Manganese-enhanced MRI of minimally gadolinium-enhancing breast tumors

Tameshwar Ganesh, Reza Bayat Mokhtari, Mosa Alhamami, Herman Yeger, Hai-Ling Margaret Cheng

Version  Post-print/accepted manuscript


Publisher’s Statement  This is the peer reviewed version of the following article: [Ganesh T, Mokhtari RB, Alhamami M, Yeger H, Cheng HL. Manganese-enhanced MRI of minimally gadolinium-enhancing breast tumors. Journal of Magnetic Resonance Imaging. 2015 Mar;41(3):806-13.], which has been published in final form at [10.1002/jmri.24608]. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.

How to cite TSpace items

Always cite the published version, so the author(s) will receive recognition through services that track citation counts, e.g. Scopus. If you need to cite the page number of the author manuscript from TSpace because you cannot access the published version, then cite the TSpace version in addition to the published version using the permanent URI (handle) found on the record page.

This article was made openly accessible by U of T Faculty. Please tell us how this access benefits you. Your story matters.
Manganese-enhanced MRI of minimally gadolinium-enhancing breast tumors

*Tameshwar Ganesh, BSc\textsuperscript{1,2}, *Reza Bayat Mokhtari, MSc\textsuperscript{3,4}, *Mosa Alhamami, MSc\textsuperscript{1,2}, Herman Yeger, PhD\textsuperscript{3,5}, Hai-Ling Margaret Cheng, PhD\textsuperscript{1,2,6,7}

* Denotes equal contributions

\textsuperscript{1}The Research Institute (Physiology & Experimental Medicine) and Diagnostic Imaging, Hospital for Sick Children, Toronto, Canada
\textsuperscript{2}Leslie Dan Faculty of Pharmacy, University of Toronto
\textsuperscript{3}The Research Institute (Developmental & Stem Cell Biology) and Pediatric Laboratory Medicine, Hospital for Sick Children, Toronto, Canada
\textsuperscript{4}Institute of Medical Science, University of Toronto, Toronto, Canada
\textsuperscript{5}Department of Laboratory Medicine & Pathobiology, University of Toronto, Toronto, Canada
\textsuperscript{6}The Institute of Biomaterials & Biomedical Engineering, University of Toronto
\textsuperscript{7}Department of Medical Biophysics, University of Toronto, Toronto, Canada

**Grant Support:** Natural Sciences and Engineering Research Council of Canada (NSERC) (#355795) and the Garron Family Cancer Centre Grant through the SickKids Foundation, all awarded to H.L.C.

**Corresponding Author:**
Hai-Ling Margaret Cheng, PhD
The Hospital for Sick Children
555 University Avenue
Toronto, Ontario
Canada M5G 1X8
Telephone: 1-416-813-5415
Email: Hai-Ling.Cheng@sickkids.ca
ABSTRACT

Purpose: To investigate the potential of manganese (Mn)-enhanced MRI for sensitive detection and delineation of tumors that demonstrate little enhancement on Gd-DTPA.

Materials and Methods: Eighteen nude rats bearing 1 to 2 cm in diameter orthotopic breast tumors (ZR75 and LM2) were imaged on a 3 Tesla clinical scanner. Gd-DTPA was administered intravenously and MnCl₂ subcutaneously, both at 0.05 mmol/kg. T₁-weighted imaging and T₁ measurements were performed pre-contrast, 10 minutes post-Gd-DTPA, and 24 hours post-MnCl₂. Tumors were excised and histologically assessed using H&E (composition and necrosis) and CD34 (vasculature).

Results: Most tumors (78%) demonstrated little enhancement (< 20% change in R₁) on Gd-DTPA. MnCl₂ administration achieved greater and more uniform enhancement throughout the tumor mass (i.e. not restricted to the tumor periphery), with R₁ changing over 20% in 72% of tumors. MnCl₂-induced R₁ changes compared to Gd-induced changes were significantly greater in both ZR75 (p < 0.01) and LM2 tumors (p < 0.05). Histology confirmed very low vascularity in both tumor models, and necrotic areas were well delineated only on Mn-enhanced MRI.

Conclusion: Mn-enhanced MRI is a promising approach for detection of low-Gd-enhancing tumors.

