Original Research

Slow Clearance Gadolinium-Based Extracellular and Intravascular Contrast Media for Three-Dimensional MR Angiography

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The objective of this study was to assess two new slow-clearance contrast media with extracellular and intravascular distribution for magnetic resonance angiography (MRA). Extracellular Gd-DTPA-BC_2glucA and intravascular Gd(DO3A)$_3$-lys$_{16}$ were developed within the European Biomed2 MACE Program and compared with two reference compounds, intravascular CMD-A2-Gd-DOTA and extracellular GdDOTA, in 12 rats. Pre- and post-contrast three-dimensional MR (TR/TE = 5 msec/2.2 msec; isotropic voxel size 0.86 mm^3) was acquired for 2 hours. Signal-to-noise enhancement (ΔSNR) was calculated. Two minutes after injection, all contrast media provided strong vascular signal enhancement. The ΔSNR for Gd-DTPA-BC_2glucA, Gd(DO3A)$_3$-lys$_{16}$, CMD-A2-Gd-DOTA, and GdDOTA were 13.0 ± 1.8, 25.0 ± 3.2, 25.0 ± 4.0, and 18.0 ± 3.4, respectively. Gd-DTPA-BC_2glucA, Gd(DO3A)$_3$-lys$_{16}$, and CMD-A2-Gd-DOTA cleared slowly from the circulation, whereas GdDOTA cleared rapidly. Vascular ΔSNR at 2 hours were 2.9 ± 0.6, 25.0 ± 3.2, 25.0 ± 4.0, and 0.4 ± 1.0. Gd(DO3A)$_3$-lys$_{16}$ provided strong vascular and minor background enhancement, and thus may be useful for MRA or perfusion imaging. Gd-DTPA-BC_2glucA produces persistent enhancement of extracellular water, and thus may allow quantification of extracellular distribution volume and assessment of myocardial viability. J. Magn. Reson. Imaging 2001;13:588–593. © 2001 Wiley-Liss, Inc.

Index terms: intravascular contrast media; extracellular contrast media; three-dimensional MR angiography; myocardial viability; gadolinium chelates

MAGNETIC RESONANCE (MR) contrast media with prolonged vascular enhancement is desirable for specific applications such as perfusion assessment (1), angiography (2), or clear delineation of the endocardial border (3).

Various contrast media for prolonged vascular enhancement have been studied. None, however, has achieved clinical approval yet. Intravascular contrast media may be classified as macromolecules with slow or rapid clearance (4,5), particles (6), or small molecules (7,8) that bind to intravascular macromolecules.

Gadolinium-labelled macromolecules have been investigated in the past (4,5). Particles such as ultrasmall supraparamagnetic iron oxide particles (USPIO) (9) have entered clinical trials and are already under investigation for MR angiography (MRA) (2,10), assessment of myocardial perfusion (1), or imaging of coronary arteries in patients (11). Moreover, small molecular compounds with high affinity to intravascular macromolecules and thus predominant intravascular distribution, such as MS325 (AngioMARK™), have been used for coronary MRA (12). These authors consistently displayed extensive portions of the native left and right coronary system with sub-millimeter in-plane resolution (12). Moreover, this contrast media provided strong blood/muscle contrast and clear definition of the myocardial border (12).

Concerns about an undesired immune response to the carrier molecule and some characteristics of deposition or retention of gadolinium ions have hindered clinical applications of gadolinium-labelled macromolecules (13–15). With USPIO, the desired T_1 shortening is associated with an undesired susceptibility effect that can deteriorate image quality and make imaging of small vessels and tissue perfusion difficult (10). This limitation becomes more important at higher field strengths, since the ratio T_1/T_2* increases with increasing field strengths. Moreover, the problem of in vivo labelling of intravascular macromolecules with small gadolinium-based compounds is that the amount of unbound low molecular contrast media that has access to the interstitial space is not clearly defined. Circulation of low molecular weight contrast media would be expected to enhance background tissue, resulting in blurring of angiograms and making perfusion assessment difficult.

