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INTERSTITIAL MAGNETIC RESONANCE LYMPHOGRAPHY WITH GADOBUTROL IN RABBITS AND AN INITIAL EXPERIENCE IN HUMANS

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ABSTRACT

The purpose of this study was first to evaluate gadobutrol as a contrast agent for interstitial Magnetic Resonance Lymphography (MRL) in rabbits, and second, to extend the study to humans, if the initial results were satisfactory. In our experiment, gadobutrol was injected into twelve white New Zealand rabbits. In nine animals, 0.5 ml of gadobutrol was subcutaneously administered through each foot pad of the hindlegs while in the remaining three animals the agent was given in each foot of the forelegs. In four of the nine rabbits, slight local massage was applied at the site of administration. Subsequently, we proceeded to administer 5 ml (4.5 ml gadobutrol mixed with 0.5 ml hydrochloride lidocaine) into the limbs of two healthy humans.

We achieved imaging of four lymph node groups (popliteal, inguinal, iliac and paraortic) in the hind-legs of the nine-rabbit group, whereas, in the forelegs of the remaining three rabbits, three lymph node groups (axillary, parasternal, mediastinal) were depicted. The flow of the contrast agent was significantly faster in the rabbits that received local massage (P<0.02). In humans, normal lymph vessels, as well as inguinal lymph nodes, were depicted in the legs. No side-effects were observed either in the rabbits or humans.

Keywords: lymphatic imaging, magnetic resonance, interstitial lymphography, gadobutrol, rabbits, normal humans

When treating lymphatic diseases, knowledge of the status of lymph vessels and lymph nodes can greatly assist the choice of therapy and help predict prognosis and possible serious complications. For these reasons many different methods of imaging and different contrast agents have been used for the diagnosis of lymphatic system disorders (1-8). So far, the widely used gold standard examination for resolution has been direct lymphography, where direct administration of the iodinated contrast agent depicts lymph vessels simultaneously with lymph nodes. Even though it is considered by many as the best method for imaging of the lymphatic system, its clinical use is limited due to technical difficulties, excessive duration of examination time, and potential side-effects which include embolism of the pulmonary artery, lungs, or other organs, allergic reactions and infection at the infusion site (9,10). Because the non-invasive clinical

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gold standard of lymphoscintigraphy (9) has limitations related to radioactive tracers, resolution of channels, and depiction of lymph nodes, we and other have turned to magnetic resonance imaging for improved delineation of the lymphatic system and differential diagnosis of lymphatic disorders in an experimental setting and to a limited extent at the clinical level.

The purpose of this study was first to evaluate gadobutrol (Gadovist 1.0, Shering AG, Berlin, Germany) as a contrast agent for interstitial Magnetic Resonance Lymphography (MRL) in rabbits, and second to extend the study to humans, if the initial results were satisfactory.

MATERIAL AND METHODS

For our study we used a contrast agent called gadobutrol (604.72 mg gadobutrol/1 ml), an inert paramagnetic substance that is used intravenously in humans for the imaging of the central nervous system circulation, but which has not been tested for subcutaneous administration (11-13). In this study, gadobutol was administered subcutaneously for MRL in animals for the second time in the international literature (first time was by Fink et al, 2002) (14). The reason for selecting gadobutrol as our contrast agent was that it has double density compared to other agents, thus allowing us to have double concentration in the same volume.

Following approval of the research protocol by the Ethical Committee of the 'Areteion' University Hospital of Athens and by the authorities of the County Veterinary Committee of Athens, we administered gadobutrol in 12 white New Zealand rabbits, weighing 6-10 Kg. All animals were under general anesthesia. The pre-anesthetic regimen included intramuscular administration of hydrochloride ketamine (Ketamine, Curamed Pharma, GmbH, Karlsruhe, Germany), hydrochloride xylazine (Rompun, Bayer, Leverkusen, Germany) and atropine (Demo, Athens, Greece) 20 minutes prior to commencement of the experiment. We then prepared the rabbits by sterilizing one of their ears for insertion of a 20 gauge venous catheter, through which a 'standard sedation solution' was given throughout the experimental procedure. Careful administration of this solution allowed the animals to maintain automatic respiration throughout the procedure. Non-invasive blood pressure, heart rate, oxygen saturation (SpO2) and electrocardiogram were recorded continuously using a monitor (Datex-Ohmeda S/5, Helsinki, Finland).

