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MR lymphangiography with intradermal Gadofosveset and human serum albumin in mice and primates

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Abstract

Purpose—Gadofosveset is a US FDA approved small molecule Gadolinium (Gd) chelate (957Da) which reversibly binds serum albumin and temporally behaves as a macromolecule. As the structure of albumin varies among species, the affinity of Gadofosveset is optimized for human albumin. In this study, Gadofosveset pre-mixed with 10% human serum albumin (HSA) was injected intradermally in mice and monkeys, and then MR lymphangiography was performed on a 3.0 T clinical scanner.

Materials and Methods—Twenty μ l of each agent was injected intradermally at both sides of the front and back paws using a 30-gauge needle into female athymic nude mice (6–8 weeks old, n=3 mice in each group). The performance of Gadofosveset-HSA was compared with Gd-labeled dendrimers (G4: 6nm, G6: 10nm) or Gd-DTPA. The target-to-muscle ratio (TMR= target signal intensity (SI) / muscle SI) was calculated at each time point. The TMRs were compared with a one-way ANOVA followed by a Bonferroni multiple comparison test.

Results—Images taken as early as 2.5 min after intradermal (id) injection depicted enhanced lymph nodes using Gadofosveset-HSA (2.41 $\pm$ 0.20). Up to 7.5 min after injection, TMRs of Gadofosveset-HSA were greater than those of dendrimers (G4 or G6-Gd-DTPA: 2.24 $\pm$ 0.10, 2.12 $\pm$ 0.11, respectively). By 15 minutes post injection TMRs of Gadofosveset-HSA (2.18 $\pm$ 0.19) were comparable to Gd-labeled dendrimers (G4-Gd-DTPA: 2.37 $\pm$ 0.15, G6-Gd-DTPA: 2.25 $\pm$ 0.18). Gadofosveset-HSA and Gd labeled dendrimers resulted in satisfactory MR lymphography in mice and monkeys.

Conclusion—Since both Gadofosveset and HSA are approved for human use and Gadofosveset clears rapidly through the kidneys, this method has advantages over Gd-dendrimers and could be used for visualizing lymphatic drainage and detecting lymph nodes.

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Keywords

Gadofosveset; MR lymphangiography; sentinel lymph node; contrast agents; lymphatic drainage

Introduction

The lymphatic circulation is a complex network of vessels and lymph nodes that serves an important role in host defense. In many types of cancer, the presence of regional lymph node metastasis is a key prognostic factor. Most imaging criteria for abnormal nodes are based on short axis node size (1–9). Subcentimeter lymph nodes may harbor cancer (10) and large lymph nodes can be inflammatory and therefore, a size criterion has proven insensitive for the detection of lymph node metastases.

In contrast to systemically administered agents, there are several techniques that rely on interstitial intradermal injection. Macromolecular agents, injected intradermally, are taken up rapidly by the lymphatics draining the site injected. These agents are employed clinically in the assessment of lymphedema and detection of sentinel lymph nodes. Examples include the interstitial injection of radionuclide-labeled sulfur colloid or albumin (11–14).

Macromolecular MRI contrast agents (15), including Gd-labeled dendrimers, have been previously employed as interstitial lymphangiographic agents (16, 17). One class of agents, Gd-labeled dendrimers, have been investigated extensively for nodal and lymphatic imaging (18). Gadofosveset, is a small molecular weight Gd-chelate that reversibly binds albumin making it an MR blood pool agent (19–22). In its albumin-bound form, Gadofosveset is a macromolecule making it suitable for visualizing the lymphatic system after interstitial injection (23, 24). However, this is difficult to study in animals because Gadofosveset is optimized to bind human albumin whereas the affinities for rodents is lower (20, 25, 26).

The purpose of this study is to develop and optimize a new method of MR lymphangiography with Gadofosveset, an FDA-approved, protein-binding small molecular MRI contrast agent in mice. Additionally, we verify the optimal preparation, Gadofosveset pre-mixed with 10% has, also similarly work in monkeys.