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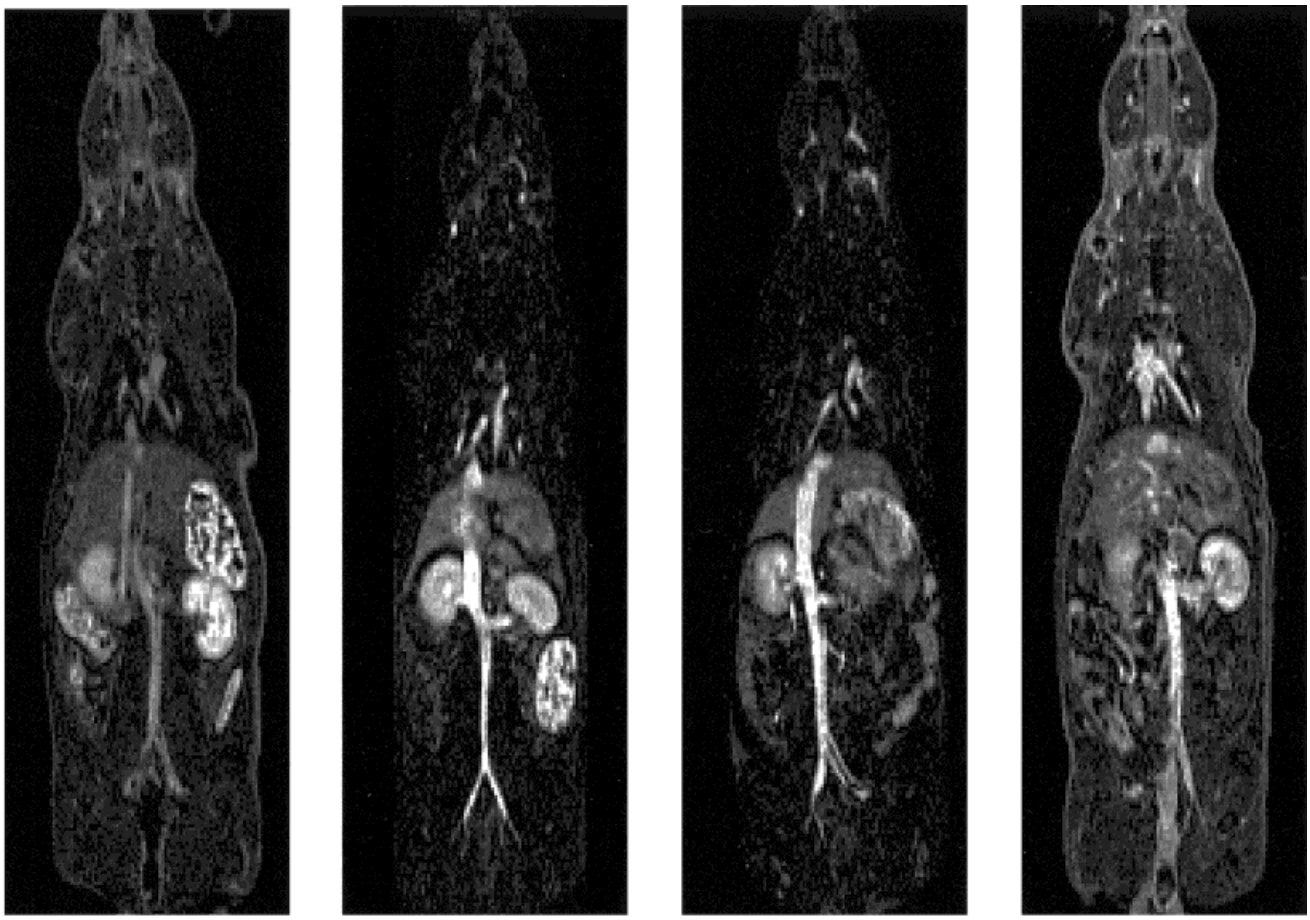

Figure 1. Representative source images acquired 2 minutes after injection of contrast media. Note that all images are displayed with identical window and center settings. All contrast media provided strong vascular enhancement. GdDTPA- BC_2glucA and GdDOTA produced considerable background enhancement. Contents of the gastrointestinal tract with short T1 can be seen.