We subcutaneously administered 0.5 ml of gadobutrol in the area of the skinfold of each foot pad of the hind-legs in 9 rabbits. In four of the nine rabbits, we performed a slight 20-second local massage at the infusion site immediately after administration of gadobutrol in order to compare the flow rate of the contrast agent once in the lymphatic system with the rate in the rabbits where massage was not performed. We then administered subcutaneously 0.5 ml of gadobutrol in the area of the skinfold of each foot pad of the forelegs of the remaining three rabbits.

Next, each rabbit was scanned with Magnetic Tomography (Gyroscan ALS-NT power trak 6000 Philips 1.5 T Netherlands) with the following characteristics: T1W 3D gradient echo TR/TE 4.8 msec/1.4 msec, Flip angle: 30° FOV 179 x 512. Images were obtained at 2,4,6,8,10,15,20,25,30,45 and 60 minutes after the subcutaneous administration of gadobutrol in order to determine the type of lymph nodes (popliteal, inguinal, etc) and the time of depiction of each lymph node group. Following the MRL scanning, all animals were observed for seven days to check for side effects (inflammation or allergic reactions) of gadobutrol.

Moreover, after the approval of the research protocol by the Ethical and Research Committee of the 'Areteion' University Hospital of Athens, we proceeded to infuse gadobutrol in two healthy volunteers. The criteria for the choice of the volunteers were a) their willingness to participate; b) the

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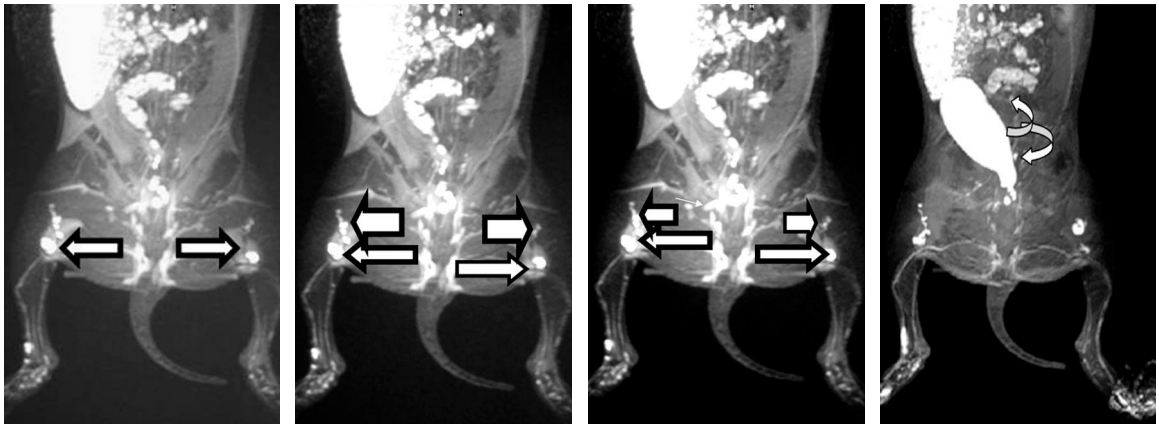

Fig.1. Images obtained at 4, 6, 8, 15 min (left to right) after administration of gadobutrol in hindlegs of rabbits with depiction of popliteal (white long arrow), inguinal (arrowhead), iliac (white short arrow), and paraaortal (curved arrow) lymph node groups.