Materials and methods

Reagents

Gadofosveset trisodium, Gadofosveset (Ablavar®; 250mM) was purchased from Lantheus Medical Imaging, Inc. (North Billerica, MA, USA). Human serum albumin (HSA) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Fetal bovine serum (FBS) was purchased from Gibco (Gaithersburg, MD, USA). A generation-6 (G6; MW 58 kDa) or a generation-4 (G4; MW 14kDa) polyamidoamine dendrimer and 30% Gd-[DTPA]-dimeglumine (Gd-DTPA, Magnevist®; 500mM) was purchased from Bayer Healthcare Pharmaceuticals (Montville, NJ, USA).

Preparation of contrast agents

Gadofosveset solutions (250mM) were diluted to a concentration of 30mM with PBS, mouse serum, 10% HSA and FBS, respectively. A G6 polyamidoamine dendrimer (G6) and a G4 polyamidoamine dendrimer (G4) coupled with the 1B4M–diethylene triamine pentaacetic acid (DTPA) bifunctional chelating agent and Gd(III) ions was synthesized to perform MR lymphangiography, respectively (G6-Gd-DTPA, G4-Gd-DTPA). The details of the dendrimer contrast agent synthesis were previously described (16, 27, 28). Physical and chemical characteristics of all MRI contrast agents used in this study are summarized in Table 1.

The concentrations of a G6-Gd-DTPA, a G4-Gd-DTPA and a Gd-DTPA were adjusted to 30mMGd with PBS.

Contrast agent administration

All in vivo procedures were carried out in compliance with the Guide for the Care and Use of Laboratory Animal Resources (1996, National Research Council) and were approved by the local Animal Care and Use Committee.

Six-to 8-week-old female homozygote athymic nude mice were purchased from Charles River (National Cancer Institute, Frederick). Mice were anesthetized by inhaled isoflurane (5% for induction and 2% for maintenance) supplemented with oxygen. Mice were placed in a mouse cradle and placed in a homebuilt MR coil in the prone position. Body temperature was maintained at ~36 °C using a heating pad. During the measurements, the breathing rates of the mice were monitored using a Biopac System MP150 (Biopac Inc., Goleta, California). Respiration rate was maintained at 25–30 respirations per minute. Twenty μ l of 30mM contrast agents were prepared and were injected intradermally at both sides of the front and back paws using a 30-gauge needle. Each contrast agent injection site was gently massaged ten times.

Seven groups of nude mice (N = 3 in each group) were injected with the following agents: (1) Gadofosveset in PBS; (2) Gadofosveset mixed with mouse serum; (3) Gadofosveset in 10% HSA; (4) Gadofosveset in FBS; (5) Gd-DTPA; (6) G6-Gd-DTPA; (7) G4-Gd-DTPA.

MR lymphangiography

MR lymphangiography was performed using an Intera Achieva 3.0 T clinical scanner (Philips Medical Systems, Cleveland, OH) equipped with an in-house 1-inch saddle type dedicated mouse receiver coil array.

A coronal T1-weighted high-resolution isotropic volume examination (THRIVE) sequence, three-dimensional (3D) ultra-fast spoiled gradient MRI sequence incorporating a frequency-selective fat-saturation pulse, was performed (TR/TE = 6.4/3.2, flip angle = 20°, number of slices = 83; section thickness = 0.6 mm, intersection gap = 0.3 mm, bandwidth = 393 Hz/pixel, NSA = 3, field of view (FOV) = 80×30 mm, matrix = 268 × 266, and acquisition time = 145 seconds).

After an unenhanced THRIVE sequence was performed, contrast agents were administrated and then same MR sequences were repeated 6 times every 150 seconds.

Image analysis

Regions of interest (ROIs) were drawn by one author (T.N with 5 years experience) around the axillary lymph nodes, kidney cortex and left ventricle of the heart on the coronal image. ROIs of the back muscle at each time point were used as reference tissue. The target-to-muscle ratio (TMR) was calculated at each time point as follows: $TMR = \text{target SI} / \text{muscle SI}$, where SI is the signal intensity as measured by ROI.