The purpose of the current study was to assess the value of two novel slow clearance intravascular and extracellular contrast media for MRA.

MATERIALS AND METHODS

Contrast Media

Gd-DTPA-BC_2glucA was synthesized as previously described by Colet et al (16) by adding glucose moieties to the extracellular contrast agent Gadopentetate dimeglumine (GdDTPA; Magnevist™, Schering, Germany). This compound is filtrated in the glomerula and almost completely recovered by reabsortion in the renal tubules (16). Pharmacokinetic studies previously demonstrated a long blood half life of 289 minutes (16). Contrast media was administered at a dose of 0.1 mmol/kg bodyweight.

$Gd(DO3A)_3$-lys_{16} is a slow clearance blood pool agent and was prepared starting from diethyl squarate. The synthesis was based on the ability of the squaric acid moiety to act as a linker between the DO3A chelate and the polylysine (17). The relaxivity at 20 MHz and 25°C is 15.6 mM^{-1} s^{-1}.

CMD-A2-Gd-DOTA (P717, a research compound from Guerbet, France) is a slow clearance blood-pool agent comprised of a carboxymethyl-dextran polymer substituted with the paramagnetic macrocyclic complex Gd-DOTA using an amino spacer (18,19). The molecular weight is 50.5 kDalton, the relaxivities measured at 60 MHz, 37°C are r_1 = 9.4 and r_2 = 15 mM^{-1} x s^{-1} (19). The elimination half life is > 180 minutes. This contrast media was tested at a dose of 30 μmol/kg bodyweight.

GdDOTA (Meglumin-godaterate, commercially available DOTAREM; Guerbet, Aulnay-Sous-Bois, France) is a standard extracellular contrast media frequently used for contrast-enhanced MRA. The molecular weight is 0.56 kDalton, the relaxivities measured at 60 MHz and 37°C are r_1 = 2.9 and r_2 = 4.5 mM^{-1} x s^{-1}, respectively.

Experimental Protocol

All experimental procedures were performed in accordance with the Federal Veterinary Office guidelines for humane handling of animals and received prior ap-

