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High resolution 3D MRI of mouse mammary glands with intra-ductal injection of contrast media

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Abstract

The purpose of this study was to use high resolution 3D MRI to study mouse mammary gland ductal architecture based on intra-ductal injection of contrast agents. Female FVB/N mice age 12–20 weeks ($n = 12$), were used in this study. A 34G, 45° tip Hamilton needle with a 25uL Hamilton syringe was inserted into the tip of the nipple. Approximately 20–25uL of a Gadodiamide/Trypan blue/saline solution was injected slowly over one minute into the nipple and duct. To prevent washout of contrast media from ducts due to perfusion, and maximize the conspicuity of ducts on MRI, mice were sacrificed one minute after injection. High resolution 3D T1-weighted images were acquired on a 9.4T Bruker scanner after sacrifice to eliminate motion artifacts and reduce contrast media leakage from ducts. Trypan blue staining was well distributed throughout the ductal tree. MRI showed the mammary gland ductal structure clearly. In spoiled gradient echo T1-weighted images, the signal-to-noise ratio of regions identified as enhancing mammary ducts following contrast injection was significantly higher than that of muscle ($p < 0.02$) and significantly higher than that of contralateral mammary ducts that were not injected with contrast media ($p < 0.0001$). The methods described here could be adapted for injection of specialized contrast agents to measure metabolism or target receptors in normal ducts and ducts with *in situ* cancers.

Keywords

mouse mammary gland; 3D MRI; ductal injection

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1. Introduction

Transgenic mouse models of human breast cancer provide important insights in the pathways and mechanism of breast cancer initiation and progression. Jansen et al. recently reported on serial magnetic resonance imaging (MRI) of murine mammary cancer in SV40 Tag mice as it progressed from the *in situ* to the invasive stage [1, 2]. This previous work relied on conventional imaging including contrast enhanced MRI to detect cancers. However, due to limited MRI spatial resolution, it was difficult to visualize the mouse mammary gland ductal architecture in those studies.

‘Ductography’ using both X-ray and MRI has been frequently used in clinical practice to increase sensitivity to ductal anatomy, cancer and other pathologies [3, 4]. Techniques for intra-ductal injection in mice are well worked out and have been used for injection of cancer cells to produce *in situ* cancers [5]. In addition, intra-ductal injections of dyes have been used in conjunction with optical imaging [6], microscopy [7], and digital photography to evaluate duct architecture, and changes in architecture associated with various pathologies [8]. Ducts have also been imaged in genetically-engineered mice expressing fluorescent proteins using Olympus OV100 Fluorescence Imaging [9], as well as using neutral red dye and a variety of fluorescent dyes [10]. In addition, an IVIS bioluminescence system has been used to visualize inflammation in glands and ducts [11].

Previous approaches to ‘ductography’ in mice have not involved high resolution three-dimensional (3D) MRI. High resolution volumetric imaging is important because of the complexity of the ductal-alveolar tree that converges into a central duct at the nipple. In addition, the excellent soft tissue contrast of MRI can provide important information regarding the relationship of ducts and *in situ* cancers to surrounding tissue including blood vessels and lymph nodes. Therefore, high resolution 3D MRI ductography has the potential to provide unique and important information.

The goal of the present work was to develop and test MRI methods that will later be used to study the development of early *in situ* cancers growing in mammary ducts. Serial imaging following intra-ductal injection of contrast media could provide information concerning cancer initiation and progression, as well as the normal development of mammary glands and changes during the menstrual cycle. As a first step, the pilot study reported here was performed on non-transgenic FVB/N mice that do not have detectable cancer.

Previous in this laboratory demonstrated contrast media exchange between blood vessels and the lumens of mammary ducts [2]. This exchange is expected to reduce contrast media concentration in lumens after intraductal injection. In this pilot study, the goal was to develop and evaluate methods for imaging mammary ducts with intraductal injection. Therefore it was preferable to maximize the concentration of contrast media in the ducts during imaging, by scanning mice in the absence of washout due to perfusion. For this reason, mice were imaged immediately after sacrifice. This insured that most of the contrast media remained in the ducts, and also allowed acquisition of high resolution images without motion artifacts. In the future, we plan to extend the methods reported here to allow

monitoring of contrast media distribution and leakage from ducts *in vivo*, and to study murine models of breast cancer, such as the SV40 Tag-expressing FVB/N [12, 13].