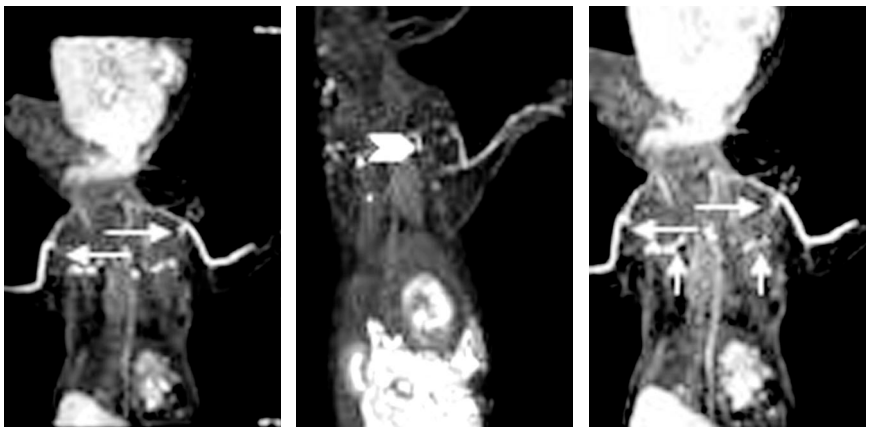

Fig. 2. Images obtained at 4 (left, center) and 15 (right) min after administration of gadobutrol in forelegs of rabbits with depiction of axillary (white long arrow), parasternal (arrowhead), and mediastinal (white short arrow) lymph node groups.

signing of an informed consent form; c) absence of allergic history; d) absence of pregnancy; and e) absence of contraindications to undergo an MRI (e.g., pacemaker). One of the volunteers was 65 years of age and weighed 77 Kg, while the other was 52 years of age and weighed 81 Kg. Both subjects had no vascular disorders in their limbs. We administered into the right limb of one of the volunteers and into the left limb of the other 5 ml of solution containing 4.5 ml gadobutrol and 0.5 ml hydrochloride lidocaine. We prepared this by filling a syringe with 4.5 ml from the special commercial preparation of gadobutrol (1 vial of 30 ml gadobutrol) followed by filling with an additional 0.5 ml of hydrochloride lidocaine (1 vial of 2%, 20 mg/ml, AstraZeneca, Monts, France). Approximately 1 ml of solution was administered subcutaneously 5 times in the dorsal area of the foot and especially in each skinfold between the toes using a 26 gauge needle. Immediately after the administration, local massage was performed for five minutes and MRL was performed using the same equipment as with the rabbit study (Magnetic

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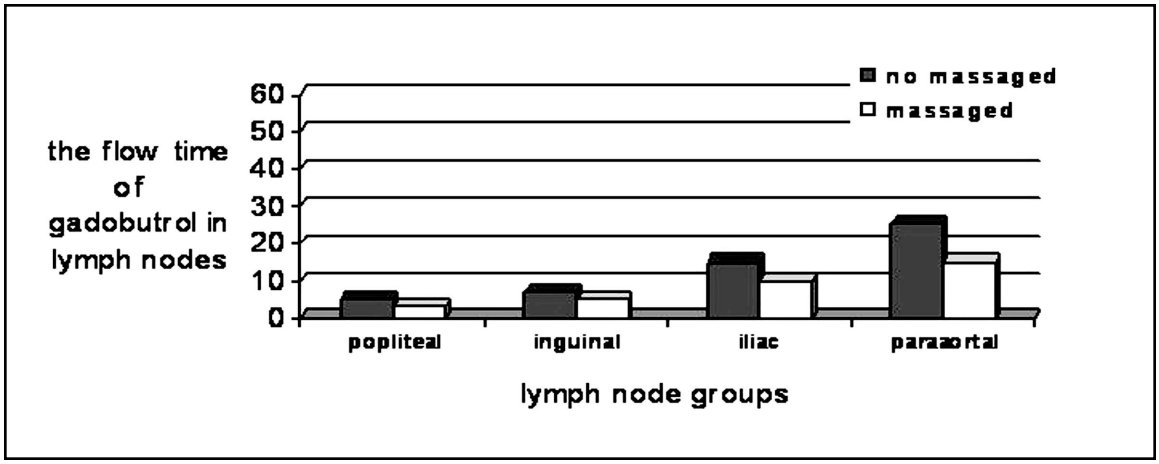