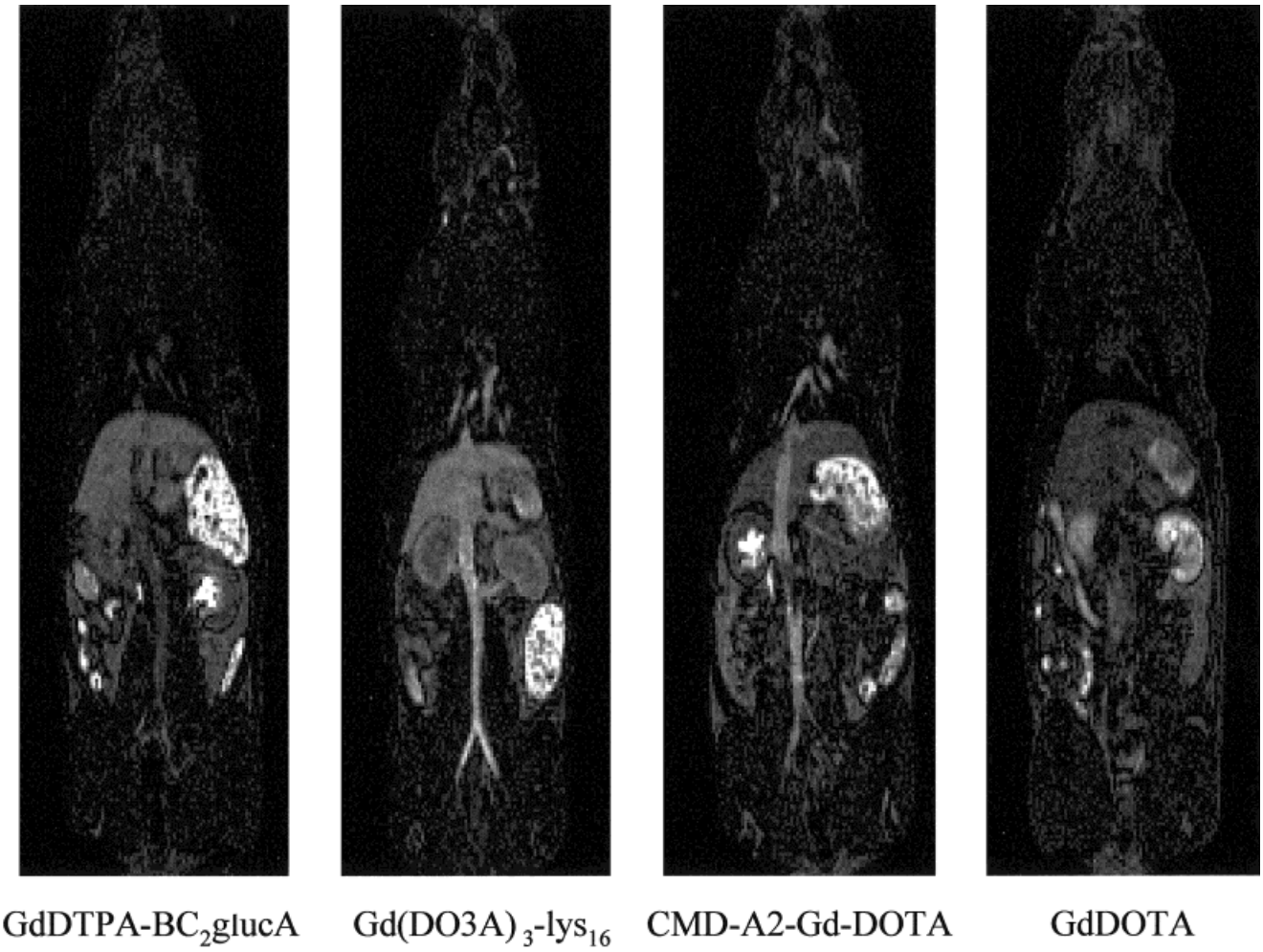

Figure 2. Representative source images acquired 122 minutes after injection of the contrast media. Note that all images are displayed with identical window and center settings. Persistent vascular enhancement was seen with GdDTPA- BC_2glucA, $Gd(DO3A)_3$-lys_{16}, and CMD-A2-Gd-DOTA, whereas GDDOTA had cleared from the circulation.

proval from the committee of animal research at the University of Basel. Twelve female Sprague-Dawley rats (270–340 g; RCC Ltd, CH-4414 Füllinsdorf) were anesthetized with pentobarbital (50 mg/kg). A tail vein was cannulated with a Butterfly™-23 winged needle infusion set for application of contrast media during experiments and a lethal dose of pentobarbital (100 mg/kg) was given upon completion of MR experiments.

Magnetic Resonance Imaging

Magnetic resonance imaging was performed using a phased array extremity coil with a 1.5-Tesla magnet (Magnetom Vision; Siemens Erlangen, Germany). Parameters of the three-dimensional ungated spoiled gradient-echo imaging sequence were as follows: TE = 2.2 msec, TR = 4.98 msec, flip angle 40°, 8 acquisitions, matrix 96 × 256, slab thickness 60 mm, 70 partitions, voxel size 0.86 × 0.86 × 0.86 mm^3, field of view (FOV) 82.5 × 220 mm, 70 contiguous partitions, acquisition time 4.27 minutes. Acquisition was done in the coronal plane of the animals to cover a large volume of the major vessels in the plane of the partitions. Three-dimensional MR images were acquired before contrast admin-

