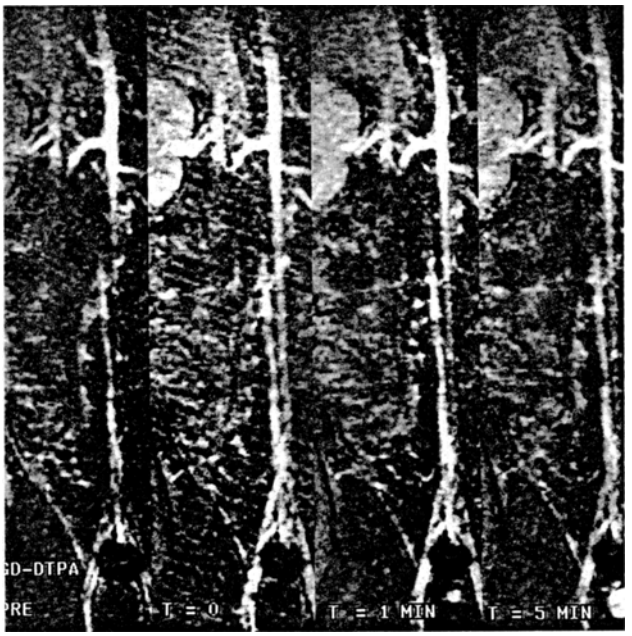
Samples of gadolinium–DTPA (Magnevist, Berlex, Wayne, NJ) and the various copolymeric chelates were prepared in phosphate-buffered saline (PBS) and 10% bovine serum (Sigma, St. Louis, MO) at concentrations of 0.032, 0.0625, 0.25, 0.5, 1.0, and 2.0 mmol of gadolinium. The samples were tested at 1.5 Tesla on a Sigma scanner (GE, Milwaukee, WI) using T1-weighted and T2-weighted pulse sequences. T1-weighted pulse sequences held the time of echo (TE) constant at 15 ms while varying the time of repetition (TR) to TR = 100, 300, 450, 600, 900, 1200, 1500, 1800, 2500, and 3500 ms. T2-weighted pulse sequences held TR constant at 3500 ms and varied the TE = 50, 70, 90, 120, 150, 180, 200, 210, 230, and 250 ms. Using standard formulas, the signal intensity of the samples was measured on each of the images, and the T1 and T2 was calculated for each concentration of the different contrast agents. Relaxivities ($r_1 = 1/T1$, $mM^{-1}\ s^{-1}$; $r_2 = 1/T2$, $mM^{-1}\ s^{-1}$) were then calculated by plotting r_1 and r_2 as a function of concentration followed by determination of the slope.

2.1.5. In vivo characterization

MRA was performed on New Zealand white rabbits with a Signa scanner (GE) at 0.5 Tesla. Coronal, 2-D, time-of-flight MRA was performed at TR = 32 ms, TE = 7 ms, 60° flip angle, 2-mm slice, 18-cm FOV, 52-s image acquisition time. The rabbits received rapid IV bolus injections via ear vein of 0.1 mmol kg^{-1} Gd in the form of Gd–DTPA or Gd–poly-DTPA–PPG, followed by rapid flush of 1.0 cc of saline.



(C)



(D)

Fig. 6. (*Continued*)

Image acquisitions were performed before contrast, immediately after IV bolus injection of gadolinium ($T=0$), and at 1, 3, 5, 10, 15, and 20 min after IV injection of contrast.

