Off-Resonance Saturation Method to Enhance SPIO Contrast in Molecular Imaging of Cancer

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Introduction

The growing use of superparamagnetic iron oxide nanoparticles (SPIO) in molecular imaging, cell tracking and biomolecular detection has resulted in the rapid development of novel SPIO formulations and imaging acquisition methods.[1] Compared to the low molecular weight, paramagnetic metal chelates such as Gd-DTPA (T_1 contrast agent), SPIO nanoparticles are considered T_2-negative contrast agents with substantially higher T_1 and T_2 relaxivity. Conventionally, SPIO particles are evaluated by T_2- or T_2*-weighted MR imaging for their abilities in dephasing water signals. Image void at high SPIO concentrations and requirement of a pre-contrast scan limit the accuracy and specificity for SPIO detection. Here we describe the development and application of an off-resonance saturation (ORS) method[2,3] to allow for on-and-off imaging of $\alpha_v\beta_3$-targeted SPIO-loaded polymeric micelles for molecular imaging of solid tumors.

Experimental Methods

SPIO-loaded polymer micelles with and without surface functionalization of cyclic Arg-Gly-Asp (cRGD) ligand were produced following a published procedure.[4] SPIO phantoms were prepared by suspending SPIO-micelles in the PBS buffer. The ORS study was carried out using a spin-echo (SE) pulse sequence modified by the addition of frequency-selective Gaussian-shaped pre-saturation pulse at B_1 powers of 52 - 104 Hz and at frequency offsets of ±2 kHz, ±1 kHz, ±800 Hz, ±600 Hz, ±400 Hz and ±200 Hz (relative to the water). Reference images were collected using identical parameters except without the pre-saturation pulse.

Results and Discussion

The ORS effect was quantified as the ratio of Mz/Mz^o, where Mz and Mz^o are the magnitudes of water z-magnetization with and without a pre-saturation pulse, respectively. A steady-state z-magnetization model was developed describe the ORS contrast. In agreement with theoretical simulations, SPIO phantom studies showed that increasing the *B1* pulse power and duration, from 52 to 104 Hz and 0 to 500 ms, respectively, caused the SPIO-loaded wells to progressively darken (data not shown).

To demonstrate the ORS effect *in vivo,* a human tumor xenograft mouse model was used. Figure 1A shows the post-injection image without the pre-saturation pulse. In total, five SPIO-micelle injections were administered intratumorally into tumor xenografts on the lower abdominal area of the mouse: three injection sites (indicated by the three arrows in the left of Fig. 1A) in the left tumor with cRGD-encoded SPIO-micelles and two injections in the right tumor with non-cRGD encoded SPIO–micelles (the two remaining arrows in Fig. 1A). All five injections were precisely planned at well-separated locations but within one single axial plane. The SPIO injection sites were barely visible in the post-injection reference images (Fig. 1A). Saturation at off-resonance frequency Ω = 1 ppm (Fig. 1B) resulted in darkening of all SPIO injection sites. This difference made the SPIO sites appear bright in the ratio image ($Mz|_{\Omega=1kHz}/\ Mz|_{\Omega=0.2kHz}$) (Fig. 1C). Importantly, the 3 injection sites on the left tumor from cRGD-encoded micelles appear brighter and larger than the 2 sites on the right tumor from non-cRGD encoded micelles, suggesting a higher retention of cRGD-micelles inside the tumors. This result agrees with our previous discoveries that cRGD-encoded micelles effectively target and internalize in $\alpha_v\beta_3$-expressing tumor endothelial cells[5] that are mostly found in tumor vasculature.

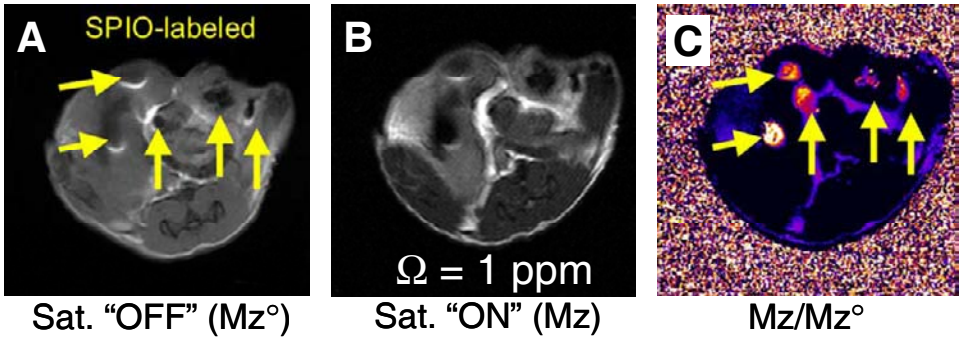

Figure 1. *In vivo* ORS imaging of SPIO-micelles injected in a subcutaneous mouse tumor xenograft. (A) Post-injection MR image without the pre-saturation pulse; (B) post-injection MR image with pre-saturation pulse at an off-resonance frequency at Ω = 1 ppm; (C) the ORS Mz/Mz^o ratio image obtained by dividing image (B) by image (A). Other imaging parameters: TR = 2s, TE = 12ms, FOV 40x40mm; data matrix = 128x128, slice thickness = 3 mm;. B_1 =104 Hz and t =500 ms.

Conclusion

This unique pre-saturation (ORS) strategy demonstrates the proof-of-principle where on-and-off MR images can be obtained to enhance the SPIO contrast in the detection of tumors. This method potentially permits accurate image subtraction with only post-contrast images to visualize specific tumor markers (e.g. $\alpha_v\beta_3$ integrins) in molecular imaging applications.

References

1. Bulte *et al*, *NMR Biomed.* **2004**, *17*, 484-499. 2. Hu et al, MRM. 2006, 56, 726. 3. Ren *et al*, poster in 5th annual meeting of Society of Molecular Imaging. 4. Gao *et al*, *Nano Letters*, in press. 5. Gao *et al*, *Angew Chem.Int.Ed.* **2004**, *43*, 6323-6327.