Fig. 3: The flow time (in minutes) of gadobutrol to the nodes of rabbits demonstrates a significantly faster flow (lower flow time) in the rabbits which received massage for every lymph node group (p<0.02) .

Tomography Philips 1.5T) with the following characteristics: T1W 3D gradient echo TR/TE 4.8 msec/1.4 msec, Flip angle: 30° FOV 179 x 512. Images were obtained before and every 5,10,15,20,30,45 and 60 minutes after the subcutaneous administration at the level of the foot, calf, thigh, and pelvis. Following the MRL scanning, human subjects were observed for seven days to document any side effects (inflammation, pain or allergic reactions) of gadobutrol.

RESULTS

Gadobutrol was quickly absorbed by the lymphatic system. After subcutaneous administration of the contrast agent in the hindlegs of the nine rabbits, four lymph node groups were depicted (popliteal, inguinal, iliac, and para-aortal lymph nodes) *(Fig. 1)*, whereas in the forelegs of the three rabbits, three lymph node groups were depicted (axillary, parasternal and mediastinal lymph nodes) *(Fig. 2)*. In the rabbits without massage, the time of depiction of popliteal lymph nodes was 4-6 minutes, inguinal lymph nodes 6-8 min, iliac lymph nodes 15 min, and that of para-aortal lymph nodes was 25 min.

In contrast, in the rabbits which received massage, the time of depiction of popliteal lymph nodes was 2-4 min, inguinal lymph nodes 4-6 min, iliac lymph nodes 10 min, and that of para-aortal lymph nodes was 15 min *(Fig. 3)*. We analyzed statistically (Pearson method) the time of depiction of lymph nodes on the hindlegs of rabbits with and without massage, and we found that imaging of the lymphatic system was significantly faster (displayed as a lower time of flow to image in *Fig. 3*) in the rabbits who received massage (p<0.02). Moreover, the time of depiction of axillary lymph nodes was 2-4 min, while that of parasternal and mediastinal lymph nodes were 15-20 min *(Fig. 3)*.

In addition, it is worth noting that the bladder of the rabbits was depicted immediately after the administration of gadobutrol (in 2-4 min). During the seven-day observation period following subcutaneous administration of the contrast agent, no side effects such as inflammation or allergic reactions were observed in any of the animals.

In humans following the administration of gadobutrol, lymphatic vessels from the foot to the inguinal lymph nodes were imaged within 60 min while only the inguinal lymph nodes, but not the popliteal lymph nodes,

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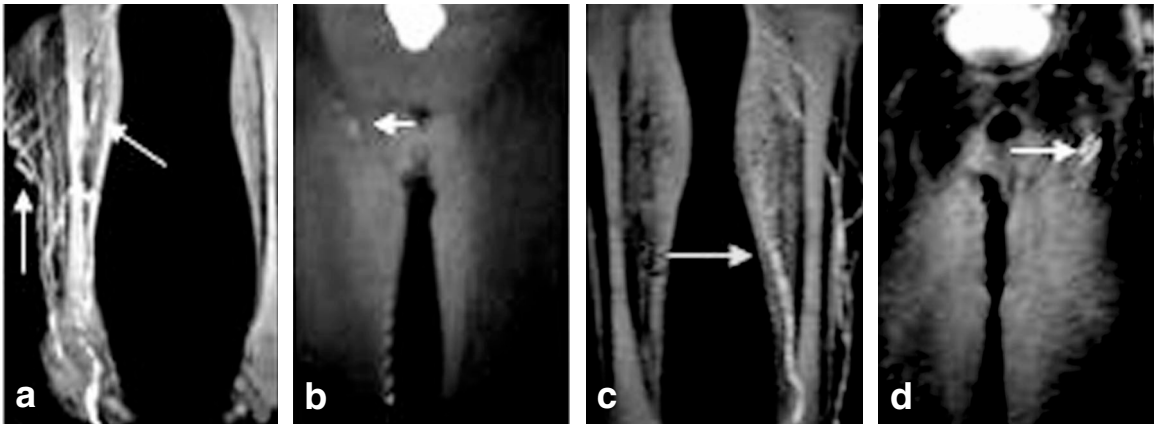