Monkey study

For further investigation of MR lymphangiography, three squirrel monkeys (bodyweight: 0.8kg) were purchased from Keeling Center for Comparative Medicine and Research (The University of Texas MD Anderson Cancer Center, Bastrop, TX) and fully anesthetized with a solution containing 10% ketamine hydrochloride (0.7 ml/kg ketamine; Sanofi-Ceva, Düsseldorf, Germany) and inhaled 2% isoflurane supplemented with oxygen.

MR lymphangiography of both hind legs was performed after sterile injection of both dorsal hind pads of each monkey. Subsequently, 0.8 ml of Gadofosveset (30mM) diluted by 10% HSA was administered intradermally into the interdigital skin fold of leg pad. This dose corresponded to 0.03 mmol/kg of body weight. After each injection the site was gently massaged for approximately 10 times and MR lymphangiography was acquired with a coronal THRIVE sequence repeatedly; TR/TE = 7.0/3.3, flip angle = 20°, number of slices = 160; section thickness = 0.8 mm, intersection gap = 0.4 mm, bandwidth = 289 Hz/pixel, NSA = 2, FOV = 256×72 mm, matrix = 640 × 180, and acquisition time = 220 seconds. The thoracic ducts were assessed visually in all three monkeys using an Philips MRI electronic workstation.

Statistical analysis

The TMRs were expressed as the mean $\pm$ S.E.M. The TMRs of axillary LNs and LN-to-kidney ratios were compared with a one-way ANOVA followed by a Bonferroni multiple comparison test. Especially, the LN-to-kidney ratios were focused on the differences between Gd-DTPA, as a small molecule, and other agents. Asterisks in the figures indicate the statistical significance as follows: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

Results

Gadofosveset-HSA showed lymph nodes with high contrast on early, mid and late phases of MR lymphography (Figure 1)

The first phase of images were obtained at 2.5 min after injection and depicted lymph nodes as high signal with high contrast compared to muscle using Gadofosveset-HSA (2.41 ± 0.20 , mean $\pm$ S.E.M.) while dendrimers showed minimal enhancement (G4-Gd-DTPA: 1.91 ± 0.08 , G6-Gd-DTPA: 1.97 ± 0.10). At 7.5 min after injection, the TMRs of Gadofosveset-HSA (2.29 ± 0.15) were superior to those of dendrimers (G4-Gd-DTPA:

2.24 $\pm$ 0.10, G6-Gd-DTPA: 2.12 $\pm$ 0.11). At 15 minutes Gadofosveset-HSA continued to demonstrate high TMRs (2.18 $\pm$ 0.19) but by this time dendrimer enhanced lymphatics were seen as well (G4-Gd-DTPA: 2.37 $\pm$ 0.15, G6-Gd-DTPA: 2.25 $\pm$ 0.18).

In contrast to Gadofosveset-HSA and dendrimers, Gd-DTPA by itself, only transiently was noted in the lymph nodes, which were barely visible. Gadofosveset mixed in by PBS, FBS and mouse serum had similar behavior to Gd-DTPA with poor visualization of the lymphatics (Figure 2AB).

The LN-to-kidney ratios with Gadofosveset-HSA (ranging 1.22 to 1.85) were higher than those of the other tested mixtures (ranging 0.73 to 1.31, $P < 0.05$). The signal intensity of the kidney (ranging 1.29 to 1.84) and heart (ranging 1.40 to 1.64) with Gadofosveset-HSA were not higher than the signals of lymph nodes (ranging 2.18 to 2.40) at any time points. The other combinations tested agents demonstrated reversed TMRs between LNs and kidneys and the TMR of Gd-DTPA showed even lower ratios of LN to kidney suggesting that the agent was absorbed rapidly in the vasculature and excreted by the kidneys.

