Characterization of Adiabatic Pulse Prepared Cell Imaging of Iron Oxide Nanoparticles

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Introduction

Cellular imaging using superparamagnetic iron-oxide nanoparticles (SPIOs) and magnetic resonance imaging (MRI) has drawn interest for applications ranging from monitoring normal biological and disease development using gene reporters to targeting biomarkers expressed in cancer cells [1]. While iron-oxide nanoparticles traditionally produces regions of signal loss in T2*- and T2-weighted images, *in vivo* applications involve inhomogeneous tissues with physiological process such as blood flow that can lead to signal loss that is not associated with the presence of nanoparticles. To this end a number of techniques have been proposed to enhance the signal in the volume surrounding the SPIOs usually by manipulating the macroscopic magnetic field [2]. A method using an adiabatic preparation pulse with a contrast dependent on the failure of the adiabatic condition [3] for spins diffusing in the microscopic magnetic field gradients surrounding the nanoparticles has been presented and supported by numerical simulation and phantom experiments [4]. We extend the adiabatic pulse preparation technique to SPIOs taken up by cells and an *in vivo* model of tumor angiogenesis imaging using $\alpha_v\beta_3$ targeted, cRGD-encoded SPIO loaded polymeric micelles [5].

Methods

Human glioma U87 cells were cultured and then incubated in serum-free RPMI media with a 0.1 concentration of small peptide RGD conjugated SPIO (10 nm, core size), targeting $\alpha_v\beta_3$ integrin overexpressed in U87 cells, for two hours at room temperature. Cells were washed with PBS and then scraped from the flasks. Collected cells were re-suspended in 1 mL of 2% agarose gel in tubes containing different numbers of cells for MRI. Binding of RGD-SPIO to U87 cells was confirmed using Prussian blue staining for iron. Cell imaging was preformed on a 3 Tesla Siemens Magnetom Trio (Siemens Medical Solutions, Malvern, PA, USA) using a spin-echo sequence with a 10ms adiabatic full passage hyperbolic secant preparation pulse: TE: 15ms, TR: 10sec, 128x128 slice=2mm, FOV=100x100mm. For the tumor model, HT1080 (fibrosarcoma) cells delivered by subcutaneous injection into the dorsal flank of a nude mouse and allowed to grow. cRGD encoded, SPIO-loaded micelles were then injected intravenously and allowed to circulate for 1.5 hours. The tumors were excised, fixed in formalin and suspended in PBS for imaging. After imaging, histological examination was performed and sections were stained for iron by Prussian Blue. In order to achieve higher spatial resolution the tumors were scanned on a 9.4 Tesla Bruker BioSpec 94/20 (Bruker BioSpin Corporation, Billerica, MA, USA): TE: 10.205ms, TR: 10sec, 128x128 slice=1.00mm, FOV=30x30mm. B_1 power to achieve adiabaticy was determined experimentally in the absence of nanoparticles, and adiabatic contrast images where calculated by subtracting the adiabatic pulse prepared image from the image without preparation and then normalizing to the non-prepared image.

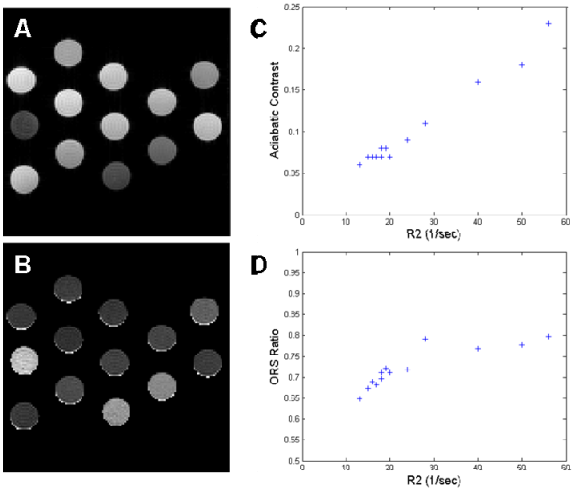

Figure 1: *T2-weighted imaging of cell samples shows varying degrees of signal loss associated with SPIO loading (A), and the adiabatic contrast image shows an inverse correlation of signal intensity with the T2-weighted image (B). Adiabatic contrast is linearly correlated with the R2 of the cell sample (C), while the off-resonance saturation (ORS) remains linear over the lower region with saturation at higher values (D).*

Results

Cell samples with a greater decrease in signal on T2-weighted imaging (Fig. 1A) show a greater the intensity in the adiabatic contrast image (Fig. 1B). The R2 of each cell sample was calculated and compared to the measured adiabatic contrast due to its linear relationship with number of iron loaded cells [6]. These data show that the linear correlation of iron concentration and adiabatic contrast reported in the phantom studies appears similar in the cell samples (Fig. 1C). For comparison, an off-resonance saturation (ORS) was applied as another method of producing increasing signal with increasing iron concentration [7]. For this method, while the technique shows a linear correlation with R2 over low concentrations the signal saturates for higher values, complicating quantification of SPIO.

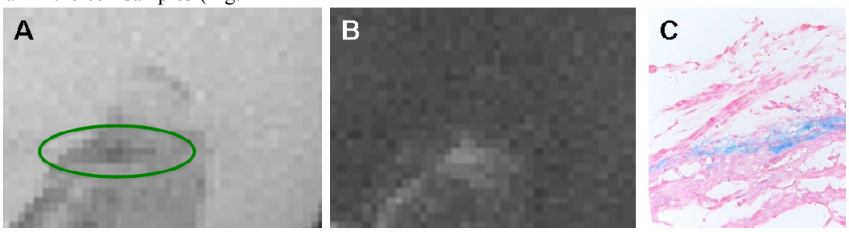

Figure 2: *The spin-echo image of the tumor shows the inhomogeneous enhancement of the tumor tissue (A) and the adiabatic contrast image shows a region of enhancement with suppression of the PBS and tumor (B). Histological examination of this section with Prussian Blue staining shows the presence of SPIO (C) in this region highlighted in (A) by a green oval.*

Adiabatic contrast images of the tumor model show suppression of background signal covering much of the tumor tissue with areas of signal enhancement. One area of interest marked by the green oval in the spin-echo image (Fig 2A) appears brighter than the nulled background signal from the tumor tissue and PBS in the adiabatic contrast image (Fig. 2B). Histology shows that this region contained an accumulation of nanoparticles consistent with the change in signal on T2-weighted imaging as well as the increase in adiabatic contrast.

Discussion

The cell and tumor images show that the adiabatic preparation technique for imaging SPIOs may be extended into *in vitro* and *in vivo* models of pathology. This method holds several advantages over R2 mapping including the ability to rapidly acquire prepared and non-prepared images without the need for multiple echoes, and the suppression of the background where the nanoparticles are not present. Secondly, this method appears to remain linearly correlated with R2 over the range of iron loading tested, which is a major advantage for quantification. Further studies are needed to characterize the effects of the adiabatic pulse on other off-resonance components within the *in vivo* environment and to optimize the frequency sweep and amplitude of the applied adiabatic pulse. The potential speed of acquisition, background suppression and linear quantification of the range of cellular SPIO loading are promising for *in vitro* and *in vivo* applications.

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