Key Words: non-enhancing; low vascularity cancer; manganese chloride; gadolinium chelate; contrast-enhanced imaging; rat breast tumor model
INTRODUCTION

Oncological imaging routinely requires the administration of exogenous contrast agents (1). Contrast-enhanced images, whether on MRI, CT, or ultrasound, generally provide improved tumor detection and delineation, as the increased vascularity of many tumors results in higher contrast agent accumulation compared to that in surrounding normal tissue (2,3). In MRI, the agent routinely used for the diagnosis, treatment planning, and monitoring of cancers in the breast, prostate, head and neck is extracellular gadolinium chelate (Gd)-DTPA (4). Contrast enhancement from Gd-DTPA arises mainly from parenchymal accumulation as a result of its distribution from the intravascular to the interstitial (i.e. extracellular) space. However, reliance on the vasculature for Gd-DTPA distribution implies that tumors with low vascularity or poor perfusion may not enhance appreciably relative to surrounding normal tissue. This may be observed in tumors that inherently present as non-enhancing, such as some low-grade gliomas (5), or ones that become non-enhancing following anti-angiogenic treatment (6). For low- or non-enhancing tumors, a more sensitive approach to detection and delineation might be needed.

Manganese (Mn) is another MRI contrast agent that has been used for imaging tumors, although to a far lesser extent (7-9). Unlike Gd-DTPA, Mn is an endogenous metal that can enter cells. It is, therefore, a cellular contrast agent and enhances tumors cells. The precise mechanism for its cellular uptake is still not fully understood, but amongst the few reports on the application of manganese chloride (MnCl₂) to cancer studies, some have noted a difference in Mn uptake between malignant and normal cells (10-13). This ability of Mn to label and provide higher signal contrast to tumor cells suggests its potential for sensitive tumor detection and delineation. Comparisons between Mn-enhanced and Gd-enhanced imaging have been made in studies of the
retina (14), mouse brain mapping (15), and myocardial infarction (16). However, the relative advantages of MnCl₂ administration for cancer imaging remain to be determined.

The aim of this study was to investigate the potential of Mn-enhanced MRI to provide detection and delineation of tumors that demonstrate little enhancement on Gd-DTPA.

MATERIALS AND METHODS

Tumor Induction in Rats

All procedures were approved by our institutional animal care committee and conducted in accordance with the national standards on animal care. Human breast cancer cell lines ZR-75-1 and 231/LM2-4, hereafter referred to as ZR75 and LM2, respectively, were used. The ZR75 cell line, a luminal A ductal carcinoma (17,18), was obtained from ATCC (American Tissue Culture Collection, Manassas, VA, USA). The LM2 cell line, a highly metastatic variant of the triple negative adenocarcinoma MDA-MB-231, was obtained after two rounds of metastasis selection in mice (19). Descriptions of methods hereafter pertain to both cell lines. Cancer cells were maintained in Dulbecco’s Modified Eagle Medium (Gibco, Burlington, ON, Canada). The medium was supplemented with 15% heat-inactivated fetal bovine serum, 100 IU/ml penicillin, and 100 ug/ml streptomycin (all from Gibco) at 37°C and 5% CO₂. Fresh supplemented medium was added every other day. Cells were harvested by washing 80% confluent flasks with phosphate buffered saline (PBS) and adding 0.05% trypsin EDTA (Gibco) to detach cells. Cells were then harvested, counted, resuspended in PBS, and prepared in a solution containing 25% matrigel (v/v).
Eighteen healthy 6-weeks-old female immunodeficient rats (Harlan Laboratories) were inoculated with approximately $1 \times 10^7$ cells in the mammary fat pads while anesthetized with 1.5% isoflurane. Estrogen pellets for 60-day release (Innovation Research of America, Sarasota, FL, USA) were inserted subcutaneously into the back of the neck for the estrogen-dependent cell line ZR75.