The Gadofosveset-HSA revealed the highest LN-to-kidney ratio at 2.5 min ($P < 0.0001$ vs Gd-DTPA, $P < 0.05$ vs other pre-mixed Gadofosveset) and decreased over time but still demonstrated significant differences with the other agents tested ($P < 0.05$). As expected, all combinations with Gadofosveset other than HSA (+PBS, +FBS + mouse serum) were excreted by the kidney so that their TMRs were reduced and no significant difference was seen with Gd-DTPA (Figure 3).

Enhancement in the blood pool and at the injection site

The TMRs from left ventricle with any mixture of Gadofosveset (+PBS, +FBS, + mouse serum, and +HSA) was approximately 1.8 or less. In contrast Gd-labeled dendrimers (G4, G6) demonstrated even lower enhancement of the left ventricles.

Dendrimers and Gadofosveset-HSA showed minimal signal at the injection site due to high T2 relaxivities of these contrast agents. Injection sites of other preparations of Gadofosveset were shown as high T1 signals (Figure 4).

Gadofosveset-HSA MR lymphangiography in monkeys

The intradermal injection of Gadofosveset pre-mixed with 10% HSA at hind leg pads clearly depicted infra-diaphragmatic portion of the deep lymphatic duct in monkeys. The 3D-THRIVE images clearly visualized lymphatic duct 4 min after intradermal injection or later (Figure 5). Images of MR lymphangiography were consistent in all three monkeys. For both mice and monkeys the final injection dose was 0.03mmol Gd/kg

Discussion

MRI has several desirable properties for visualization of the lymphatic channels and lymph nodes. It has higher contrast and temporal resolution than other techniques, such as radionuclide-labeled sulfur colloid or albumin and is capable of defining even small lymphatic channels using three-dimensional images. It does not involve ionizing radiation

and unlike radiopharmaceuticals, MR contrast agents have long shelf lives. MR lymphangiography could be used for sentinel lymph node detection, or for diagnosing lymphatic vessel abnormalities or injury (4, 7, 15, 29, 30).

Gadofosveset pre-mixed with human serum albumin successfully depicted lymphatic drainage after intradermal injection in mice and monkeys. Because Gadofosveset is a small molecule similar to Gd-DTPA, when it was injected without HSA, it quickly entered the vascular network and only faintly opacified the lymphatics. However, when albumin was premixed with Gadofosveset it behaved in a manner similar to dendrimers with regard to depicting the lymphatics. Moreover, in comparison to dendrimers, it was more rapidly seen in the lymphatic system (23, 26).

The R1 relaxivity of Gadofosveset is increased after binding to albumin. At 1.0 T there is a 10 fold difference in signal between albumin bound and unbound Gadofosveset (20). Therefore, premixing with HSA would be expected to markedly increase the signal associated with Gadofosveset improving lymphatic imaging though the injection dose was much lower than the dose for vessel imaging. The interstitial injection of 30 mM Gadofosveset was sufficient to define the draining lymphatic system and lymph nodes. However, after 20 minutes, the injected site became difficult to visualize probably due to dissociation of the albumin from the Gadofosveset, resulting in dispersion of the agent locally. This is actually a desirable property as it enables the rapid clearance from the injection site with subsequent renal excretion. This is in contrast to dendrimers that only slowly disperse at the injection site and then are slowly excreted from the liver. Thus, Gadofosveset is likely a safer alternative than dendrimer MR lymphography (20).

There are two potential limitations for translating our optimal method evaluated in this study toward the clinical application. Since mice weigh approximately 25g, and the physical size of their lymphatics are smaller, it is important to test the method in larger animals. Therefore, although further studies for optimizing dosing methods and evaluating toxicity might be needed toward clinical translation, we examined our optimized method in monkeys and validated the success. Per standard animal research guidelines, the animals were anesthetized. Therefore we do not have information regarding the pain of injection. Although we anticipate such a subdermal injection to be well tolerated in humans, we might have to co-inject contrast agents with local anesthetics.