2. Materials and methods

2.1. Ductal injections

All procedures were carried out in accordance with our institution's Animal Care and Use Committee approval. A total of 12 female FVB/N mice (Charles River Laboratories, Inc.) age 12 – 20 weeks, were used in this study. Mice were anesthetized in a rodent 'knockdown box' under 3–5% isoflurane and 1L O₂, then transferred to a nosecone and kept anesthetized at 1.5–2.5% isoflurane. The fur was shaved off the skin around the body and legs. The body was wiped with alcohol to remove loose fur and to visualize the nipples. The excess loose skin around the nipple was removed with fine tip forceps and approximately < 1 mm at the top of the nipple was removed. A 34G, 45° tip Hamilton needle attached to a 25uL Hamilton syringe was then inserted in the tip of the nipple. Approximately 20 – 25uL of a Gadodiamide/Trypan blue solution (0.8 mM Gd chelate; 10% of Trypan Blue) (Omniscan; GE Healthcare Novation, Princeton, NJ), 10% of Trypan Blue (Sigma Aldrich, St. Louis, MO) in saline was injected slowly over one minute into the nipple and duct. A control solution contained Trypan blue but no Gadolinium. In this study, Trypan blue served as a dye to stain the ducts so that each ductal injection could be easily and rapidly evaluated by eye.

2.2. MRI experiments

The mouse was sacrificed one minute after injection by cervical dislocation and imaged afterwards to allow acquisition of high resolution (78 μ m) motion-free MR images on a 9.4 Tesla small animal scanner (Bruker, Billerica, MA) with 11.6 cm inner diameter, actively shielded gradient coils (maximum constant gradient strength for all axes: 230 mT/m). The mouse was placed supine on an animal holder and inserted into a 30 mm diameter quad coil (RAPID MR International, Columbus, Ohio). Axial multi-slice RARE (Rapid Acquisition with Relaxation Enhancement) spin echo T2-weighted (T2W) images with fat suppression were acquired first (TR/TE_{effective} = 4000/20.3 ms, field of view (FOV) = 25.6 mm, matrix size = 256², slice thickness = 0.5 mm, RARE factor = 4, number of excitations (NEX) = 2). Then the 3D T1-weighted (T1W) images with fat suppression were acquired with the FLASH (fast low angle shot), spoiled gradient-echo sequence (TR/TE = 30/5.7 ms, flip angle = 30°, FOV = 30×20×40 mm, matrix size = 384×256×512, NEX = 2).

To provide more detailed pictures of ductal architecture, we also performed 3D MRI for a single gland *ex vivo* from two additional mice. The same ductal injection procedure as above was used, the mouse was sacrificed, and the gland with attached skin was immediately cut out. To prevent the skin and gland from folding, the tissue was placed in between two pieces of histology foam, kept moist with fomblin, and wrapped with clear plastic wrap to prevent it from drying out. The wrapped gland was placed on a homebuilt 8-leg low-pass half-open birdcage coil (3 cm length × 3 cm width × 2 cm height). Fat suppressed 3D T1W images were acquired with the FLASH spoiled gradient-echo sequence (TR/TE = 30/5.7 ms, flip angle = 30°, FOV = 30×20×5 mm, matrix size = 384×256×64, NEX = 16).

2.3 Data analysis

To assist with visualizing the duct architecture, 3D visualization and analysis software Amira (FEI Visualization Sciences Group, Burlington, MA) was used for volume rendering. In addition, the data were processed and analyzed quantitatively using software written in IDL (Exelis VIS, Inc., Boulder, CO). The signal-to-noise ratio (SNR) over regions-of-interest (ROIs) in muscle, mammary glands without contrast, and mammary glands with contrast were calculated by selecting an axial slice that was about 2.5 mm from the injection site. For the mammary glands injected with contrast media, only pixels with signal intensity greater than three times the average intensity in the gland without contrast media injection, excluding pixels in lymph nodes, were used to calculate SNR. This was done to limit the calculation to mammary ducts. Finally, a paired t-test was performed to compare the SNR in muscle and the contrast-injected mammary glands. A p-value less than 0.05 were considered significant.

3. Results

Figure 1 shows a diagram of mouse mammary glands (a) and the ductal tree of a mouse inguinal gland injected with 25 μ L of Trypan Blue/Gadolinium solution (b). Trypan blue dye was well distributed throughout the ductal tree. Figure 2 shows three axial slices through a mouse post-injection. The slices on the right are from 3D T1W MR datasets and show cross sectional views of the ducts demonstrating strong enhancement due to Gadolinium injection (arrows on the right). In comparison, the gland on the left that was not injected with contrast media shows no enhancement. Injection of a control solution that contained Trypan blue but no Gadolinium did not produce significant enhancement on T1W images (not shown here). The 3 slices on the left are from a 2D T2W scan. These images also show signal enhancement in the mammary ducts injected with the contrast media solution relative to ducts that were not injected.

Volume rendering of the T1W images provides an excellent 3D view of the geometry of mouse mammary ducts. Figure 3 shows volume rendered images for three different mice with injections at three different locations: (a) the top pectoral nipple, (b) top inguinal nipple, and (c) lower inguinal nipple. The red contrast identifies the ductal tree. The surrounding organs and tissue appear as blue and green in the background. Each mammary gland has a slightly different ductal tree structure depending on the location on the body.