**In-vivo MRI**

Rats were imaged when tumors reached a size of 1 to 2 cm in diameter. Imaging was performed on a 3-Tesla clinical MR scanner (Achieva 3.0 T TX, Philips Medical Systems, Best, The Netherlands), using an eight-channel wrist coil for signal detection. Rats were induced on 2% isoflurane in pure oxygen (2 L/min flow rate) and maintained on 1.5% isoflurane during imaging. Rats were placed prone within the coil, resting on top of a water-blanket maintained at 36 degrees Celsius (HTP-1500, Adroit Medical Systems, Loudon, TN, USA). A 24-gauge angiocath was inserted into the lateral tail vein for contrast injection, and this was connected through a 1-mL line tubing to a 3-way stop-cock through which a contrast agent and saline could be delivered separately. Gd-DTPA (Magnevist, Bayer, Wayne, NJ, USA) was injected as a bolus at a dose of 0.05 mmol/kg followed by 2 mL of saline. A $T_1$-weighted spin-echo scan and $T_1$ mapping were acquired before and 10 minutes after Gd-DTPA injection. The $T_1$-weighted spin-echo scan used a 2D acquisition with the following parameters: repetition time (TR) = 724 ms, echo time (TE) = 14 ms, number of signal averages (NSA) = 3, 10 cm field-of-view (FOV), twenty 1-mm thick slices, and $0.6 \times 0.6$ mm in-plane resolution. $T_1$ mapping consisted of a 3D spoiled fast field echo (T1-FFE) sequence repeated at flip angles of $2^\circ$, $10^\circ$, and $20^\circ$; other
parameters were TR = 6.2 ms, TE = 3.2 ms, NSA = 8, 10 cm FOV, twenty 1-mm thick slices, and 0.6 × 0.6 mm in-plane resolution; $B_1$ mapping was performed using dual-angle 2D non-selective excitation (20). At the end of the experiment, 0.05 mmol/kg of manganese(II) chloride tetrahydrate (Sigma-Aldrich Canada Inc., Oakville, ON, Canada) was injected subcutaneously at the back of the neck, and animals returned the next day for 24-hour post-MnCl$_2$ imaging.

**In-vitro MRI**

Cell pellets were also prepared and imaged to assess uptake and retention of MnCl$_2$. Medium containing 1.0 mM of MnCl$_2$ was added to cells growing in the exponential growth phase for one hour, after which cells were rinsed with fresh medium and trypsinized. Cells were centrifuged at 440 g for 10 minutes to create cell pellets in borosilicate glass tubes (Life Science Products Inc., Frederick, CO 80530, USA). Imaging and $T_1$ mapping were performed as described above. Three independent measurements were made for each cell line at each time-point.

**Data Analysis**

MRI data was transferred to an independent workstation for quantitative data analysis using in-house software developed in Matlab (v.8.1) (MathWorks, Natick, MA).

In-vivo data were first analysed for $T_1$ relaxation times before and after contrast administration. $T_1$ maps were generated by calculating $T_1$ relaxation times, corrected for $B_1$ variations, on a pixel-wise basis on every slice in the 3D imaging volume using a previously described method (20). Regions of interest (ROIs) were drawn on $T_1$ maps to encompass the entire tumor; the mean $T_1$ value was then determined in the ROI. The change in relaxation rate $R_1$
(1/T₁) post-contrast injection relative to baseline (i.e. pre-contrast injection) was determined for each tumor following both MnCl₂ and Gd-DTPA administration.

In-vitro data was also analysed on a pixel-wise basis for R₁ relaxation rates in cell pellets immediately, 24 hours, and 72 hours after incubation with MnCl₂. The average R₁ was calculated in each cell pellet.

**Statistical Analysis**

Changes in R₁ in tumors following MnCl₂ and Gd-DTPA administration were compared using a paired-sample t-test. For the in-vitro study, two-way analysis of variance (ANOVA) was performed, with the two main effects being the cell line and time post-incubation; post-hoc Tukey-Kramer testing for multiple comparisons was then performed at the 95% confidence level. Significance is reported at a p-value of 5% unless otherwise stated.

**Histology and Immunohistochemistry**

Tumors were excised anywhere between 3 to 10 weeks post-tumor induction, depending on their growth rate in vivo. Samples were fixed in 10% neutral buffered formalin at room temperature and processed for histopathology. This involved embedding in paraffin, sectioning into 5 μm thick slices, and staining with hematoxylin and eosin (H&E) to assess composition and necrosis and CD34 to assess tumor vascularization (21,22). Light microscope images (Olympus BX60, Olympus Canada Inc., Richmond Hill, Ontario, Canada) were taken to assess tumor composition, structure, and vascularity (H.Y. with over 30 years of experience examining the histopathology of xenografts versus patient tumors).
RESULTS