In conclusion, Gadofosveset pre-mixed with HSA produced excellent imaging of the lymphatic system after interstitial injection in both mice and monkeys. Gadofosveset-HSA resulted in rapid visualization of the lymphatics, both lymphatic channels and draining lymph nodes, that persisted for 20 – 30 minutes. Gadofosveset-HSA also rapidly excreted by the kidneys compared with macromolecular agents covalently conjugated with Gd-chelates, that is favorable factors for potential clinical translation. It is unclear whether Gadofosveset will need to be pre-mixed with HSA in humans, but even if this proves to be the case both components are approved by global health authorities also favoring clinical translation. Thus, Gadofosveset and HSA could be readily used for visualizing lymphatic drainage and detecting sentinel lymph nodes in clinical practice.

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References

1. Suga K, Shimizu K, Kawakami Y, et al. Lymphatic drainage from esophagogastric tract: feasibility of endoscopic CT lymphography for direct visualization of pathways. *Radiology*. 2005; 237:952–960. [PubMed: 16304114]
2. Suga K, Yuan Y, Okada M, et al. Breast sentinel lymph node mapping at CT lymphography with iopamidol: preliminary experience. *Radiology*. 2004; 230:543–552. [PubMed: 14699178]
3. Minato M, Hirose C, Sasa M, Nishitani H, Hirose Y, Morimoto T. 3-Dimensional Computed Tomography Lymphography-Guided Identification of Sentinel Lymph Nodes in Breast Cancer Patients Using Subcutaneous Injection of Nonionic Contrast Medium: a Clinical Trial. *Journal of computer assisted tomography*. 2004; 28:46–51. [PubMed: 14716231]
4. Misselwitz B. MR contrast agents in lymph node imaging. *European journal of radiology*. 2006; 58:375–382. [PubMed: 16464554]
5. Notohamiprodjo M, Weiss M. MR lymphangiography at 3.0 T: Correlation with Lymphoscintigraphy. *Radiology*. 2012; 264:78–87. [PubMed: 22523325]
6. Hama Y, Bernardo M, Regino CAS, et al. MR lymphangiography using dendrimer-based contrast agents: a comparison at 1.5T and 3.0T. *Magnetic resonance in medicine*. 2007; 57:431–436. [PubMed: 17260373]
7. Kobayashi H, Kawamoto S, Sakai Y, et al. Lymphatic drainage imaging of breast cancer in mice by micro-magnetic resonance lymphangiography using a nano-size paramagnetic contrast agent. *Journal of the National Cancer Institute*. 2004; 96:703–708. [PubMed: 15126607]
8. Sena LM, Fishman SJ, Jenkins KJ, et al. Magnetic resonance lymphangiography with a nano-sized gadolinium-labeled dendrimer in small and large animal models. *Nanomedicine*. 2010; 5:1183–1191. [PubMed: 21039196]
9. Kobayashi H, Kawamoto S, Star RA, Waldmann TA, Tagaya Y, Brechbiel MW. Micro-magnetic Resonance Lymphangiography in Mice Using a Novel Dendrimer-based Magnetic Resonance Imaging Contrast Agent. *Cancer research*. 2003; 63:271–276. [PubMed: 12543772]
10. Harisinghani MG, Barentsz J, Hahn PF, et al. Noninvasive detection of clinically occult lymph-node metastases in prostate cancer. *The New England journal of medicine*. 2003; 348:2491–2499. [PubMed: 12815134]
11. Quon A, Gambhir SS. FDG-PET and beyond: molecular breast cancer imaging. *Journal of clinical oncology*. 2005; 23:1664–1673. [PubMed: 15755974]
12. Veronesi U, De Cicco C, Galimberti VE, et al. A comparative study on the value of FDG-PET and sentinel node biopsy to identify occult axillary metastases. *Annals of oncology*. 2007; 18:473–478. [PubMed: 17164229]
13. Zornoza G, Garcia-Velloso MJ, Sola J, Regueira FM, Pina L, Beorlegui C. 18F-FDG PET complemented with sentinel lymph node biopsy in the detection of axillary involvement in breast cancer. *European Journal of Surgical Oncology (EJSO)*. 2004; 30:15–19.
14. Wagner JD, Schauwecker D, Davidson D, et al. Inefficacy of F-18 fluorodeoxy-D-glucose-positron emission tomography scans for initial evaluation in early-stage cutaneous melanoma. *Cancer*. 2005; 104:570–579. [PubMed: 15977211]
15. Lucarelli RT, Ogawa M, Kosaka N, Turkbey B, Kobayashi H, Choyke PL. New approaches to lymphatic imaging. *Lymphatic research and biology*. 2009; 7:205–214. [PubMed: 20143919]
16. Kobayashi H, Kawamoto S, Bernardo M, Brechbiel MW, Knopp MV, Choyke PL. Delivery of gadolinium-labeled nanoparticles to the sentinel lymph node: comparison of the sentinel node visualization and estimations of intra-nodal gadolinium concentration by the magnetic resonance imaging. *Journal of controlled release*. 2006; 111:343–351. [PubMed: 16490277]