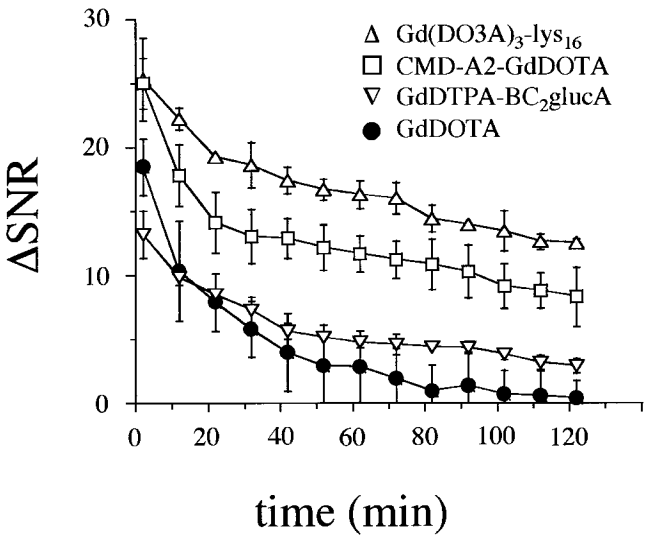

Figure 3. ΔSNR of blood plotted over time after injection of $Gd(DO3A)_3$-lys_{16}, CMD-A2-Gd-DOTA, GdDTPA-BC_2glucA, or GdDOTA in a tail vein (N = 3 each compound).

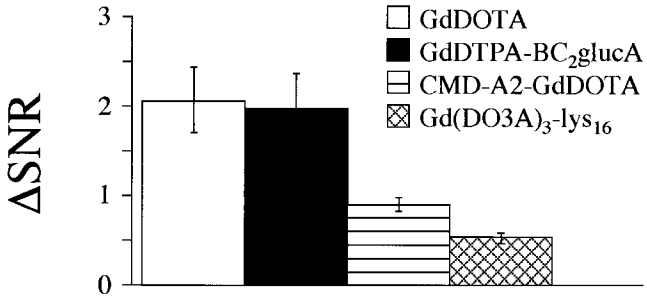

Figure 4. ΔSNR of skeletal muscle 2 minutes after injection of the contrast media. GdDOTA ($N = 3$) and GdDTPA-BC_2glucA ($N = 3$) provided substantial enhancement of skeletal muscle, which is consistent with extracellular distribution. CMD-A2-Gd-DOTA ($N = 3$) and $Gd(DO3A)_3$-lys_{16} ($N = 3$) produced only minor background enhancement, which is consistent with a predominantly intravascular distribution.

istration and at 2, 12, 22, 32, 42, 52, 62, 72, 82, 92, 102, 112, and 122 minutes after contrast injection.

Data Analysis

For quantitative data analysis, the signal intensity (SI) in defined regions of interest (ROI)was measured on source images before and after contrast administration. The ROI in vessels contained at least five pixels to avoid partial volume effects. In skeletal muscle, ROI were chosen to contain a minimum of 50 pixels, and visible vascular structures were avoided. Noise was defined as SI in an ROI contained within the FOV, but outside the animal. The signal-to-noise ratio (SNR) was calculated as signal of a given tissue divided by the standard deviation of the noise. ΔSNR was calculated as SNR_{post} minus SNR_{pre} contrast administration. All values are reported as mean ± standard error of the mean. Differences in SI and SNR were evaluated statistically by means of a paired Student's t-test. A P value of < 0.05 was considered significant.

RESULTS

All four tested contrast media were well tolerated by all animals. Figures 1 and 2 show representative source images at 2 minutes and 2 hours after injection of the four different gadolinium-based contrast agents. Images were not processed and are displayed with identical window and level settings.

