HIGH SENSITIVITY DETECTION OF TARGETED PFOB NANOPARTICLES BINDING IN A CARCINOMA MOUSE MODEL USING A NEW DIFFUSION-WEIGHTED SPECTROSCOPY SEQUENCE

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Introduction

Molecular imaging with MRI targeted contrast agents (CA) has emerged as a promising diagnostic approach in cancer research by detecting associated biomarkers. Thanks to the lack of ^{19}F background signal, ^{19}F CA may be an interesting candidate to detect tumor angiogenesis. We recently developed a multi spin echo (MSE) MRI sequence dedicated to perfluorooctylbromide (PFOB), a perfluorocarbon well-known for its high biocompatibility but yielding a complex multipeak spectrum due to J-coupling. This sequence takes full advantage of NMR properties of PFOB and yields an excellent sensitivity thanks to cancellation of J-modulation by selective refocusing and T_2 enhancement by using short interpulse delay **[1]**. Thus, we could previously detect tumor angiogenesis in a U87 glioblastoma mouse model by comparing concentrations in mice receiving functionalized PFOB nanoparticles (PFOB NP) targeting $\alpha_v\beta_3$ integrins and in mice receiving non-functionalized PFOB NP **[2]**. However, detection of bound targeted ^{19}F CA is challenging due to the presence of unbound agents in the blood pool. In this context, diffusion-weighted spectroscopy (DW-MRS) could selectively detect binding of angiogenesis-targeted ^{19}F CA by spoiling signal coming from flowing agents **[3]**. In this preliminary work, in order to detect sparse expression of $\alpha_v\beta_3$ biomarkers, we developed an original DW-MRS sequence dedicated to PFOB, combining the robustness of a diffusion-weighted LASER (Localization by Adiabatic SElective Refocusing) sequence **[4]** with a MSE acquisition scheme close to the one of our MSE MRI sequence. We demonstrate here the increased sensitivity of this sequence compared to conventional spectroscopic acquisition, and show that this strategy allows specific detection of angiogenesis in a CT26 carcinoma mouse model for an individual case.

Materials and Methods

MR acquisitions: NMR experiments were performed on a 7T Bruker rodent scanner with a homemade 3.2-cm linear birdcage coil. Previously described DW-LASER sequence, yielding insensitivity to anisotropy and cross-terms **[4]**, was modified by replacing the long acquisition by a spin echo train (30 echoes), as shown in Figure 1. All 180° adiabatic pulses performed selective refocusing of the CF_3 group. Echo time was 80 ms for the LASER module, and interpulse delay was 30 ms in the subsequent echo train. Each echo was sampled over 25.5 ms (64 effective complex points, 4000 Hz spectral bandwidth). Final spectrum was the complex sum of the 30 echoes.

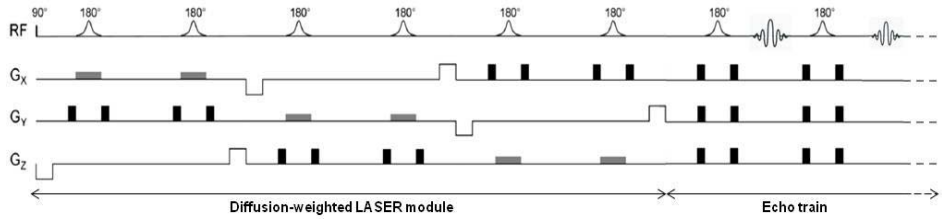

Figure 1. Volume selective diffusion-weighted LASER_MSE sequence.

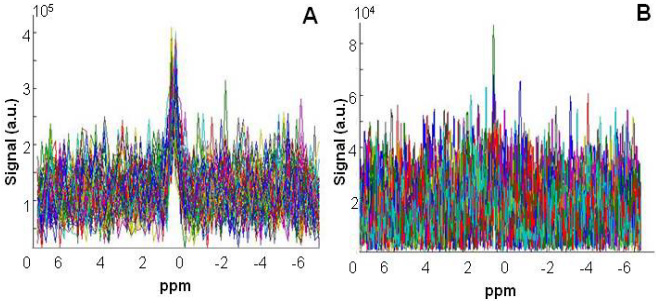

Figure 2. Array of 64 spectra acquired with the LASER_MSE sequence (A) and with the LASER sequence (B) on the low concentrated phantom.