In-vivo contrast-enhanced $T_1$-weighted spin-echo images acquired post-injection of MnCl$_2$ or Gd-DTPA are shown for both ZR75 and LM2 cancers in Figure 1. Administration of MnCl$_2$ resulted in substantial enhancement of ZR75 tumors upon visual inspection, a cell line that consistently demonstrated visually very low to negligible enhancement on Gd-DTPA. In LM2 tumors where more noticeable enhancement on Gd-DTPA was sometimes observed, enhancement from MnCl$_2$ remained greater. It is also seen that MnCl$_2$ can reveal tumor morphology, in this case a small hypointense centre, not easily appreciated on Gd-enhanced images (Figure 1B).

To quantify the increased tumor enhancement provided by MnCl$_2$ for low Gd-enhancing tumors, Figure 2 superimposes $T_1$ maps on the images shown in Figure 1. It is seen in Figures 2A and B that MnCl$_2$ reduced tumor $T_1$ more than did Gd-DTPA, and that this effect was generally greater in ZR75 tumors than in LM2 tumors. Overall, nearly all tumors demonstrated a larger increase in $R_1$ on MnCl$_2$ than on Gd-DTPA (Figure 2C). MnCl$_2$ resulted in $R_1$ increases over 20% in 72% of tumors, whereas Gd-DTPA achieved similar increases in only 22% of tumors. This larger $R_1$ increase on MnCl$_2$ was significant whether we considered the cancer cell lines individually or in combination (Figure 3).

To determine if differences existed in MnCl$_2$ uptake and retention between the cancer cell lines, Figure 4 shows in-vitro measurements of $R_1$ in cells from 0 to 72 hours after exposure to 1.0 mM MnCl$_2$. Mn-induced effects were significantly greater in LM2 cells, but they returned to baseline 24 hours after exposure whereas ZR75 cells retained contrast much longer.
Four different cases are shown in Figures 5 to 8 to illustrate the capabilities of Mn-enhanced MRI, using histological assessment to guide us in our interpretation of features identified on imaging. In all cases, low or poor vascularity was confirmed by CD34 positive staining of capillaries on histology. Figure 5 shows a ZR75 tumor that appears patchy on Mn-enhanced MRI (Figure 5A). Gross pathology (Figure 5B) revealed a uniform appearance throughout the tumor, but H&E (Figure 5C) showed necrotic patches in agreement with imaging findings. CD34 staining (Figure 5D) confirmed very few blood vessels were present and were located mainly in the periphery. Figure 6 again shows a ZR75 tumor, this one with a less patchy appearance on both Mn-enhanced MRI (Figure 6A) and H&E (Figure 6C). CD34 (Figure 6D) again confirmed the presence of very few blood vessels. Figure 7 shows a LM2 tumor with a central non-enhancing core (Figure 7A) that matched a white necrotic center on gross pathology (Figure 7B). This core was better delineated on Mn-enhanced MRI compared to Gd-enhanced MRI. H&E revealed a distinct transition from viable tumor to a necrotic core devoid of cells (Figure 7C). CD34 revealed very few but large calibre blood vessels localized in the periphery (Figure 7D). Figure 8 shows another LM2 tumor, one where a small empty hole seen on gross pathology (Figure 8B) was identified only on Mn-enhanced MRI (Figure 8A). H&E showed the presence of many red blood cells, which would explain the particularly high Gd-enhancement observed in this tumor. CD34 also confirmed the presence of more blood vessels compared to the other tumors (Figure 8D). Note that the histology shown focuses on areas exhibiting features of apoptosis (pyknotic nuclei) and/or overt necrosis among viable epithelioid cell islands with a glandular-like arrangement typical of the histology shown for many breast cancer lines.
DISCUSSION

Sensitive detection and delineation of tumors currently relies on the tumor blood supply to deliver contrast-enhancing imaging agents. Where tumors are hypervascular, this approach is, indeed, very sensitive. However, where tumors have low vascularity or a poorly formed vasculature that would impair the delivery of contrast agents, detection sensitivity can be compromised. In this study, we investigated the potential of Mn-enhanced MRI, which is a cellular, not vascular, imaging technique, to sensitively detect and delineate tumors that exhibit relatively low enhancement on Gd-DTPA. It was shown in nude rats that two different orthotopic breast cancer models, both confirmed on histology to have low vascularity, enhanced significantly more on MnCl₂ compared to Gd-DTPA, with MnCl₂ producing an additional $R_1$ increase of 15% on average over that from Gd-DTPA. As the Mn ion accumulates inside cells and effectively highlights areas where cells are present, Mn-enhanced MRI offers a unique capability of accurately delineating necrotic tumor regions. The greatest advantage perhaps is unambiguous tumor localization, as enhancement on MnCl₂ applies to the entire tumor mass and is not restricted to select regions, such as the border, that generally have higher vascularity.

