Magnetic Resonance Lymphography of the Thoracic Duct after Interstitial Injection of Gadofosveset Trisodium: A Pilot Dosing Study in a Porcine Model

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Abstract

Background-rationale: To investigate whether interstitial injection of gadofosveset trisodium (Ablavar®, Lantheus Medical, North Billerica, MA) would be suitable for thoracic duct (TD) imaging in a pig model.

Methods and Results: Gadofosveset trisodium alone or premixed with 10% human serum albumin (HSA) was administered intradermally in the extremities of pigs at varying doses to visualize the TD by MRI. Two blinded readers evaluated MRIs for TD visibility. The inter-observer variability for all MR imaging sessions was assessed using the Spearman rank correlation test. MR lymphography using gadofosveset trisodium premixed with HSA yielded superior visualization of the TD compared to gadofosveset trisodium alone, with a high inter-observer agreement (correlation coefficient of 0.88 ($p=0.00000115$)).

Conclusions: We demonstrate that gadofosveset trisodium (premixed with 10% HSA) can be injected intradermally in order to perform MR lymphography of the thoracic duct. Since this agent is already FDA approved for MR imaging, the off-label use of it for imaging of the thoracic duct in humans is feasible, and the approach may prove to be beneficial for patients with TD abnormalities.

Introduction

THE THORACIC DUCT (TD) is the largest lymphatic vessel, and receives lymphatic drainage from over three-quarters of the body. The TD can be damaged during cardiac, thoracic, and major neck surgeries, leading to lymphatic leaks or obstruction.¹ Complications include chylothorax and chylopericardium, which eventually result in protein malnutrition, cardiac failure, electrolyte imbalance, immune abnormalities, respiratory impairment, and cardiac failure.² However, the TD is difficult to visualize on conventional computed tomography (CT) or magnetic resonance imaging (MRI). Lymphoscintigraphy has insufficient spatial resolution, and its main role is in sentinel lymph node imaging.³ X-ray lymphography involves cannulation of a lymphatic vessel on the dorsum of the foot with the injection of an oil-based iodinated contrast agent. Although the lower lymphatic vessels are visualized, the TD is often incompletely seen. Because this method is invasive and uncomfortable for patients and in-

cludes a risk of pulmonary embolism, it is rarely performed.⁴ Ultrasound microbubbles can be used for imaging local lymphatic drainage, but visualization of the TD is not possible. Without adequate visualization of the TD, repair is difficult. Therefore, identifying the TD anatomy and the site of injury prior to surgery is vital to successful outcomes.

For these reasons, investigators have pursued other interstitially injected contrast agents that are taken up by the lymphatics and drain into the TD. Since the lymphatic vessels are highly selective for nano-sized molecules, the agents used are generally in the 6–20 nm range of diameter.^{5–8} Sena et al.⁵ evaluated a 17 nm sized gadolinium (Gd) labeled dendrimer for MR lymphography both in small and large animal models and successfully visualized the deep lymphatic vessels (including the TD) with a low Gd dose (1 μ mol Gd/kg). Unfortunately, dendrimer-based contrast agents are not approved for human use and demonstrate slow clearance from the body.

Gadofosveset trisodium (Ablavar® Lantheus Medical, North Billerica, MA) is an FDA approved Gd chelate for

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intravenous use with a prolonged intravascular half-life secondary to its reversible binding to serum albumin and resultant increase in molecule size.⁹ Gadofosveset trisodium is currently used as a blood pool agent for MR angiography studies with similar safety profile to existing clinically available Gd chelates.¹⁰ Herborn et al. evaluated the pre-FDA-approved version of gadofosveset trisodium (known as MS-325) for interstitial lymphography in a rabbit model and reported its utility in visualizing lower extremity and abdominal lymphatic vessels and lymph nodes.¹¹ In this study, we investigated whether the clinically approved version of gadofosveset trisodium would be suitable for TD imaging in a pig model, which is a size appropriate model for humans, as a prelude to human studies.

Materials and Methods

Contrast agent

All studies were approved by the National Heart Lung and Blood institutional Animal Care and Use Committee. Gadofosveset trisodium (Ablavar® Lantheus Medical Imaging, Billerica, MA, USA) was purchased commercially; it has a Gd concentration of 250 mM. Gadofosveset trisodium is designed to bind human serum albumin (HSA) with high affinity; however, its binding to porcine albumin is thought to be lower. Therefore, initial studies focused on interstitial injections (into the hind foot of the pig) of gadofosveset trisodium with and without the addition of albumin (premixed with phosphate buffered serum (PBS) (Mediatech Inc, Manassas, VA, USA) or 10% HSA (Grifols Therapeutics Inc, Research Triangle Park, NC, USA)).