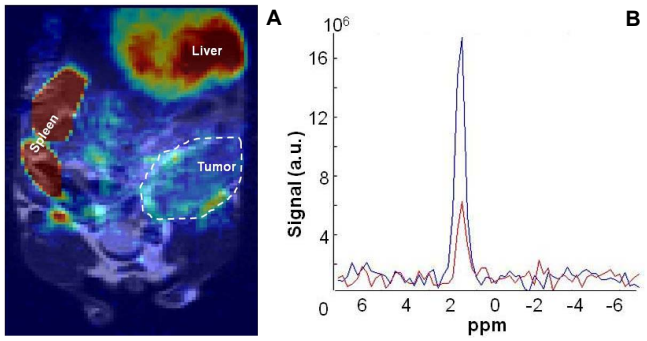

Figure 3. (A) Superposition of the ^{19}F image with the anatomical image. The tumor is delineated by the white dashed line. (B) Spectra acquired in the tumor with the LASER_MSE sequence at b=0 s/mm² (blue) and b=5000 s/mm² (red)

Phantoms experiment: 64 spectra were acquired in a diluted PFOB emulsion phantom (concentration in NP ~180 pM) with the LASER and the LASER_MSE sequence. Data processing with line broadening and integration around the peak was performed to estimate standard deviation (SD) over the 64 spectra.

Animal experiment: The tumor model was established by injecting 3.10^5 cells (murine colon carcinoma cell line CT26.WT, ATCC, Molsheim, France) subcutaneously into the right flank of an immuno-depressed "nude" mouse. Twelve days after tumor inoculation, the animal was anesthetized with i.p. injection of ketamine and domitor, was infused with 200 µL PFOB emulsion (concentration in NP ~ 90 nM) functionalized by grafting of RGD peptides at the surface of NP and placed inside the coil together with an emulsion reference (~30 µL) for signal calibration. After acquisition of anatomical ^1H images, ^{19}F acquisitions were performed with our MSE MRI sequence (TE = 15.5 ms, TR = 4000 ms, 60 echoes two 8-mm-thick slices, 0.63×0.94 mm² in-plane resolution, 30 min total acquisition time). DW-MRS was then performed using the LASER_MSE sequence in an 11×11×11 mm³ voxel positioned in the tumor (24 min acquisition).

Results and Discussion

Increased sensitivity of multi echo LASER: Despite the low concentration in NP in the sample, the CF_3 peak of PFOB is clearly visible on each of the individual 64 of the LASER_MSE sequence, unlike the LASER sequence (Figure 2). Relative SD on PFOB signal for the LASER_MSE sequence was 13%, whereas it was 47% for the LASER sequence, leading to a ~3-fold gain in sensitivity for the LASER_MSE sequence compared to the LASER sequence.

Detection of angiogenesis in vivo: As shown by Figure 3A, a strong ^{19}F signal was found in the mouse liver and spleen, and a weak diffuse signal was found in the viscera. In the tumor, ^{19}F signal is mainly visible in the rim, highlighting the rich vasculature in this region. Mean concentration in the tumor was evaluated to 240 pM in NP thanks to the emulsion reference. Figure 3B shows the spectra acquired with the LASER_MSE sequence at b=0 and b=5000 s/mm². The CF_3 peak of PFOB is clearly visible in both acquisitions. Signal loss between diffusion-weighted and non diffusion-weighted spectra was ~65%. This difference suggests that the signal seen in imaging is mainly of vascular origin, as signal coming from flowing particles is spoiled by diffusion. According to Waters et al **[3]**, the b-value of 5000 s/mm² is adequate to suppress signal coming from unbound particles. Therefore, the signal persistence at b=5000 s/mm² also suggests that a small part comes from PFOB NP unaffected by diffusion, and these particles are probably bound to $\alpha_v\beta_3$ receptors in the tumor neo-vessels. This result has to be confirmed on other animals and to be compared with the results obtained after injection of a non-targeted emulsion. However, this preliminary work shows that sub-nanomolar concentrations in PFOB NP can be detected *in vivo* with an original single-voxel spectroscopy sequence adapted to NMR constraints of PFOB. The ability of our LASER_MSE sequence to suppress ^{19}F signal from the blood pool by diffusion is encouraging to specifically detect and quantify angiogenesis on individual animals with no need to wait for blood clearance.

[1] Giraudeau et al. *MRM* 2010 ; [2] Giraudeau et al. *Proc ISMRM* 2011 ; [3] Waters et al. *MRM* 2008 ; [4] Valette et al. *Proc ISMRM* 2010