As demonstrated in a previous in-vitro study (23), cancer cells preferentially enhance due to cellular uptake of the Mn ion. Therefore, Mn-enhanced MRI is an excellent tool for localizing cancer cells. As our histology findings indicate, tumor regions that show positive contrast indicate mainly viable regions with dense packing of cells. Regions within the same tumor that do not enhance or enhance less correspond to necrotic areas that may be partially or completely devoid of cells. Note that such detailed morphological assessment, which provides important information on the status of the tumor, is difficult to achieve on Gd-enhanced MRI.
The degree of enhancement depends not only on the cellularity of the tumor but on a number of other factors. One important influence is the cancer cell type. As seen in this study, the two cell lines ZR75 and LM2 differed markedly in their uptake and release of the Mn ion as assessed by MR relaxometry. While ZR75 cells took up significantly smaller amounts of Mn ions compared to LM2, an observation previously seen in relation to other cell lines and is most likely attributed to a lower metastatic potential (23), they retained them for much longer, resulting in a sustained bright positive contrast even 24 hours after they were removed from exposure to MnCl₂. Had imaging been performed within a shorter time interval, the LM2 cells may have retained more Mn ions and thereby produced a significantly higher signal. Optimizing contrast is beyond the scope of this study, but it is important to bear in mind the characteristics of the specific cancer cell lines when designing experiments to answer different scientific questions.

Other factors that determine the degree of enhancement include the dose and mode of administering MnCl₂, the time interval post-injection for imaging, and the vascularity of the tumor. In this study, we chose a low dose of 0.05 mmol/kg and injected subcutaneously to avoid problems of elimination through liver uptake associated with intravenous injections. Subcutaneous injection effectively enabled a much longer and sustained delivery of MnCl₂. Thus, although MnCl₂ could not be efficiently delivered to the tumors due to poor tumor vascularity, the prolonged presence of MnCl₂ in the body meant there was a continuous albeit slow leakage of MnCl₂ across tumor blood vessel walls, eventually reaching the tumor center through diffusion and accumulating in tumor cells. It is also possible that in vivo when cells are exposed to MnCl₂ over a long interval, sometime during this time window some tumor cells may attain equilibrium and start releasing contrast agent while other tumor cells may continue to
accumulate contrast agent so long as MnCl₂ is present in the body. This behavior is difficult to model and study in vitro, but findings from our cell studies suggest that the most likely explanation for the in-vivo observations is that LM2 cells had already begun to release Mn ions, whereas ZR75 cells may not have reached the equilibrium point yet.

Although the two breast cancer models both displayed low enhancement on Gd-DTPA, their vascular characteristics were different. In the ZR75 model, very few blood vessels were identified on histology, and this was in agreement with the very low to negligible enhancement seen on Gd-DTPA. The LM2 model was also hypovascular, but compared to ZR75 tumors there were more blood vessels. However, these vessels were also poorly formed, leading to poor perfusion, as histology also revealed patchy, disorganized deposits of red blood cells that were inconsistent with their presence within well structured blood vessels. Through these models, this study has shown that tumors with either low vascularity or poor perfusion may benefit from Mn-enhanced MRI.

In order to optimize Mn-enhanced MRI for detecting low Gd-enhancing tumors, there are a number of considerations for future investigation. One area to investigate is the choice of imaging timepoint so that sufficient positive contrast can be achieved. It is reasonable to hypothesize that certain cell lines, such as the LM2, may offer maximum contrast less than 24 hours post-MnCl₂ administration. Finding this optimal timepoint for imaging is critical to reaping the maximum benefit from Mn-enhanced MRI. Alongside this timepoint optimization, tumors should be extracted and analyzed for absolute Mn content to support imaging findings. In this study, Mn content was not quantified, as the main focus was to reserve entire tumors for histological studies of tumor vascularization. Another important endeavor is to investigate the
The universality of this method in a wide range of brain and body tumor models and include both primary and metastatic cancers.

