Ultra-short Echo Time $^{19}F/^{1}H$ Imaging of Gadolinium-free Perfluoro-carbon Nanoparticles: A Robust Method for In Vivo Angiogenesis Imaging

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Introduction

The standard of care and outcomes for anti-angiogenic therapy could be profoundly changed by patient stratification based on MR Molecular Imaging of angiogenesis in diagnosis, treatment, and response monitoring [1]. Previously, $\alpha_v\beta_3$-integrin targeted nanoparticle (NP) emulsions [2] have been shown to detect and quantify angiogenesis for multiple tumor models in small animals. NP were visualized in pre- and post-injection ^{1}H-MRI via a Gadolinium-chelate (Gd) label [3, 4] or directly quantified using the ^{19}F-MR signal from the perfluoro-carbon core [5]. Early *in vivo* successes exploited the single resonance peak of perfluoro-crown-ether NP (PFCE; $C_{10}F_{20}O_5$) [5]. However, perfluoro-octyl-bromide (PFOB; CF_3-$(CF_2)_6$-CF_2Br) is a more clinically-relevant NP with a better-understood human safety profile, but it has a much more complex spectrum (*i.e.,* 7 ^{19}F resonance peaks and multiple relaxation conditions). Furthermore, although Gd has been shown to enhance the ^{19}F signal through T1 shortening, the absence of Gd is more attractive from a translational point of view. A new technique—dual-frequency balanced ultra-short echo time (UTE-SSFP) sequence with 3D radial readout—offers high sensitivity imaging of PFOB without the need for Gd. The current objective is to image angiogenesis in a rabbit adenocarcinoma model with the simultaneous $^{19}F/^{1}H$ UTE-SFFP technique using $\alpha_v\beta_3$-targeted PFOB nanoparticles with and without Gd to establish the feasibility of high sensitivity MR molecular imaging of Gd-free, fluorine-based, clinically-relevant contrast agents.

Methods

The study was performed on a 3T clinical whole-body scanner (Achieva, Philips Healthcare) using a dual-tuned transmit/receive surface coil (7×12 cm) and a dual $^{19}F/^{1}H$ spectrometer system [6]. Male New Zealand White rabbits (~2 kg, N=4) were implanted in the popliteal fossa of the left hind leg with 2-3 mm Vx-2 carcinoma tumors (National Cancer Institute, MD), which grew to ~15 mm within 2 weeks. Imaging was performed 3h post-injection of 1.0 ml/kg of $\alpha_v\beta_3$-targeted NP with PFOB core either with or without Gd-DTPA-bisoleate incorporated on the outer layer. The rabbits were initially anesthetized with xylazine/ketamine *i.m.* and maintained with a 20 ml/h ketamine *i.v.* infusion. All animal care protocols were in accordance with institutional guidelines. A radial 3D UTE balanced steady-state free precession (SSFP) sequence with concurrent dual-frequency RF for ^{1}H and ^{19}F (transmit and receive) was used with ^{19}F tuned to CF_2-resonance line group (5 lines, ±500 Hz range), isotropic voxel $(2.19mm)^3$, matrix 64^3, TR=1.75 ms, TE=90μs, flip angle α = 30°, pBW 900 Hz and a scanning time of 30 minutes. The radial k-space data was reconstructed at full resolution for the ^{1}H component, and a lower resolution with higher signal to noise for the ^{19}F component (Nyquist radius of 20%) [7].

Results and Discussion

In vivo imaging of angiogenesis-targeted PFOB nanoparticles was successful in all subjects, showing heterogeneous areas of neovasculature at the tumor rim (Figure 1, arrows) as expected in this established model. On ^{1}H images, the bound nanoparticles that carry Gd give rise to T1-based signal enhancement (Fig. 1d), whereas the Gd-free NP are invisible in ^{1}H MRI (a). For PFOB-NP both, with and without Gd, the ^{19}F signal is clearly detected (b/e) and anatomical localization is shown using a green overlay on ^{1}H anatomy (c/f). The majority of the fluorine nuclei (12 of 17) are found in the CF_2 resonances. Within the 90μs echo time of the UTE-SSFP sequence, dephasing does not lead to destructive superposition of the resonances, thereby maximizing the obtained signal. Although T1-shortening of Gd has been shown to be beneficial in fluorine imaging, the balanced sequence provides high signal even for PFOB-NP without Gd with a T1 of about 1 second.

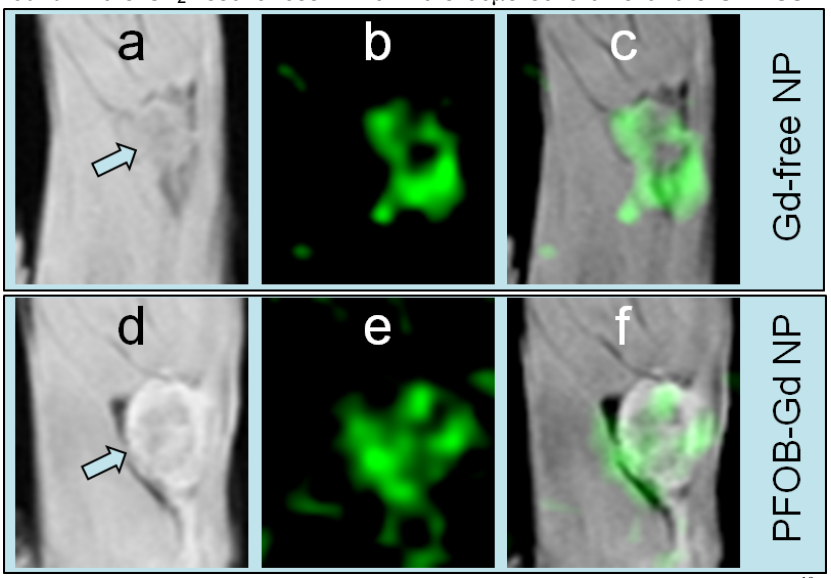

Figure 1: Molecular imaging of $\alpha_v\beta_3$-targeted NP on Vx2 tumors (arrows) in rabbits by ^{19}F MRI. Gd-free (a,b,c) or Gd-containing (d,e,f) NP with a perfluoro-octyl-bromide (PFOB) core were used and imaged with a novel UTE-SSFP based 3D radial sequence. ^{1}H images show T1-based enhancement only with Gd NP (d), while the ^{19}F signal is clearly detected in both cases (b,e). Image overlays (c/f) demonstrate the anatomical co-localization.

Conclusion

Dual frequency $^{19}F/^{1}H$ radial 3D balanced UTE-SSFP is a robust pulse sequence that allows high-sensitivity *in vivo* detection of angiogenesis-targeted PFOB-NP despite the complex resonant peak structure and extended longitudinal relaxation times. The synergistic combination of imaging technique and clinically-relevant, gadolinium-free agent should facilitate translation into clinical use.

References

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