Fig. 4: Depiction of lymphatic vessels (a and c, white long arrows) in healthy humans and inguinal lymph nodes (b and d, white short arrows) in the right (a,b) and left (c,d) lower extremities after subcutaneous administration of gadobutrol.

were seen *(Fig. 4)*. Both subjects experienced mild pain only for the duration of the infusion. A seven-day follow-up revealed no side effects (allergic reactions, pain, or local edema).

DISCUSSION

Knowledge of an abnormal lymphatic anatomy is essential for determining the prognosis of lymphatic disease, the method of therapy, and the range of possible complications. It is more difficult to obtain by imaging when we deal with the differential diagnosis of a metastatic lymph node, and this is one of the biggest focus areas of lymphatic system imaging techniques (1,10,15,16). In this experiment, we studied the efficiency of gadobutrol as a subcutaneous contrast agent for MRL in rabbits. Gadobutrol is an inert contrast agent, without allergic reactions in animals and with double density in comparison with other contrast agents. The technique of administration proved to be easy, and we were able to administer a small amount of contrast agent (0.5 ml) in the subcutaneous space of the skinfold of the foot pad of each hindleg or foreleg of rabbits. The contrast agent was absorbed quickly by the lymphatic system, mainly in the rabbits that were massaged at the area of administration, and the MRL provided satisfactory depiction of the lymph node groups of the rabbits. It seems that massage is necessary for the quick depiction of the lymphatic system as our statistical analysis indicated and as other authors have suggested (9). The quick depiction of the bladder of the rabbits (in 4-6 min) confirms that subcutaneous administration of gadobutrol was followed by absorption either from the subcutaneous space to the venous capillaries or from the lymphatic system to venous system. Our seven-day observation of the animals confirmed that subcutaneous administration of gadobutrol was not associated with negative side effects such as inflammation or allergic reactions either local or general.

The aforementioned animal experiment has shown that gadobutrol is a contrast agent that is absorbed very rapidly by the lymphatic system of rabbits without negative side effects. So we proceeded, after appropriate institutional approval and informed subject consent, with the use of gadobutrol as a contrast agent in MRL for the depiction of the lymphatic system in humans. We used 0.5 ml of gadobutrol for the depiction of the lymph node groups in rabbits, but in humans we used ten times more in order to achieve the same positive results. In addition, we

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added local anaesthesia such as hydrochloride lidocaine, since humans undergo the examination while awake and may feel pain at the injection site (17). Gadobutrol has already been used in clinical studies as an intravenous agent for the imaging of brain tumors and of the central nervous system circulation at a dose of 0.3 mmol/kg but it has never been administered subcutaneously. So we proceeded carefully to infuse gadobutrol in humans at a dose only of 0.06 mmol/kg, which is a very low amount that would pose no dangers for the health of the volunteers. Both subjects experienced mild local pain at the site of administration, only for the duration of the infusion. After the end of the infusion, all local pain disappeared completely. Finally, we achieved depiction of the normal lymphatic system of lower extremities in humans (the lymph vessels and the inguinal lymph nodes) without detecting any side effects over a seven-day follow-up period.

The results of this first pilot clinical study encourage further investigation of the efficiency and value of gadobutrol for an enhanced and painless depiction of the lymphatic system and mainly for the diagnosis of lymphatic diseases. MRL and the subcutaneous administration of gadobutrol should achieve better results with the refined determination of the dosage of gadobutrol in expanded human studies.