The greatest question perhaps is if this method can be translated to humans and have clinical benefits. There has been much debate over the safety of MnCl$_2$ due to toxicity observed in preclinical studies. However, it is important to note that these studies employed much higher doses, ranging from 0.25 to 0.50 mmol/kg or even higher for single administrations and up to 2.0 mmol/kg for cumulative dosing (24-26). The fact that Mn is found naturally in our bodies, being an essential micronutrient for proper cell function, suggests that there should be a safe threshold limit. We need to find this safe threshold to enable application in humans. In this study, greater enhancement could have been easily achieved by using a high dose of MnCl$_2$. However, our goal was to determine if tumors would enhance even at low doses. Future studies will invariably need to seek a balance between tumor detectability and dosing. Ultimately, the greatest benefits will be reaped if the method can enable not only the detection of primary lesions but also early detection of metastases that have not yet developed a significant vasculature.

There are a number of limitations of the study that need to be discussed to interpret the results in the correct context. First and foremost, the increased enhancement provided by MnCl$_2$ over Gd-DTPA averaged 15%. Although this difference was statistically significant, it is nonetheless a fairly modest improvement and whether or not it suffices to improve clinical detection remains to be answered. As mentioned previously, a number of factors that influence enhancement, such as dose and imaging timepoint, have not been optimized in this study; these certainly will need to be carefully considered to reap greater gains in detectability. Another limitation is that only two cancer cell lines were investigated, and even between these there was
a difference in enhancement gains provided by MnCl$_2$. To generalize our findings to other breast cancer subtypes and other primary cancers, other cell lines need to be included and their Mn-uptake characterized. A third limitation involves method of assessment. Because xenografts were used, their presence and locations were known a priori and the only objective method of assessing increased detectability was to compare changes in contrast-induced $R_1$. However, in the clinic, radiologists have no knowledge of whether or not a tumor even exists, and tumors are detected visually and not by quantitative changes in relaxation rates. Therefore, the most effective way to assess if our method improves detectability is to have a radiologist, one who is blinded to the location and existence of a tumor, to inspect both Gd-enhanced and Mn-enhanced MRI from a set of images whose order have been randomized.

In conclusion, in-vivo Mn-enhanced MRI is shown to detect and delineate more accurately and with greater sensitivity orthotopic breast tumors that exhibit low enhancement on Gd-DTPA. As a cellular contrast agent, MnCl$_2$ effectively enhances the entire tumor mass and depicts necrotic regions that are otherwise difficult to appreciate. The results of this study suggest that Mn-enhanced MRI may play a role in the detection and characterization of tumors.
REFERENCES


17. Osborne CK, Monaco ME, Kahn CR, Huff K, Bronzert D, Lippman ME. Direct inhibition of
growth and antagonism of insulin action by glucocorticoids in human breast cancer cells in

18. Engel LW, Young NA, Tralka TS, Lippman ME, O'Brien SJ, Joyce MJ. Establishment and
characterization of three new continuous cell lines derived from human breast carcinomas.

advanced metastatic breast cancer using combination oral UFT-cyclophosphamide

20. Cheng HL, Wright GA. Rapid high-resolution T(1) mapping by variable flip angles: accurate
and precise measurements in the presence of radiofrequency field inhomogeneity. Magn

21. Ganeshan B, Goh V, Mandeville HC, Ng QS, Hoskin PJ, Miles KA. Non-small cell lung
cancer: histopathologic correlates for texture parameters at CT. Radiology 2013;266:326-
336.

22. Goscinski MA, Nesland JM, Giercksky KE, Dhakal HP. Primary tumor vascularity in
esophagus cancer. CD34 and HIF1-alpha expression correlate with tumor progression. Histol

23. Nofiele JT, Czarnota GJ, Cheng HL. Noninvasive manganese-enhanced magnetic resonance

24. Bock NA, Paiva FF, Silva AC. Fractionated manganese-enhanced MRI. NMR Biomed

FIGURE LEGENDS

Figure 1. In-vivo Mn-enhanced and Gd-enhanced MR images of breast tumor-bearing rats at 3 Tesla. $T_1$-weighted spin-echo images acquired pre- and post-contrast injection for (A) ZR75 and (B) LM2 breast tumors. A dose of 0.05 mmol/kg of contrast agent was administered. Arrow points to tumor.

