

The Development of an MR Agent for Imaging of Malignant Micro-calcification in Breast Cancer

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Introduction

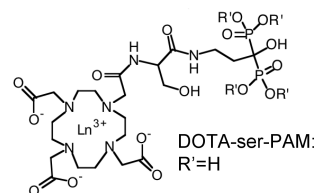
We have been interested in developing multi-modality contrast agents that have high sensitivity and specificity for the imaging of hydroxyapatite (HA), which is the most common form of micro-calcifications found in human breast cancers [1]. Our approach is based on the observation that bis-phosphonates, like pamidronate, show high affinity and high specificity for HA. We have previously reported an optical agent (near IR) [2], a SPECT agent, and a dual modality (NIR optical and SPECT) agent for imaging HA [3]. We have also developed analogous lanthanide-chelated DOTA-bisphosphonate derivatives [4] that have high affinity and high selectivity for HA. Although, there have been a number of studies describing the synthesis and characterization of gadolinium based MR contrast agents targeted to imaging HA, their success has been limited to the extent that it has been suggested that binding to HA “silences” these agents through the removal of water from their hydration sphere upon binding to HA. In separate studies we have found that the water associated with HA crystals have a relatively long T_1 and a short T_2 indicating that these waters are out of the “extreme narrowing NMR limit” and thus may not be visualized on conventional MR sequences that have relatively long TE 's. In this present report we demonstrate the application of Ultra-short TE (UTE) sequences [5] for MR-imaging of contrast agents bound to HA *in-vivo* and *in-vitro*.

Methods

UTE MR imaging. All of the MRI studies employing UTE were performed on the clinical 1.5T GE Signa scanner equipped with a custom-built small animal imaging coil.

In vitro concentration dependant MR imaging by UTE as a function of TR. 5 mg HA was taken in each of 1.5 mL plastic eppendorf tubes and 0, 0.1, 1, 10 and 100 μ M concentration of Gd-DOTA-Ser-PAM (see Structure) in 50 μ l PBS were added respectively to each tube. After 1h vortexing, RT crystals were washed with 4 \times 500 μ l PBS. 50 μ l PBS was added to each of the tubes and MR imaging performed using UTE with TR=17, 50, 200, 500, and 1000 msec and TE=100 μ sec. Other imaging parameters were: FOV=11 cm, slice thickness=5 mm, matrix size=256 \times 256, NEX=2.

In vivo imaging of HA. 50 mg of HA crystals taken in 300 μ l PBS was implanted subcutaneously at right flank of anesthetized mice. UTE MR image was taken after implantation of HA crystal using TR/TE=200 msec/ 100 μ sec, FOV=6 cm, slice thickness=5 mm, matrix size=256 \times 256. 4 μ mol of Gd-DOTA-Ser-PAM in 300 μ l saline was injected intravenously. After 6h of clearance, an MR image was taken with same parameters.



Results and discussion

As shown in Figure 1, UTE images obtained with TR=200 msec resulted in the highest imaging sensitivity, allowing visualization of HA crystals mixed with as low as 1 μ M concentration of agent. The actual concentration is at least ~40% lower, since the crystals were washed. This result points at the exceptionally high relaxivity of the agent in the bound state. As shown in Figure 2, Gd-DOTA-Ser-PAM provided high sensitivity detection of the implanted HA crystals. These results demonstrate that Gd-DOTA-Ser-PAM in combination with UTE MR imaging would constitute a MRI agent for high specificity and high sensitivity detection of micro-calcification comprised of HA *in vivo*.

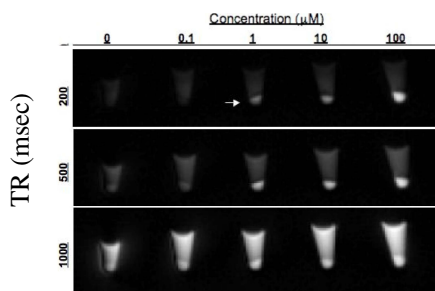


Figure 1. *In vitro* concentration dependent MR imaging of HA crystals labeled with Gd-DOTA-Ser PAM using the UTE sequence. The arrow points at successful detection of crystals labeled with 1 μ M CA using TR/TE=200 msec/100 μ sec. The same intensity scale is used in all images.

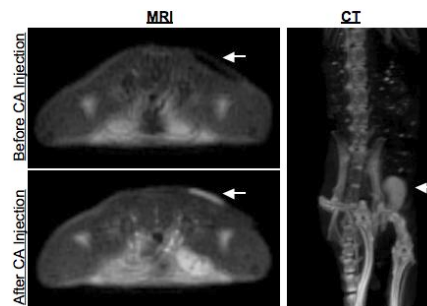


Figure 2. *In vivo* MRI study: HA crystals were implanted subcutaneously at right flank of a mouse. CT scan (right) was performed to locate implanted HA crystals (arrow). UTE MR imaging was performed before (top left) and after injection of Gd-DOTA-Ser-PAM and after 6h clearance (bottom left).

Conclusions

We have produced a family of lanthanide-chelated bis-phosphonates derivatives that are potential MRI agents for imaging HA. The Gd based compounds possess high relaxivity. We have shown that application of UTE MR sequences is crucial for the newly developed MRI agents, allowing for the first time enhanced imaging of micro-calcifications structures *in vitro* and *in vivo*. This study provides a foundation for the design and development of methods for high sensitivity MR detection of micro-calcifications in breast cancer.

References

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