17. Barrett T, Kobayashi H, Brechbiel M, Choyke PL. Macromolecular MRI contrast agents for imaging tumor angiogenesis. *European journal of radiology*. 2006; 60:353–366. [PubMed: 16930905]
18. Kobayashi H, Brechbiel MW. Nano-sized MRI contrast agents with dendrimer cores. *Advanced drug delivery reviews*. 2005; 57:2271–2286. [PubMed: 16290152]
19. Grist T, Korosec F, Peters D. Steady-state and dynamic MR angiography with MS-325: initial experience in humans. *Radiology*. 1998; 207:539–544. [PubMed: 9577507]
20. Lauffer R, Parmelee D, Dunham S. MS-325 : Albumin-targeted Contrast Agent for MR Angiography. *Radiology*. 1998; 207:529–538. [PubMed: 9577506]
21. Lauffer RB, Parmelee DJ, Ouellet HS, et al. MS-325: a small-molecule vascular imaging agent for magnetic resonance imaging. *Academic radiology*. 1996; 3(Suppl 2):S356–S358. [PubMed: 8796603]
22. Yang M, Hoppmann S, Chen L, Cheng Z. Human serum albumin conjugated biomolecules for cancer molecular imaging. *Current pharmaceutical design*. 2012; 18:1023–1031. [PubMed: 22272822]
23. Caravan P, Cloutier NJ, Greenfield MT, et al. The interaction of MS-325 with human serum albumin and its effect on proton relaxation rates. *Journal of the American Chemical Society*. 2002; 124:3152–3162. [PubMed: 11902904]
24. Normal M-C, Herborn CU, Lauenstein TC, et al. Interstitial MR Lymphography with MS-325: Characterization of Normal and Tumor-Invaded Lymph Nodes in a Rabbit Model. *American Journal of Roentgenology*. 2002; 179:1567–1572. [PubMed: 12438057]
25. Caravan P, Parigi G, Chasse JM, et al. Albumin binding, relaxivity, and water exchange kinetics of the diastereoisomers of MS-325, a gadolinium(III)-based magnetic resonance angiography contrast agent. *Inorganic chemistry*. 2007; 46:6632–6639. [PubMed: 17625839]
26. Fasano M, Curry S, Terreno E, et al. The extraordinary ligand binding properties of human serum albumin. *IUBMB life*. 2005; 57:787–796. [PubMed: 16393781]
27. Kobayashi H, Kawamoto S, Jo S-K, Bryant HL, Brechbiel MW, Star RA. Macromolecular MRI contrast agents with small dendrimers: pharmacokinetic differences between sizes and cores. *Bioconjugate chemistry*. 2003; 14:388–394. [PubMed: 12643749]
28. Kobayashi H, Sato N, Hiraga A, et al. 3D-micro-MR angiography of mice using macromolecular MR contrast agents with polyamidoamine dendrimer core with reference to their pharmacokinetic properties. *Magnetic resonance in medicine*. 2001; 45:454–460. [PubMed: 11241704]
29. Longmire MR, Ogawa M, Choyke PL, Kobayashi H. Biologically optimized nano-sized molecules and particles: more than just size. *Bioconjugate chemistry*. 2011; 22:993–1000. [PubMed: 21513351]
30. Kobayashi H, Brechbiel MW. Dendrimer-based nanosized MRI contrast agents. *Current pharmaceutical biotechnology*. 2004; 5:539–549. [PubMed: 15579043]