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REFERENCES

1. Anzai, Y, KE Blackwell, SL Hirschowitz, et al: Initial clinical experience with Dextran-coated superparamagnetic iron oxide for detection of lymph node metastases in patients with head and neck cancer. Radiology 192 (1994), 709-715.
2. Bengele, HH, S Palmacci, J Rogers, et al: Biodistribution of an ultrasmall superparamagnetic iron oxide colloid, BMS 180549, by different routes of administration. Magn. Reson. Imaging 12 (1994), 433-442.
3. Wagner, S, D Pfefferer, W Ebert, et al: Intravenous MR lymphography with superparamagnetic iron oxide particles: Experimental studies in rats and rabbits. Eur. Radiol. 5 (1995), 640-646.
4. Muhler, A, X Zhang, H Wang, et al: Investigation of mechanisms influencing the accumulation of ultrasmall superparamagnetic iron oxide particles in lymph nodes. Invest. Radiol. 30 (1995), 98-103.
5. Harika, L, R Weissleder, K Poss, et al: Lymphography with a lymphotropicT1-type MR contrast agent: Gd-DTPA-PGM. Magn. Reson. Med. 33 (1995), 88-92.
6. Misselwitz, B, J Platzek, B Raduchel, et al: Gadofluorine 8: Initial experience with a new contrast medium for interstitial MR lymphography. MAGMA 8 8 (1999), 190-195.
7. Ruehm, SG, C Corot, JF Debatin: Interstitial MR lymphography with a conventional extracellular gadolinium-based agent: Assessment in rabbits. Radiology 218 (2001), 664-669.
8. Herborn, CU, FM Vogt, TC Lauenstein, et al: Assessment of normal, Inflammatory, and tumor- bearing lymph node with contrast-enhanced interstitial magnetic resonance lymphography: Preliminary results in rabbits. J. Magn. Reson. Imaging 18 (2003), 328-335.
9. Witte, CL, WH Williams, MH Witte: Lymphatic imaging. Lymphology 26 (1993), 109-111.
10. Rudnick, MR, S Goldfarb, L Wexler, et al: Nephrotoxicity of ionic and nonionic contrast media in 1196 patients: A randomized trial. The Iohexol Cooperative Study. Kidney Int. 47 (1995), 254-261.
11. Vogler, H, J Platzek, G Schuhmann-Giampieri, et al: Preclinical evaluation of gadobutrol: A new extracellular contrast agent for magnetic resonance imaging. Eur. Radiology 21 (1995), 1-10.
12. Tombach, P, C Bremer, P Reimer, et al: Pharmacokinetics of 1M gadobutrol in patients with chronic renal failure. Invest. Radiol. 35 (2000), 35-40.
13. Staks, T, G Schuhmann-Giampieri, T Frenzel, et al: Pharmacokinetics, dose proportionality, and tolerability of gadobutrol after single

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intravenous injection in healthy volunteers. Invest. Radiol. 29 (1994), 709-715.
14. Fink, C, M Bock, F Kiessling, et al: Interstitial magnetic resonance lymphography with gadobutrol in rats: Evaluation of contrast kinetics. Invest. Radiol. 37 (2002), 656-662.
15. Vassalo P, C Matei, WD Heston, et al: Characterization of reactive versus tumor-bearing lymph nodes with interstitial magnetic resonance lymphography in a animal model. Invest. Radiol. 30 (1995), 706-711.
16. Mohr, U, R Weissleder: Lymph node diagnosis with imaging methods: An overview with special reference to recent developments in the area of MR contrast media Z Lymphology 20 (1996), 15-20.
17. Ruehm, SG, T Schroeder, F Jorg, et al: Interstitial MR lymphography with gadoterate meglumine: Initial experience in humans. Radiology 220 (2001), 816-821.

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