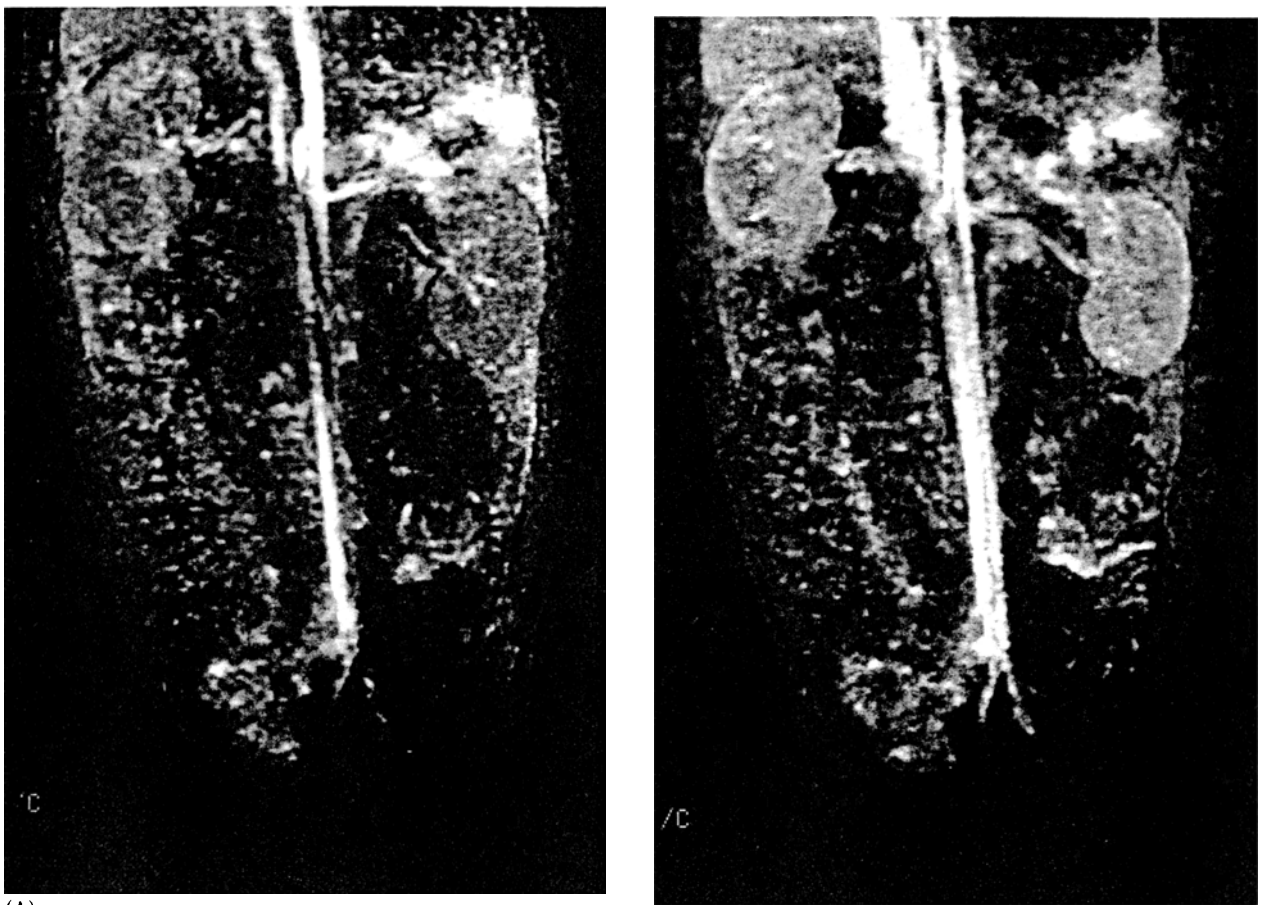
3. Results

Figs. 1–5 show representative images of the five different gadolinium-based copolymeric chelates. The calculated molecular weights of the copolymeric subunits and gadolinium content for the different structures are shown in Table 1. Relaxivities for the different compounds in PBS and serum are shown in Tables 2 and 3, respectively.

As shown in Tables 2 and 3, the gadolinium-based polymeric chelates with larger-molecular-weight linker monomers had moderately higher relaxivities than Gd–poly-DTPA–EOEA, the compound with the lowest-molecular-weight linker monomer. Gd-copolymers had up to about 50% higher relaxivity than Gd–DTPA. With the possible exception of Gd–poly-DTPA–S-PEG, relaxivity values of the Gd-copolymers showed only slight, if any, difference between PBS and serum. In vivo MRA (Figs. 6 and 7) showed markedly greater vascular enhancement for the Gd–poly-DTPA–PPG compound than Gd–DTPA. At 20 min after contrast, the vascular enhancement was maintained for the copolymeric contrast agent with no appreciable background tissue enhancement seen outside of the blood pool. In comparison, enhancement from Gd–DTPA at 0.1 mmol kg^{-1} was relatively weak and diminished by 5 min after contrast.

4. Discussion

We have previously developed manganese-based copolymeric chelates [13–17]. These prior compounds used diaminoethylene glycol linkers with EDTA to create linear copolymeric chelates. These manganese-based copolymers had at least two-fold higher relaxivity than low molecular weight chelates of manganese (e.g. Mn–EDTA–methoxyethylamine). Mn–poly-EDTA–



(A) (B)

Fig. 7. MRA with 0.1 mmol kg^{-1} Gd–poly-DTPA–PPG: (A) precontrast; (B) postcontrast at $T=0$ min; (C) $T=1$ min; (D) $T=20$ min postcontrast; and (E) composite (left to right): precontrast, $T=0$, 5, and 20 min. Note absence of contrast in bladder and sustained contrast enhancement of vasculature.