Study design

Three pigs were employed for initial studies of PBS versus HSA premixing experiments, and four pigs were used for dosing studies using HSA premixed with gadofosveset trisodium. The initial studies were conducted at 3T (Achieva, Philips, The Best, Netherlands), and the subsequent studies were performed at 1.5T (Achieva). Pigs varied in weight between 18 and 42 kg. All procedures were performed in accordance with guidelines on the use of animals in research with approval by the respective Animal Care and Use Committees (ACUC). For the initial studies, the same pig was injected with gadofosveset trisodium premixed with phosphate-buffered serum (PBS), separated by 14 days with gadofosveset trisodium premixed with 10% human serum albumin (HSA). In the follow-up experiment, pigs (n=4) received a total of six total hind foot injections (two pigs injected in both feet). Three different concentrations of gadofosveset (10 mM, 33 mM and 90 mM) were used as described below.

In vivo imaging

Similar imaging procedures were performed at 1.5T and 3.0T. After an overnight fast with access to water ad libitum,

the animal was pre-anesthetized with a combination of ketamine hydrochloride (25 mg/kg) and midazolam (0.5 mg/kg) administered intramuscularly. The animal was intubated with an appropriate size endotracheal tube and was transferred to the MRI scanner. The animal was placed in the left decubitus position while general anesthesia was maintained with inhaled 1%–2% isoflurane delivered through a vaporizer and circle absorption breathing system using an MRI compatible anesthetic machine and ventilator (Penlon, Nuffield Anesthesia Ventilator Series 200, Oxfordshire, UK or Surgivet, Smiths Medical PM, Inc., Waukesha, WI, USA). During the MR imaging, the animal's vital signs were monitored with an MRI compatible monitoring system (In vivo systems, Orlando, FL, USA).

A baseline 3D T1-weighted (T1W) sequence with fat suppression was acquired with a cardiac or torso coil (XR Torso Coil, Philips Medical Systems, Best, Netherlands). Then, gadofosveset trisodium was administered intradermally into the hind foot and four phases of post-injection T1W MRI were acquired. This was followed by gentle massaging of the injection site, and another four phases of post-injection T1W MRI were acquired. MR acquisition parameters are presented in Table 1. If an additional injection was performed, it occurred at least 30 min after the initial injection.

Image analysis

All MR image analysis was performed by two readers on a commercially available work station (Extended MR WorkSpace [EWS], Philips). For each imaging session, baseline MR images were subtracted from the post-injection follow up MR images at each phase and the degree of contrast enhancement within the TD was evaluated on both subtracted and raw MR images using a 4-point scoring system (0=no enhancement within the TD, 1=partial enhancement of the TD, 2=complete but faint enhancement of the TD, 3=complete enhancement of the TD). An overall score (0–3) was given for each imaging session. Additionally, the inter-observer variability for all MR imaging sessions was assessed using the Spearman rank correlation test.

Results

Comparison of gadofosveset trisodium in PBS vs. gadofosveset trisodium premixed with human serum albumin

Three pigs underwent six injections of 3 mL of 10 mM gadofosveset + PBS (Gd dose=1 μ mol Gd/kg) in the hind feet. The TD was completely visible (Score=2) in two animals, was partially visible (Score=1) in three animals, and was not visible (Score=0) in one animal for reader 1. The TD was completely visible (Score=2) in two animals, was partially visible (Score=1) in two animals, and was not visible (Score=0) in two animals for reader 2.

TABLE 1. MR IMAGING PARAMETERS USED IN THE CURRENT STUDY

	TR/TE (msec)	Matrix	Field of view (mm)	Slice thickness (mm)	Flip angle	Voxel size (mm×mm×mm)	Acquisition time (sec)
3 Tesla	3.6/1.73	372×293	370	0.7	10	0.7×0.7×0.7	223
1.5 Tesla	9.3/4.3	416×415	350	1	10	0.84×0.84×1	201

Three pigs (six injections) underwent MR lymphography with 3 mL of 10 mM gadofosveset trisodium premixed with 10% HSA (Gd dose = $1 \mu\text{mol Gd/kg}$). Among the six injections, the TD was completely visible (Score = 3) in two animals, partly visible (Score = 2) in three animals, and poorly visible (Score = 1) in one animal for reader 1. The TD was completely visible (Score = 3) in two animals, partly visible (Score = 2) in two animals, and poorly visible (Score = 1) in two animals for reader 2. Serial image analysis revealed that enhancement did not improve after massage. Therefore, initial studies documented that MR lymphography with gadofosveset trisodium + 10% HSA was superior to gadofosveset trisodium alone for depicting the TD (Fig. 1).

