The role of ^{19}F diffusion weighted spectroscopy for specific and sensitive detection of molecularly targeted perfluorocarbon nanoparticles

E. A. Waters[1], X. Yang[1], G. M. Lanza[1], S. A. Wickline[1], and J. Chen[1]
[1]Cardiology, Washington University Medical School, Saint Louis, MO, United States

Introduction

Molecular imaging with targeted nanoparticle contrast agents presents opportunities for simultaneous detection and treatment of disease. However, real-time detection of targeted delivery of a molecular agent is challenging because it requires separating the signal of bound agent from the background signal of the unbound agent circulating in the bloodstream. Previously, we reported that ^{19}F signal from perfluorocarbon (PFC) nanoparticles internalized in stem cells can be detected specifically by using ^{19}F diffusion weighted MR spectroscopy (DW_MRS) to suppress the ^{19}F signal of nontargeted nanoparticles.[1] By analogy, we hypothesized that ^{19}F DW_MRS may provide a unique tool for *real-time detection* of the binding of $\alpha_v\beta_3$ integrin targeted PFC nanoparticles to angiogenesis *in vivo*. The objective of this study was to characterize the specificity and sensitivity of ^{19}F DW_MRS for detecting the binding of PFC nanoparticles to angiogenesis in the ears of a transgenic mouse model (K14-HPV16) of multi-stage epidermal squamous carcinoma with abundant upregulated angiogenesis.

Methods

8-month-old male K14-HPV16 mice (n=5) were compared, with age-matched non-transgenic littermates (n=3) as controls. Mice were anesthetized with 50 mL/kg of a ketamine-xylazine cocktail. They were then injected with 1 mL/kg of avb3-targeted perfluoro-15-crown-5 ether (20 mol%) nanoparticle emulsion. MRI was performed on an 11.7T Varian Inova MR system, with the mouse's left ear positioned inside a single-turn solenoid coil tuned to 470 MHz. An MR compatible small animal heating and monitoring system (SA instruments, NY) was used to keep the body temperature at 37°C and to ensure physiologic respiratory rates. The ear was localized with scout ^{1}H images. Subsequently, slice-selective ^{19}F DW_MRS was performed using half-sine shaped diffusion gradients applied equally in three orthogonal directions. ^{19}F spectra were acquired with interleaved arrays of b-values ranging from 0 to 16000 s/mm^2 for transgenic mice and 0 to 4650 s/mm^2 for controls. Acquisitions were respiratory-gated with 128 signal averages, TR=2s, and scan time of ~21 minutes. TE ranged from 0.019s to 0.045s depending on the maximum diffusion weighting for each acquisition. For each ^{19}F spectrum, the area under the single peak that corresponds to the fluorine signal from crown ether was numerically integrated in Matlab. The area under each peak was then normalized to the area of the largest peak in its arrayed diffusion weighted acquisition.

Results

In K14-HPV16 transgenic mice, a small initial signal decay was observed from b=0 to b=~2000 s/mm^2, corresponding to suppression of ^{19}F signal from nanoparticles that were still flowing in the bloodstream, as expected. However, a substantial portion of ^{19}F signal persists even at very high diffusion weighting (b=15778) (Fig. 1A). This persistent diffusion weighted ^{19}F signal likely corresponds to a population of nanoparticles bound to $\alpha_v\beta_3$ integrin on the endothelium of neovasculature that is known to populate the ears of K14-HPV16 mice. In control mice (i.e., no specifically-bound nanoparticles), the ^{19}F signal decayed rapidly with increasing diffusion weighing. The ^{19}F signal was undetectable by b=111 s/mm^2, indicating that minimal binding had occurred and confirming that PFC nanoparticles were predominantly circulating in blood (Fig. 1B). This is consistent with the expected lack of angiogenesis (and molecular binding sites) in the normal ear. Immunohistological staining confirmed nanoparticle binding in the ears of K14-HPV16 mice (Fig. 2). Comparison of the results for transgenic and control animals indicate that the rapid decay of the ^{19}F signal in the control population is well-differentiated from the slower decay and persistent ^{19}F signal in the transgenic population (Fig. 3).

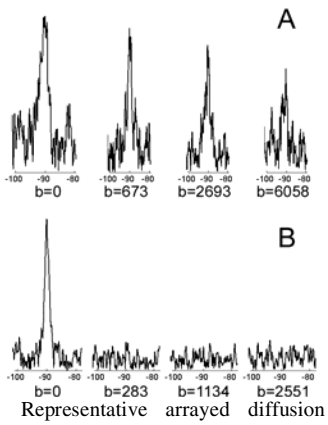

Figure 1: Representative arrayed diffusion weighted acquisitions for (A) K14-HPV16 and (B) control mice. Note contrast between persistent signal corresponding to bound nanoparticles in (A) and the rapid signal decay in (B).

Discussion and Conclusions

The lack of background ^{19}F signal in living tissue enables robust diffusion weighted spectroscopy in vivo. The results of this study show that ^{19}F DWS can be used for real-time and specific *in vivo* detection of bound PFC nanoparticles by selective suppression of the ^{19}F signal from flowing PFC nanoparticles in the bloodstream.

References

[1]Chen *et al*. Proc. Intl. Soc. Mag. Res. Med. 14 (2006) p. 359

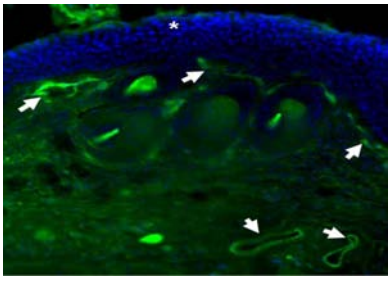

Figure 2: Fluorescent histological image showing FITC-labeled $\alpha_v\beta_3$ targeted PFC nanoparticles bound to neovasculature. Arrowheads indicate vessels.

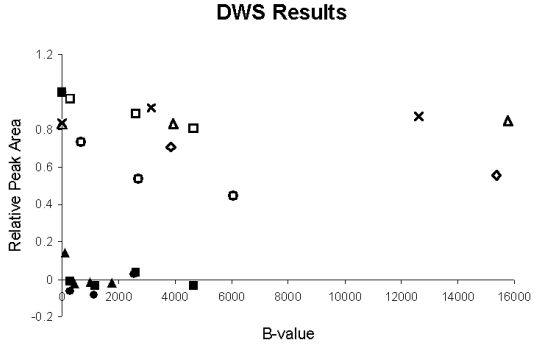

Figure 3: Results of numerical integration of arrayed diffusion weighted acquisitons. Open symbols: data from K14-HPV16 mice; filled symbols: data from controls. Note persistent signal in all transgenic mice even at very high diffusion weighting in contrast to rapid signal decay in controls.