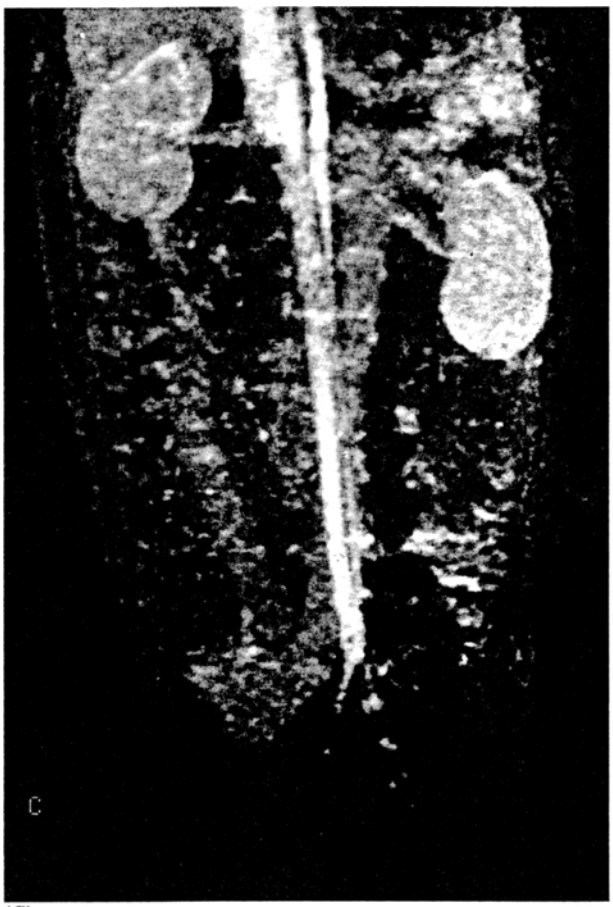
EOEA-DP was shown in preclinical imaging to enhance signal from heart, liver, and urinary bladder.

Whereas gadolinium has only one valence state, manganese has several and may exchange more rapidly than gadolinium with endogenous biological ligands [19–22]. Because of this difference, it may be somewhat easier to strongly chelate gadolinium than manganese. Designing a polymeric gadolinium-containing chelate could result in a more stable blood pool agent. This was part of our motivation for developing copolymeric chelates based on gadolinium.

Unlike the manganese-based copolymers which showed an appreciable increase in relaxivity compared with the monomeric metal chelates, our gadolinium-based copolymers show a more modest increase in in vitro relaxivity relative to the monomeric chelates of gadolinium (e.g. Gd–DTPA). Gd–poly-DTPA–EOEA and Mn–poly-EDTA–EOEA use the same linker monomer between the chelates, yet the manganese compound shows an increase in proton relaxation enhancement (PRE), while the gadolinium compound does not. Only Gd-copolymers prepared with larger-molecular-weight linker monomers showed this increase in relaxivity. Manganese is perhaps more conducive to relaxation enhancement effects due to slower correlation times. The electron spin correlation time for a manganese ion is somewhat closer to the proton Larmour frequency than gadolinium and may account for the more modest increase in PRE shown on in vitro relaxivity studies with the gadolinium-based copolymers [23].

With an almost 10-fold increase in the molecular weight of the linker monomer in the PEG linker, the relaxivity of Gd–poly-DTPA–NH–PEG was increased about 80% compared with the relaxivity of Gd–poly-DTPA–EOEA. PEG is known to be a hydrophilic molecule that exhibits random thermal motion in the fast regime on the NMR time scale. The mobility of the PEG may account for the relatively modest increase in PRE which otherwise might be expected to be higher from a slower-tumbling, larger molecule. This may provide an explanation for the relatively modest increase in PRE in the in vitro studies. Previous studies of

gadolinium-based copolymeric chelates prepared with polyethylene linkers demonstrated an even greater increase in PRE relative to PEG-containing Gd-copolymers, up to a maximum of 12 methylene units in the