Typical features of extracellular contrast media were observed with Gd-DTPA-BC_2glucA and GdDOTA at 2 minutes after injection. At 2 minutes after injection, both compounds provided strong vascular enhancement (ΔSNR_{Ao} = 13.0 ± 1.8 and 18.0 ± 3.4, respectively) and diffuse background enhancement of skeletal muscle (ΔSNR_{SM} = 2.0 ± 0.4 and 2.1 ± 0.4, respectively) and subcutaneous tissue. Two hours after injection, Gd-DTPA-BC_2glucA provided persistent enhancement, whereas GdDOTA-enhanced three-dimensional-MRA had almost returned to baseline values (ΔSNR_{Ao} = 2.9 ± 0.6 and 0.4 ± 1.0) (Fig. 2). Within 2 hours, ΔSNR_{Ao} of Gd-DTPA-BC_2glucA and GdDOTA enhanced 3D-MRA declined by 22.3% and 97.8%, respectively (Fig. 3).

$Gd(DO3A)_3$-lys_{16} and CMD-A2-Gd-DOTA demonstrated a typical intravascular distribution pattern with strong vascular (ΔSNR_{Ao} = 25.0 ± 3.2 and 25.0 ± 4.0) and only minor background (ΔSNR_{SM} = 0.5 ± 0.0 and 0.9 ± 0.1) enhancement (Fig. 4). Both contrast media cleared slowly from the circulation. Two hours after injection, ΔSNR_{Ao} were 14.0 ± 0.3 and 8.3 ± 2.3, which were 56.0% and 33.2% of 2 minute values, respectively. Strong vascular and little background enhancement allowed clear delineation of vascular structures on maximum intensity projections (Fig. 5).

DISCUSSION

Intravascular Contrast Media

$Gd(DO3A)_3$-lys_{16} and CMD-A2-Gd-DOTA provided strong and prolonged enhancement of the blood pool with minor enhancement of background tissue such as skeletal muscle. Among the macromolecular carriers available for coupling with contrast agents, dextran derivatives have many advantages such as biocompatibility, solubility, versatility of chemical activation, and the availability of different molecular weights. On the other hand, the main disadvantage of the dextrans is their polydispersity. In the current study, CMD-A2-Gd-

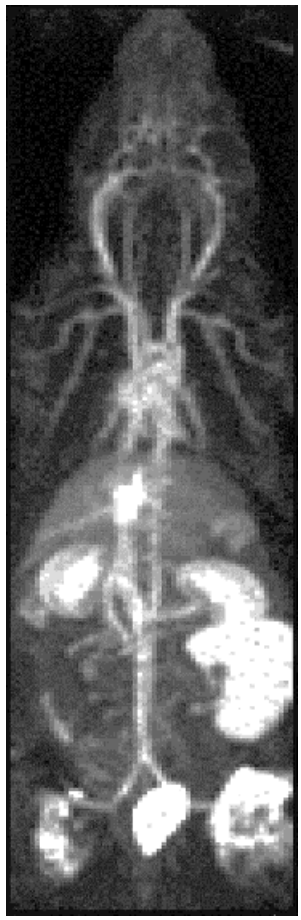

Figure 5. Maximum projection image from three-dimensional MR acquired 2 minutes after injection of $Gd(DO3A)_3$-lys_{16}.

DOTA showed some background enhancement that may be ascribed to the polydispersity of the compound (18). Several pathways for transport through the vascular endothelium are possible. Small molecules are generally transported via intercellular junctions, but as the molecular weight increases, other pathways come into play, such as vesicular transport (20). Dextran is mainly eliminated through the kidneys. Molecules with molecular weights lower than about 15 kDalton pass freely through glomerular filtration with a clearance equal to that of endogenous creatinine. The passage of larger molecules is more restricted, and dextrans with molecular weights above 50 kDalton are practically not excreted, but are completely eliminated from the blood stream by biodegradation. CMD-A2-Gd-DOTA may be useful for MRA. However, its value for qualitative assessment of the intravascular distribution volume or perfusion is limited because distribution volume and arterial input function are not clearly defined, since some gadolinium-labelled dextran polymers have access to the interstitial space, while others do not.