Figure 2. $R_1$ increase in tumors following MnCl$_2$ and Gd-DTPA administration. $T_1$ maps superimposed on anatomical $T_1$-weighted spin-echo images for (A) ZR75 and (B) LM2 breast tumors. Arrow points to tumor. (C) Relative $R_1$ increase post-contrast is compared between MnCl$_2$ and Gd-DTPA for all tumors.

Figure 3. Contrast-induced relative $R_1$ increase in tumors. $R_1$ increase relative to pre-contrast injection levels averaged across all tumors or across the tumor subtype is compared for MnCl$_2$ and Gd-DTPA. Shown are mean values ± SD. Significance is indicated: * $p < 0.05$, ** $p < 0.01$.

Figure 4. Cellular retention of MnCl$_2$ in vitro. Measurements of $R_1$ in cancer cell pellets at various times post-incubation with MnCl$_2$. Shown are mean values ± SD. A decrease in $R_1$ is significant at 24 hours post-incubation only for LM2 cells: * $p < 0.05$.

Figure 5. Case study 1. A low Gd-enhancing ZR75 tumor is shown on (A) contrast-enhanced MRI, (B) gross pathology, (C) H&E (×4 and ×20 magnification), and (D) CD34 (×4 and ×20 magnification). Histology confirmed very low vascularity (arrowheads) and patchy necrosis (arrows) consistent with Mn-enhanced MRI.
**Figure 6. Case study 2.** A low Gd-enhancing ZR75 tumor is shown on (A) contrast-enhanced MRI, (B) gross pathology, (C) H&E (×4 and ×20 magnification), and (D) CD34 (×4 and ×20 magnification). Histology confirmed very low vascularity (arrowheads).

**Figure 7. Case study 3.** A LM2 tumor with a non-enhancing core is shown on (A) contrast-enhanced MRI, (B) gross pathology, (C) H&E (×4 and ×20 magnification), and (D) CD34 (×4 and ×20 magnification). Necrotic center (arrows) essentially devoid of cells is well depicted on Mn-enhanced MRI. Few dilated blood vessels are seen (arrowheads).

**Figure 8. Case study 4.** A LM2 tumor with a hypointense center seen only on Mn-enhanced MRI is shown on (A) contrast-enhanced MRI, (B) gross pathology, (C) H&E (×4 and ×20 magnification), and (D) CD34 (×4 and ×20 magnification). Small hole observed on gross pathology was identified only on Mn-enhanced MRI. Histology revealed the presence of patchy, disorganized deposits of red blood cells (arrowheads).
Figure 1. Mn-enhanced MRI improves detection sensitivity and/or delineation of tumor structure compared to Gd-DTPA-enhanced MRI. In-vivo $T_1$-weighted spin-echo images at 3 Tesla acquired in rats with a (A) ZR75 breast tumor and a (B) MDA-MB-231 variant breast tumor. Note necrotic core in (B) is barely seen on Gd-enhanced MRI but clearly delineated on Mn-enhanced MRI.
Figure 2. Contrast-induced T1 decreases in tumors following contrast administration. T1 maps superimposed on $T_1$-weighted spin-echo images at 3 Tesla acquired in rats with a (A) ZR75 breast tumor and a (B) MDA-MB-231 variant breast tumor. (C) The decrease in T1 following MnCl$_2$ injection is generally higher than that following Gd-DTPA in all tumors.
Figure 3. Relative T1 decrease following contrast administration in ZR75 and LM2 breast tumors. The change following injection of MnCl$_2$ is significantly higher than that following Gd-DTPA in both low-enhancing ZR75 tumors (p < 0.01) and in LM2 tumors (p < 0.05). Shown are mean values and SD across all tumors.
Figure 4. xxx.
Figure 5. ZR75 tumor 5 – typical low vascularity tumor
Figure 6. ZR75 tumor 1 – low vascularity with necrotic and hemorrhagic areas
Figure 7. LM2 tumor 1 – distinct necrotic white core
Figure 8: LM2 tumor 2 – necrotic with a hole in the center