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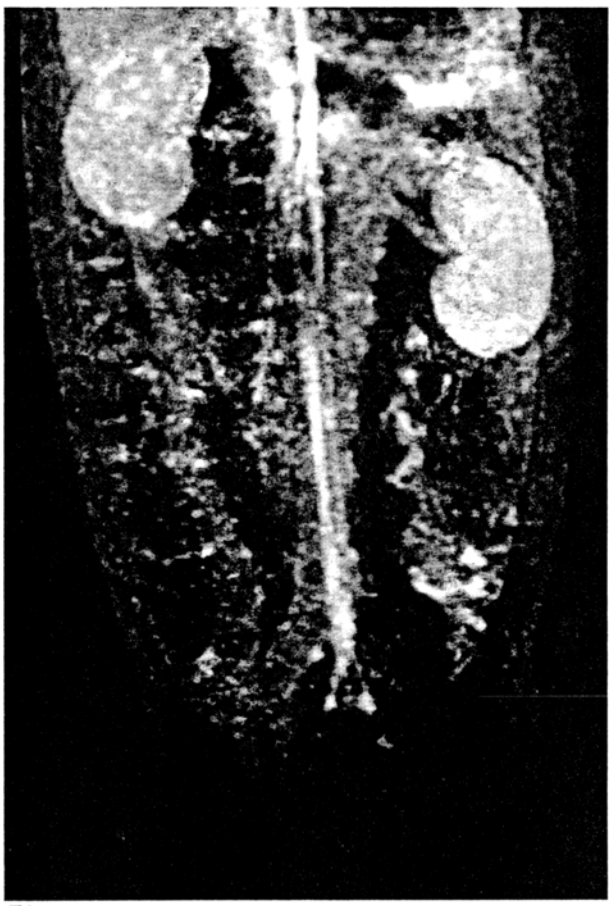

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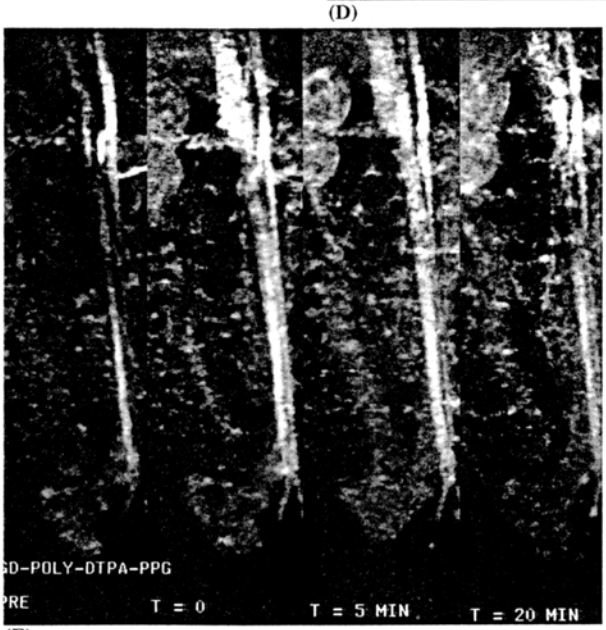

(E)

Fig. 7. (*Continued*)

linker monomer [24]. Based on the above discussion, making a copolymer with a more rigid chain could help to immobilize the complex, thereby making the spin diffusion of the complex's nuclei more efficient. This in turn could transfer relaxation effects more efficiently to the solvent proton milieu [25].

Linkers composed of multiple methylene groups are much more hydrophobic than PEG subunits. Because of their hydrophobicity, these methylene-containing linkers may form tertiary structures within the copolymeric chelate backbone when exposed to an aqueous milieu. Consequently, these tertiary structures may motionally constrain the linker monomers and their paramagnetic centers and indeed, the overall copolymeric chelate, such that the correlation time is slowed, and relaxivity is increased [21]. Linker monomers composed of PEG, however, are not as highly hydrophobic and, in fact, are somewhat amphiphilic. Thus, PEG in an aqueous milieu may indeed exhibit rapid random motion on the NMR time scale such that the effects of correlation time on relaxivity may not be as prominent in vitro, in PBS or 10% serum. Therefore, introduction of higher-molecular-weight PEG linker monomers would be expected to result in only a moderate increase in relaxivity.

In vitro relaxivity studies are interesting and important but may be misleading. In vivo MRA with Gd–poly-DTPA–PPG showed a dramatic increase in blood signal compared with Gd–DTPA even though the in vitro relaxivity of the copolymeric contrast agent was only moderately greater than Gd–DTPA. Confinement to the vascular space may largely account for the markedly greater blood volume enhancement of the Gd-copolymer compared with Gd–DTPA. On the other hand, rheology of blood is different than PBS or 10% serum. The macromolecular contrast agents based on PEG- or PPG-containing copolymers may become even more effective MR contrast agents in the milieu of blood than Gd–DTPA as suggested by our MRA studies using poly-Gd–DTPA–PPG.

PEG is known to be inert and relatively well-tolerated biologically. Although the in vitro relaxivity of these gadolinium-based copolymeric chelates prepared from PEG-linker monomers is only moderately higher than Gd-DTPA, these copolymeric agents prepared with hydrophilic linkers still function as effective blood pool contrast agents. Although preliminary results are encouraging, additional studies are needed to assess the potential of these gadolinium-based copolymeric contrast agents.

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