Figure 4 shows the average SNR of T1W images calculated in a selected slice from each mouse ($n = 12$), about 2.5 mm from the injection site for muscle, for a mammary gland with intra-ductal contrast injection, and for the opposing mammary gland without intra-ductal injection ROIs. The SNR of muscle was about four times that of the mammary gland without contrast agent, but 30% less than the SNR calculated for enhanced areas in the ducts injected with contrast media. The paired t-test showed that the SNR for areas identified as enhancing ducts in mammary glands after intra-ductal contrast media injection was significantly higher than that of muscle ($P < 0.02$). Comparison between mammary glands with and without contrast media injection is biased due to the method used to identify signals from enhanced ducts (please see 'Methods'). However, the average SNR of regions

identified as enhanced ducts is approximately six times higher than the average SNR in the non-enhanced ducts ($P < 0.0001$).

Finally, Figure 5 shows a photograph of a single gland (a) and a maximum intensity projection (MIP) of the *ex vivo* MR image (b). The Trypan Blue distribution matches the enhanced ducts on MRI and demonstrates that the contrast agent solution spread through the entire ductal tree, reaching even very small ducts far from the injection site. Although the smaller ducts farther from the injection site were not clearly detected by MRI in this study, the Trypan Blue results suggest that with improved SNR, imaging sooner after intraductal injection, and optimization of pulse sequences, a larger number of ducts could be detected by MRI.

4. Discussion

To our knowledge, these are the first 3D MR images of contrast media injected directly into mouse mammary ducts. Intra-ductal injection of contrast media allows non-invasive high resolution imaging of ductal architecture in 3-dimensions. The complexity of the ductal-alveolar tree can be clearly seen in the volume-rendered T1-weighted images. In this pilot study, we imaged 12 mice after sacrifice. This was done so that normal ductal architecture could be evaluated at high resolution without motion artifacts. In addition, immediate sacrifice following intra-ductal injection may have minimized leakage of contrast media out of the ducts, and therefore provided a sharper image.

The observed enhancement of ducts on T2-weighted images suggests that fluid injected into ducts may be detected directly without contrast media injection. However, the results from this study are difficult to interpret and were not analyzed quantitatively because of concerns that the high intraductal concentration of contrast media shortened T2 and bias the results. In future studies we plan to test the use of intraductal injections of saline to evaluate water exchange across the ductal epithelium and into *in situ* cancers.

It was essential to keep injection volume low, i.e. 25 μ l or less and inject slowly (over 1 minute) to avoid damage to ducts. Larger injection volumes and/or rapid injection resulted in significant damage that could be identified both visually and on MRI based on extravasation of contrast media and Trypan Blue.

Based on the number of experiments that show substantial filling of ducts the success rate for ductal injections is currently about 80%. The unsatisfactory results (20% of all injections) were due to accumulation of fluid subcutaneously under nipples, inverted nipples that were too small to be injected, or ducts that were injected too quickly or filled with too much volume.

In these pilot studies mice were sacrificed soon after intraductal injection and imaged *post mortem* to minimize contrast media washout due to perfusion, and maximize the conspicuity of ducts on MRI. In future studies, we plan to extend this approach to *in vivo* serial imaging of ducts that develop *in situ* cancer. This will allow serial measurements of the rate of washout of contrast media from ducts due to changes in perfusion and ductal permeability.

Previous work by Jansen et al. demonstrated that contrast media injected I.V. leaks into ducts containing *in situ* cancer [2] and suggested that the permeability of ducts may be a marker for developing cancers. With intra-ductal injection, leakage of relatively high concentrations of contrast media out of mammary ducts could in principle be monitored with higher SNR and spatial resolution. In addition, the methods described here could be adapted for injection of specialized contrast agents to study metabolism or target receptors in normal ducts and *in situ* cancers.

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Figure 1.

(a) Diagram of mouse mammary glands: 1 - Axillary, 2 - Cranial thoracic, 3 - Caudal thoracic, 4 - Abdominal, 5 - Inguinal [14]. (b) Photograph of Trypan blue dye distributed throughout the ductal tree.

Figure 2.

Three MRI axial slices (2 mm apart) of a mouse acquired after ductal injection: left panel - T2W images, right panel - T1W images. Only the right gland was injected with contrast media, not the left gland. The arrow indicates the site of injection at the nipple. The display FOV for each image was 25 mm $\times$ 15 mm.

Figure 3.

Volume rendered ductal trees (orange) for three different mice with the contrast injection was performed at (a) the axillary nipple, (b) the abdominal nipple, and (c) the inguinal nipple.

Figure 4.

The average SNR plus standard deviation in T1W images for ROIs in mammary glands without contrast agent, ROIs in muscle, and ROIs in mammary glands with contrast agent. The analysis was done for a selected axial slice in each mouse ($n = 12$) about 2.5 mm from the injection site. The average SNR for the mammary glands injected with contrast media represents only signal from enhancing ducts.

Figure 5.

(a) Photograph of a single mouse mammary gland after injection of the Gadolinium/Trypan blue solution. (b) The MIP of MRI for the same gland. The display FOV for each image was 20 mm $\times$ 25 mm.