Comparison of different concentrations of gadofosveset trisodium premixed with human serum albumin

Four pigs underwent MR lymphography at varying concentrations of gadofosveset + 10% HSA resulting in six injections. Two injections of dose 1 (3 mL of 10 mM Ablavar + 10% HSA (Gd dose = $1.5 \mu\text{mol Gd/kg}$) resulted in scores of 1 and 2 for both reader 1 and 2. Two injections of

Dose 2 (3 mL of 33 mM Ablavar + 10% HSA (Gd dose = $5 \mu\text{mol Gd/kg}$)) was given, resulting in scores of 2 and 3 for reader 1 and scores of 1 and 3 for reader 2. Finally, two injections of dose 3 which was a lower injection volume but higher Gd concentration, thus holding the Gd dose constant (1 mL of 90 mM gadofosveset + 10% HSA (Gd dose = $5 \mu\text{mol Gd/kg}$)) resulted in scores of 2 and 3 for reader 1, and scores of 2 and 2 for reader 2.

Thus, MR lymphography with gadofosveset + HSA at a Gd concentration of 33 mM revealed the optimal contrast enhancement and visibility of the TD in comparison to lower Gd concentrations (10 mM). The higher concentration (90 mM) enabled a lower injection volume (1 mL vs. 3 mL) with a similar TD visibility and diagnostic quality and thus did not seem advantageous from a visualization viewpoint (Fig. 2 and Table 2).

The Spearman rank correlation test yielded a correlation coefficient of 0.88 ($p = 0.00000115$), suggesting a substantial agreement between readers 1 and 2 for TD visibility assessment.

Discussion

This study demonstrates the feasibility of using an FDA-approved albumin-binding gadolinium chelate to visualize the thoracic duct. Interstitial injections of gadofosveset mixed with 10% human serum albumin reliably depicted the thoracic duct with sufficient clarity in a normal pig. Gadofosveset trisodium is expected to bind HSA following *ex vivo* mixing,

FIG. 1. Sagittal maximum intensity projection MR image obtained at 3T demonstrates the thoracic duct in a 35 kg pig after injection of $1 \mu\text{mol Gd/kg}$ (3 mL/10 mM gadofosveset trisodium premixed with HSA). Thoracic duct visibility was rated as 3 for both readers for this injection and imaging session.

FIG. 2. Sagittal maximum intensity projection MR image obtained at 1.5T demonstrates the thoracic duct in a 20 kg pig after injection of $5 \mu\text{mol Gd/kg}$ (3 mL/33 mM gadofosveset trisodium premixed with HSA). Thoracic duct visibility was rated as 3 for both readers for this injection and imaging session.

TABLE 2. THORACIC DUCT (TD) VISIBILITY ASSESSMENT SCORES FOR READERS 1 AND 2 IN ALL INJECTIONS

<i>Injected Agent</i>	<i>Injected volume (ml)</i>	<i>Reader 1</i>	<i>Reader 2</i>
1 μ mol Gd/kg (10mM gadofosveset trisodium premixed with 10%HSA)	3	3	3
1 μ mol Gd/kg (10mM gadofosveset trisodium premixed with 10%HSA)	3	2	2
1 μ mol Gd/kg (10mM gadofosveset trisodium premixed with 10%HSA)	3	3	3
1 μ mol Gd/kg (10mM gadofosveset trisodium premixed with 10%HSA)	3	1	1
1 μ mol Gd/kg (10mM gadofosveset trisodium premixed with 10%HSA)	3	2	2
1 μ mol Gd/kg (10mM gadofosveset trisodium premixed with 10%HSA)	3	2	1
1 μ mol Gd/kg (10mM gadofosveset trisodium premixed with PBS)	3	0	0
1 μ mol Gd/kg (10mM gadofosveset trisodium premixed with PBS)	3	2	2
1 μ mol Gd/kg (10mM gadofosveset trisodium premixed with PBS)	3	1	1
1 μ mol Gd/kg (10mM gadofosveset trisodium premixed with PBS)	3	1	1
1 μ mol Gd/kg (10mM gadofosveset trisodium premixed with PBS)	3	2	2
1 μ mol Gd/kg (10mM gadofosveset trisodium premixed with PBS)	3	1	0
1.5 μ mol Gd/kg (10mM gadofosveset trisodium premixed with 10%HSA)	3	1	1
1.5 μ mol Gd/kg (10mM gadofosveset trisodium premixed with 10%HSA)	3	2	2
5 μ mol Gd/kg (33mM gadofosveset trisodium premixed with 10%HSA)	3	3	3
5 μ mol Gd/kg (33mM gadofosveset trisodium premixed with 10%HSA)	3	2	1
5 μ mol Gd/kg (33mM gadofosveset trisodium premixed with 10%HSA)	1	2	2
5 μ mol Gd/kg (33mM gadofosveset trisodium premixed with 10%HSA)	1	3	2

