Gd(III)DTPA-*L*-Cystine-PEG Copolymers: A Biodegradable Macromolecular Agent for Blood Pool MR Imaging

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Synopsis. Biodegradable, PEGylated macromolecular Gd(III)DTPA-*L*-cystine copolymers were prepared and tested as a blood pool contrast agent on mice. The macromolecular contrast agent demonstrated superior contrast enhancement in the heart and blood vessels as compared to the low molecular weight control agent. At fifteen minutes, the PEGylated macromolecular agent still showed prominent enhancement. Little contrast enhancement by the control agent was detectable in the vasculature.

Introduction. Macromolecular gadolinium (III) magnetic resonance contrast agents are well suited for blood pool imaging because of their long plasma retention time. However, current polymeric contrast agents have a less complete elimination than their low molecular weight counterparts allowing for the possible toxic accumulation of Gd(III) ions. Therefore, we have incorporated a biodegradable disulfide bond into the polymer backbone, which is degraded via the disulfide-thiol exchange reaction with endogenous thiols. Here we report the modification of Gd(III)DTPA-*L*-cystine with monomethoxy-poly(ethylene glycol)amine (MPEG-NH_2) to the carboxylic groups of *L*-cystine. Polyethylene glycol is a nontoxic, non-antigenic and biocompatible polymer that has a large hydrodynamic radius rendering itself ideal for use in blood pool imaging.

Materials and Methods. DTPA-cystine copolymers were synthesized from *L*-cystine and DTPA dianhydride using condensation polymerization in water followed chelating with $Gd(OAc)_3$. MPEG-NH_2 (MW = 2000) was synthesized as described by Zalipsky. Conjugation of MPEG-NH_2 was accomplished with EDC and NHS in water. Free MPEG-NH_2 was removed by centrifugation filtration. MR images were taken pre-injection and at 1, 5,10 and 15 minutes post-injection on a Siemens Trio 3T scanner. Imaging parameters were 1.74 ms TE, 4.3 ms TR, 25° RF tip angle, 120 mm FOV, 1.6 mm coronal slice thickness.

Results. The average molecular weight was determined by size exclusion chromatography using FPLC with poly(*N*-2-hydroxypropylmethacrylamide) calibration. DTPA-cystine copolymers were determined to be M_w =12.2 kDa (M_w/M_n = 1.06) and after chelation of Gd(III) the apparent M_w of the polymer was 12.0 kDa (M_w/M_n =1.07). Conjugation of MPEG-NH_2 increased the apparent polymer size to M_w = 18.7 kDa (M_w/M_n = 1.20). The Gd(III) content, determined by inductively coupled plasma (ICP) spectroscopy, was 0.095 mmol Gd(III)/g copolymers. The PEGylated polymeric contrast agent was incubated with *L*-cysteine for 2 h at 37º to demonstrate the degradability of the polymer. MALDI-TOF was then performed on the sample. The spectrum was analyzed and showed the low molecular weight degradation products. The agent was then injected into mice via the tail vain. Immediately after injection, significant contrast enhancement of the heart, aorta and femoral arteries by the macromolecular agent was observed (Figure 1). After fifteen minutes, enhancement by the macromolecular agent was still observed in the heart. Little contrast enhancement by the control agent was shown.

Discussion. PEGylation of Gd(III)DTPA-*L*-cystine copolymers produced significant contrast of the vasculature. PEG most likely imposes a steric restriction on the rotation of the polymers resulting in the increased signal intensity. The prolonged retention of the macromolecular agent may stem from the steric inaccessibility of the disulfide bond caused by PEG. A detailed investigation is currently underway to fully characterize the macromolecular contrast agent including a study to characterize the relaxivity of PEGylated Gd(III)DTPA-*L*-cystine copolymers.

Conclusion. Gd(III)DTPA-*L*-cystine-PEG shows promise as a MR blood pool imaging agent because of its large signal intensity.

References. 1) Z.-R. Lu, et al. Poly(L-glutamic acid) Gd(III)-DOTA conjugate with a degradable spacer for magnetic resonance imaging. Bioconjugate Chem. 14, 715-719 (2003). 2) J.M. Harris, et al. Chemistry for peptide and protein PEGylation. Adv. Drug Del. Rev. 54, 459-476 (2002). 3) S. Zalipsky et al. Attachment of drugs to polyethylene glycols. Eur. Polym. J. 19, 1177-1183 (1983).

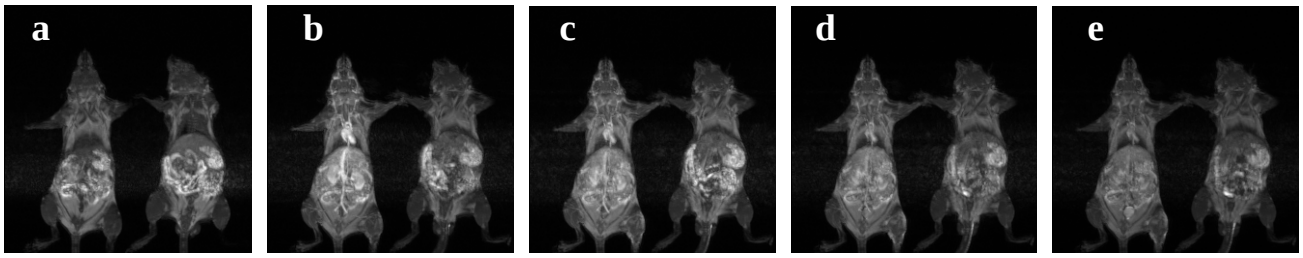

Figure 1. Contrast enhanced 3D (MIP) MR images of mice using Gd(III)DTPA-*L*-Cystine-PEG copolymers (left mouse in figures, 0.03 mmol Gd/Kg) and Gd-(DTPA-BMA) (right mouse in figures, 0.1 mmol Gd/Kg). The images were taken pre-injection (a) and 1, 5, 10 and15 (b-e, respectively) minutes post-injection of contrast agent.