Micro-Magnetic Resonance Mammo-Lymphangiography using a Nano-size Contrast Agent to Image Lymphatic Drainage of the Breast Cancer in Mice

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Synopsis

There is need for precise identification of sentinel lymph nodes in patients with breast cancer. Improvements to current imaging methods using MRI employing a newly synthesized nano-size, paramagnetic particle are an attractive goal. A four dimensional method of micro-MR mammo-lymphangiography using a nano-size, paramagnetic contrast agent (G6; 9nm/ 240 kDa) is developed to visualize the lymphatics and lymph nodes draining mouse breast cancers. The conventional MR contrast agent, Gd-[DTPA]-dimeglumine (<1 kDa), failed to depict the lymphatics. MR mammo-lymphangiography using nano-size paramagnetic contrast agents is far more powerful in the localization of sentinel nodes in breast cancer than conventional methods.

Introduction

The presence of lymph node metastases has major prognostic implications in breast cancer patients and is the major criterion for determining the need for adjuvant chemotherapy. Sentinel lymph node (SLN) biopsy has become increasingly routine because the disease status of the SLN accurately reflects the status of more distant axillary lymph nodes. Because of the advantages of SLN biopsy over axillary lymph node dissection in regard to post-operative complications, development of an accurate, reliable non-invasive method of lymph node assessment would be of great clinical benefit to breast cancer patients. In this study, a dynamic micro-MR mammo-lymphangiography (dmMRML) method to visualize breast lymphatic flow was developed and tested using a dendrimer-based nano-size paramagnetic contrast agent in mouse models.

Methods

Contrast agent: A polyamidoamine (PAMAM)-G6 dendrimer (58 kD) based MRI contrast agent coupled with 2-(*p*-isothiocyanatobenzyl)-6-methyl-diethylenetriamine-pentaacetic acid (1B4M) containing 1024 Gd(III) (240 kD) ions was synthesized. ***Animal models:*** Thirty one week-old heterozygous BALB-*neu*T mice transgenic for the rat HER-2/*neu* oncogene under the control of the mouse mammary tumor virus promotor (MMTV) were used for a spontaneous breast cancers and lymph node metastasis model. Nude mice with PT-18 tumor xenografts, a murine mast cell line, were used for inplanted tumor and metastasis model. Normal nude and Balb/c mice were used for normal mammary gland studies. ***Dynamic3D-micro-MR mammo-lymphangiography:*** All mice were anesthetized and injected intracutaneously with 0.1 μmolGd of PAMAM-G6 or 0.3 μmolGd of Gd-[DTPA]-dimeglumine into mammary tissue surrounding a tumor. All dynamic micro-MR images were obtained using a 1.5-tesla superconductive magnet unit (Signa LX, General Electric Medical System) with a 1-inch round surface coil (Birdcage type) fixed by an in-house constructed coil holder. A 3D-fast spoiled gradient echo [3D-fastSPGR (efgre3d package); TR/TE 28.5/7.9; TI 65 msec; 31.2 kHz, flip angle 30°, 4 NEX; scan time 7'36"] with chemical fat-suppression was used 6, 12, 18, 24, 30, 36, 42, and 48 min after injection of the contrast agents. The coronal images were reconstructed with 0.6-mm section thickness with 0.3-mm overlap (two 512 matrix Zips). FOV was 6 x 3 cm and the size of matrix was 512 x 256.

Results

NORMAL MICE; 1. Three lymph nodes: axillary, lateral thoracic, and superficial cervical, with their draining lymphatic vessels were visualized by dmMRML with the G6 contrast agent. The axillary lymph node and its lymphatic vessels were visualized at the initial (6 min) time point in all 10 mice. However, two other lymph node groups and their lymphatic vessels appeared later. 2. Draining lymph nodes and lymphatic vessels were clearly visualized only with the G6 agent but not Gd-[DTPA]-dimeglumine (Fig. 1). This result indicates the advantage of the G6 nano-size contrast agent over Gd-[DTPA]-dimeglumine in visualizing lymphatic drainage using dmMRML.

BREAST-TUMOR BEARING MICE; In both BALB-*neu*T transgenic mice and PT-18 mast cell tumor xenograft models, flows within the draining lymphatic vessels from breast tumors to metastatic lymph nodes was readily visualized. Lymph nodes containing a solid tumor showed filling defects or rim-enhancement after contrast administration due to the absence of normal lymph node tissue that enabled diagnosis of micro-metastasis (Fig. 2).

Conclusion

We developed a novel method for dmMRML, which was able to visualize both draining lymph nodes and lymphatic vessels from breast tissue in mice using a G6 dendrimer-based nano-size macromolecular paramagnetic contrast agent. This four-dimensional imaging method helped us visualize the lymphatic flow over time on a 3-D display. The superior temporal and spatial resolution of this method should allow wide applications to the study of tumor lymphatics and lymphatic metastasis in both experimental animals and clinical medicine.

Fig. 1 comparison of dmMRML with Gd-DTPA and G6 agent.

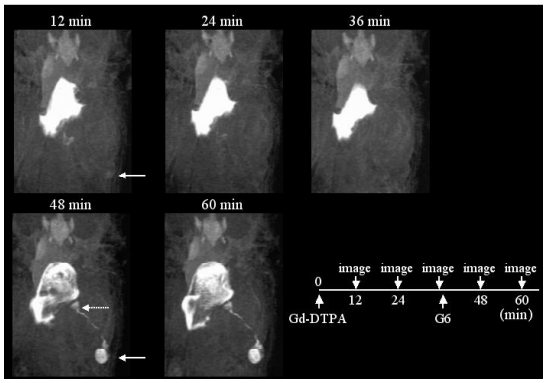

Fig. 2 mMRMLs of normal and metastatic LN with PT-18 tumor