TD visibility assessment scores: 0=no enhancement within the TD, 1=partial enhancement of the TD, 2=complete but faint enhancement of the TD, 3=complete enhancement of the TD.

and its size is expected to increase to least 8.5–11 nm due to the bound albumin.¹² The gadofosveset trisodium HSA conjugate was preferentially taken up by the lymphatics after intradermal injection.^{8,13} Gadofosveset injected without HSA, however, failed to reliably depict the TD, likely due to vascular extravasation and low binding affinity of the low molecular weight agent for porcine albumin.

The intradermal injection of the gadofosveset-HSA resulted in increased interstitial pressure accelerating uptake of the agent into the lymphatics. In this regard the location of the injection needle is critical for a successful study; if the needle enters the subcutaneous space, insufficient pressure is generated to fill the lymphatics, whereas intradermal injection results in sufficient interstitial pressure to force the agent into the lymphatics. Massage over the injection site, although expected to facilitate transport of the contrast agent within the lymphatics, did not materially affect the results.¹³

A variety of MR-based imaging agents have previously been explored for lymphography. Staatz et al. investigated perfluorinated Gd chelates in a pig model. This agent has lipophilic properties with an estimated diameter of 3.9 nm. They reported positive enhancement of the lymph nodes and lymphatic vessels; however, their injected volumes were in the range of 8.2 mL to 60 mL, depending on the size of the animal.¹⁴ This volume of intradermal injection could cause damage to the skin in humans. Sena et al. evaluated a nano-sized Gd-labeled dendrimer, also in a porcine model. This, macromolecular MRI agent, successfully visualized the deep lymphatic system, including the TD even after a low dose (1 μ mol Gd/kg), low volume (3 cc) intradermal injection.⁵ However, in spite of these favorable results, this contrast agent is not approved clinically and therefore, is unavailable for human use. Herborn et al. evaluated the performance of gadofosveset trisodium alone in the detection of regional lymph nodes after interstitial injection in a tumor-bearing rabbit model. They injected 0.5 mL 250 mM gadofosveset trisodium (Gd dose=30 μ mol Gd/kg) interstitially and reported successful visualization of the lymph nodes and the deep

lymphatic vessels using the preapproved version of the agent, known as MS-325.¹¹ In the current study, MR lymphography with premixed gadofosveset trisodium (as clinically packaged) and HSA revealed a better success rate for complete visualization of the TD compared to gadofosveset trisodium alone. There was high intra-observer agreement regarding visualization of the TD. Moreover, this was achieved with lower injected Gd doses in our study compared to Herborn et al. (1–5 μ mol vs. 30 μ mol Gd).¹¹ Importantly, since gadofosveset trisodium is now approved for human use, this preliminary study paves the way for a human trial with this agent.

A potential concern regarding interstitial injections is local skin necrosis. As is known from intravenous contrast media extravasations, this effect is related to the volume of injection. We were able to show that even smaller volumes (1 mL vs. 3 mL) could still successfully depict the TD. We believe this volume should be well tolerated in humans as it corresponds to small amounts of extravasation during intravenous injections.

Our study has several other limitations. This study included a relatively small number of animals as it was considered a pilot feasibility trial; nonetheless, the results were consistent among animals. Additionally, we did not specifically address whether gadofosveset could be used to identify sentinel nodes. However, anecdotally we were able to see the uptake of the gadofosveset trisodium+HSA within the popliteal and inguinal lymph nodes in several animals in whom the lower leg was scanned. Thus, this technique can potentially be used for sentinel lymph node imaging and this also should be investigated, however, it appears to be important that high pressures be generated at the injection site to facilitate lymphatic uptake.

In conclusion, we demonstrate that gadofosveset trisodium (premixed with 10%HSA) can be injected intradermally in order to perform MR lymphography of the thoracic duct. It is unclear whether the agent will have to be premixed when used clinically as the agent is designed specifically to bind

endogenous human albumin and further studies in humans will need to be performed. Since this agent is FDA approved for MR imaging, translating it for imaging of the thoracic duct appears feasible for research purposes, with suitable institutional permissions for off-label use, and may prove to be of benefit for patients with TD abnormalities.

Author Disclosure Statement

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