Figure 1.

Serial coronal MR lymphangiography images after intradermal injection of 3 different contrast agents (top: Gadofosveset-HSA, middle: G4-Gd-DTPA, bottom: Gd-DTPA) into both sides of the front and back paws. MR lymphangiography images were obtained with T1-weighted 3D-Thirve sequence every 2.5 min after injection of contrast agents. The axillary LNs (arrows) were clearly depicted with both Gadofosveset-HSA and G4-Gd-DTPA. In contrast, the axillary LNs (arrows) were not visualized clearly with Gd-DTPA. The background signals in normal structures minimally increased after injection of G4-Gd-DTPA, however, visibly increased after injection of both Gadofosveset-HSA and Gd-DTPA.

Figure 2.

Target-to-muscle ratios (TMRs) of T1 signals in dynamic contrast MR lymphangiography after injection of various preparations of Gadofesveset and different contrast agents are shown. Serial TMRs in axillary LNs (red line), left ventricles (green) and kidney cortexes (purple) were plotted every 2.5 min after injection of contrast agents (A). Gadofesveset-HSA and dendrimers showed superior TMR in axillary LNs to Gd-DTPA or Gadofesveset mixed with PBS, FBS, and mouse serum, which poorly visualized the lymphatic system (B).

Figure 3.

Axillary LN-to-kidney ratios of T1 signals in dynamic contrast MR lymphangiography after injection of various preparations of Gadofesveset and different contrast agents are plotted. Axillary LN-to-kidney ratios with Gadofesveset-HSA are higher than those of Gadofesveset other preparations or Gd-DTPA. Statistical differences are analyzed with a one-way ANOVA followed by a Bonferroni multiple comparison test (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ for Lymph node-to-Kidney ratio compared to Gd-DTPA

Figure 4.

Maximum intensity projection (MIP) images of MR lymphangiography 2.5 and 15 min after injection of different contrast agents; Gadofosveset, Gadofosveset-HSA, Dendrimers (G4-Gd-DTPA and G6-Gd-DTPA) and Gd-DTPA are shown. The axillary LNs (arrows) and the injection sites in both paws (arrowheads) were depicted. Dendrimers and Gadofosveset-HSA showed minimal signal at the injection site due to high local concentration and superior T1 and T2 relaxivities of these contrast agents. Injection sites of Gadofosveset or Gd-DTPA were shown as high T1 signals.

Figure 5.

The intradermal injection of Gadofosveset pre-mixed with 10%HSA (Gadofosveset-HSA) at a hind leg pad clearly depicted deep lymphatic vessels in monkeys. The 3D-THRIVE images clearly visualized lymphatic vessel 4 min after intradermal injection and lasted up to 30 min after injection.

Table

Chemical and physical characteristics of MRI contrast agents used in this study.

Contrast agents	MW (Da)	Size (nm)	Gd/agent	r1 (/s/mM)
Gadofosveset in PBS	957	<1	1	5
Gadofosveset in 10% HSA	68000 (+HSA)	7	2–3	8
Gadofosveset in serum	various	various	ND	8
G4-Gd-DTPA	58000	6	64	12
G6-Gd-DTPA	230000	10	200	15
Gd-DTPA	938	<1	1	4