Extracellular Contrast Media

Both Gd-DTPA-BC_2glucA and GdDOTA provided strong vascular and diffuse background enhancement. Unlike GdDOTA, however, Gd-DTPA-BC_2glucA cleared slowly from the circulation, which is in agreement with previous reports (16). Thus, the addition of sugar moieties to the extracellular contrast agent GdDTPA efficiently reduces the rate of its renal excretion (16). This effect is modulated by the length of the linker separating the sugar from the DTPA (16).

The substantial background enhancement of Gd-DTPA-BC_2glucA may not be ideal for MRA. Its slow clearance, however, is favorable for measurement of extracellular space and assessment of myocardial viability. Arheden et al (21,22) and Pereira et al (23,24) demonstrated that the increase of the fraction of nonviable myocytes is paralleled by an expansion of the extracellular distribution volume from 18% in normal myocardium and $>$ 80% in infarcted myocardium (21). Measuring the extracellular space with a rapidly clearing standard extracellular contrast agent, however, is difficult (25,26). If the exchange rate between infarction, normal myocardium, and blood is slower than the clearance rate, equilibrium distribution may not be achieved. Thus, Gd-DTPA-BC_2glucA, with slow clearance from the circulation, would be expected to allow better quantification of the extracellular distribution volume than GdDOTA.

CONCLUSIONS

Both Gd$(DO3A)_3$-lys_{16} and Gd-DTPA-BC_2glucA are slow clearance contrast media. Gd$(DO3A)_3$-lys_{16} provides strong vascular and minor background enhancement, and thus may be helpful for three-dimensional MRA. Gd-DTPA-BC_2glucA, on the other hand, distributes in the extracellular space and may be useful for quantification of the extracellular distribution volume and assessment of myocardial viability.

REFERENCES

1. Panting JR, Taylor AM, Gatehouse PD, et al. First-pass myocardial perfusion imaging and equilibrium signal changes using the intravascular contrast agent NC100150 injection. J Magn Reson Imaging 1999;10:404–410.
2. Anzai Y, Prince MR, Chenevert TL, et al. MR angiography with an ultrasmall superparamagnetic iron oxide blood pool agent. J Magn Reson Imaging 1997;7:209–214.
3. Wagenseil JE, Johansson LO, Lorenz CH. Characterization of t1 relaxation and blood-myocardial contrast enhancement of NC100150 injection in cardiac MRI. J Magn Reson Imaging 1999; 10:784–789.
4. Ogan MD, Schmiedl U, Moseley ME, Grodd W, Paajanen H, Brasch RC. Albumin labeled with Gd-DTPA. An intravascular contrast-enhancing agent for magnetic resonance blood pool imaging: preparation and characterization. Invest Radiol 1987;23:665–571.
5. Schmiedl U, Sievers RE, Brasch RC, et al. Acute myocardial ischemia and reperfusion: MR imaging with albumin-Gd-DTPA. Radiology 1989;170:351–356.
6. Kellar KE, Fujii DK, Gunther WH, Briley-Saebo K, Spiller M, Koenig SH. 'NC100150', a preparation of iron oxide nanoparticles ideal for positive-contrast MR angiography. MAGMA 1999;8:207–213.
7. Grist TM, Korosec FC, Witte S, et al. Steady-state and dynamic MR angiography with MS-325: initial experience in humans. Radiology 1998;207:539–544.
8. Lauffer RB, Parmelee DJ, Dunham SU, et al. MS-325: albumin-targeted contrast agent for MR angiography. Radiology 1998;207: 529–538.
9. Bremerich J, Roberts TP, Wendland MF, et al. Three-dimensional MR imaging of pulmonary vessels and parenchyma with NC100150 injection (Clariscan). J Magn Reson Imaging 2000;11:622–628.
10. Engelbrecht MR, Saeed M, Wendland MF, Canet E, Oksendal AN, Higgins CB. Contrast-enhanced 3D-TOF MRA of peripheral vessels: intravascular versus extracellular MR contrast media. J Magn Reson Imaging 1998;8:616–621.
11. Taylor AM, Panting JR, Keegan J, et al. Safety and preliminary findings with the intravascular contrast agent NC100150 injection for MR coronary angiography. J Magn Reson Imaging 1999;9:220–227.
12. Stuber M, Botnar RM, Danias PG, et al. Contrast agent-enhanced, free-breathing, three-dimensional coronary magnetic resonance angiography. J Magn Reson Imaging 1999;10:790–799.
13. Bourne MW, Margerun L, Hylton N, et al. Evaluation of the effects of intravascular MR contrast media (gadolinium dendrimer) on 3D time of flight magnetic resonance angiography of the body. J Magn Reson Imaging 1996;6:305–310.
14. Adam G, Neuerburg J, Spuntrup E, Muhler A, Scherer K, Gunther RW. Gd-DTPA-cascade-polymer: potential blood pool contrast agent for MR imaging. J Magn Reson Imaging 1994;4:462–466.
15. Moseley ME, White DL, Wang SC, et al. Vascular mapping using albumin-(Gd-DTPA), an intravascular MR contrast agent, and projection MR imaging. J Comput Assist Tomogr 1989;13:215–221.
16. Colet JM, Laurent S, Muller RN. Renal reabsorption, a new strategy in the developement of vascular contrast agents. In: Proceedings of the 8th Annual Meeting of ISMRM, Denver, 2000.
17. Aime S, Botta M, Geninatti Crich S, Giovenzana G, Palmisano G, Sisti M. Novel paramagnetic macromolecular complexes derived from the linkage of a macrocyclic Gd(III) complex to polyamino acids through a squaric acid moiety. Bioconjug Chem 1999;10: 192–199.
18. Corot C, Schaefer M, Beaute S, et al. Physical, chemical and biological evaluations of CMD-A2-Gd-DOTA. A new paramagnetic dextran polymer. Acta Radiol Suppl 1997;412:91–99.
19. Corot C, Violas X, Robert P, Port M. Pharmacokinetics of three gadolinium chelates with different molecular sizes shortly after intravenous injection in rabbits: relevance to MR angiography. Invest Radiol 2000;35:213–218.
20. Paaske WP, Sejrsen P. Transcapillary exchange of 14C-inulin by free diffusion in channels of fused vesicles. Acta Physiol Scand 1977;100:437–445.
21. Arheden H, Saeed M, Higgins CB, et al. Reperfused rat myocardium subjected to various durations of ischemia: estimation of the distribution volume of contrast material with echo-planar MR imaging. Radiology 2000;215:520–528.

22. Arheden H, Saeed M, Higgins CB, et al. Measurement of the distribution volume of gadopentetate dimeglumine at echo-planar MR imaging to quantify myocardial infarction: comparison with 99mTc-DTPA autoradiography in rats. Radiology 1999;211:698–708.
23. Pereira RS, Prato FS, Lekx KS, Sykes J, Wisenberg G. Contrast-enhanced MRI for the assessment of myocardial viability after permanent coronary artery occlusion. Magn Reson Med 2000;44:309–316.
24. Pereira RS, Prato FS, Sykes J, Wisenberg G. Assessment of myocardial viability using MRI during a constant infusion of Gd-DTPA: further studies at early and late periods of reperfusion. Magn Reson Med 1999;42:60–68.
25. Tong CY, Prato FS, Wisenberg G, et al. Measurement of the extraction efficiency and distribution volume for Gd-DTPA in normal and diseased canine myocardium. Magn Reson Med 1993;30:337–346.
26. Diesbourg LD, Prato FS, Wisenberg G, et al. Quantification of myocardial blood flow and extracellular volumes using a bolus injection of Gd-DTPA: kinetic modeling in canine ischemic disease. Magn Reson Med 1992;23:239–253.