Brain tumor angiogenesis can be imaged by ^{19}F MRI: high sensitivity detection of targeted PFOB emulsion in U87 human glioblastoma mouse model

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Introduction

Due to the absence of NMR-visible ^{19}F in living tissues, ^{19}F MRI has the strong advantage over ^{1}H MRI to specifically detect administered ^{19}F-containing compounds without background signal, and to yield a linear relationship between signal and concentration of contrast agent. These properties, combined with the fact that ^{19}F is the most sensitive nucleus after ^{1}H, have raised much enthusiasm about ^{19}F MRI emerging as a potential substitute to PET imaging. A very promising application would be the detection and characterization of brain tumors. However, only a few studies have taken advantage of ^{19}F MR imaging or spectroscopy to study tumors [1], and this has never achieved in the brain. In this context, designing a high sensitivity ^{19}F MRI strategy may be critical to assess the ability of ^{19}F MRI to detect brain tumors.
Due to their high payload in ^{19}F nuclei, perfluorocarbon (PFC) emulsions yield a significant increase in sensitivity (per mole of contrast agents) and are therefore frequently used for ^{19}F MRI. Among PFCs, perfluorooctylbromide (PFOB) emulsions are of particular interest for translational and clinical research, since PFOB has undergone clinical trials. In the present work, we used functionalized PFOB nanoparticles (PFOB NP) targeting $\alpha_v\beta_3$ integrins - which are involved in the process of tumor angiogenesis – in a U87 glioblastoma mouse model. In order to take full advantage of the contrast agent for ^{19}F MRI, we used our recently developed PFOB-dedicated multi spin echo (MSE) sequence allowing cancellation of J-modulation and T_2 enhancement, and yielding an excellent sensitivity [2]. It is demonstrated that our strategy allows *in vivo* detection of angiogenesis in brain tumors.

Methods

Tumors were induced by i.c. injection of U87 human glioma cells (2 µL, ~ 10^5 cells) in the right cerebral hemisphere of immuno-depressed male nude mice. NMR experiments were performed on a 7T Bruker rodent scanner with a homemade 2.6-cm linear birdcage coil. An emulsion reference was placed in the coil for signal calibration. Two weeks after implantation, mice were anesthetized with i.p. injection of ketamine and domitor. Three mice were infused with 200 µL PFOB emulsion (20% w/w, concentration in NP ~ 50 nM) grafted with RGD peptides, and containing polyethylene glycol (PEG, 5% w/w) for stealth and rhodamine for fluorescence. Two other mice were infused with the control emulsion without RGD. Anatomical coronal ^{1}H images were first acquired with a multislice multi spin echo sequence (TE = 8 ms, TR = 2500 ms, 12 echoes, nine 0.7-mm-thick slices, 0.3×0.3 mm^2 in-plane resolution). About one hour after injection, ^{19}F images were acquired with our optimized MSE sequence (TE = 10.5 ms, TR = 3000 ms, 60 echoes, one 7-mm-thick slice, 0.5×0.5 mm^2 in-plane resolution, 90 min total acquisition time). Briefly, this sequence allows imaging the CF_3 group of PFOB during a long train of spin echoes selectively refocusing the CF_3 group, which results in J-coupling suppression and T_2 enhancement and provides a ~5-fold gain in sensitivity compared to conventional short TE/TR sequences [2]. After imaging, mice were sacrificed and brains were removed, frozen and sectioned for fluorescence microscopy.

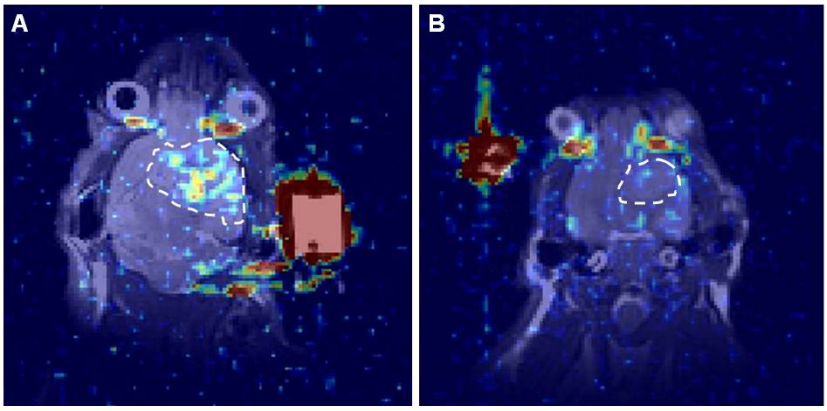

***Fig. 1**. Superposition of ^{19}F image with anatomical ^{1}H image of mice infused with the targeted **(A)** and control **(B)** PFOB emulsions. Tumors are delineated by the white dashed line. ^{19}F signal is clearly visible in the tumor in A, whereas only noise is seen in B. The red spot is the emulsion reference.*

Results and Discussion

The three mice infused with the targeted emulsion show enhanced ^{19}F signal localized in the tumor's region as seen in Fig. 1.A. Signal distribution within the tumor is not homogeneous. This result is corroborated by fluorescence microscopy presented in Fig. 2, showing intense fluorescent signal essentially localized around and inside the tumor. The heterogeneous pattern of enhancement observed either in MRI and microscopy is consistent with the known heterogeneity of tumor vasculature in this model that over-express $\alpha_v\beta_3$ integrin on the endothelial cells [3]. SNR in the region delineated by the white dashed line is 6, which leads to a mean concentration in PFOB NP in the tumor of ~ 120 ± 10 pM. SNR in the contralateral hemispheres is lower than 2. ^{19}F signal in the tumor of the mice infused with the control emulsion is hardly higher than in the contralateral hemisphere (Fig. 1.B) and yields a concentration in PFOB NP of 40 ± 5 pM. The fact that we see neither vascular signal in the normal brain nor signal from non-targeted PFOB NP in the tumor can be ascribed to strong spoiling effects on flowing spins during the long echo train of our MSE sequence. Therefore, our hypothesis is that only $\alpha_v\beta_3$-bound PFOB NP are detected in the brain, allowing the specific detection of angiogenesis. Note that large retro-orbital veins consistently exhibited ^{19}F signal for all five animals, presumably due to the very large blood volume where some ^{19}F signal could persist despite spoiling.

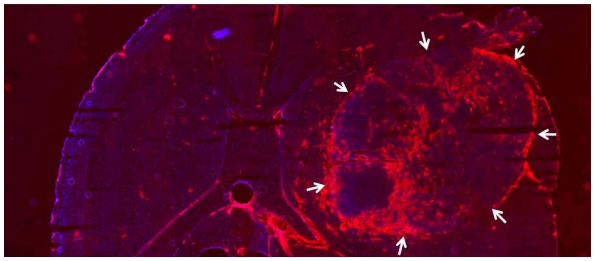

***Fig. 2**. Fluorescence microscopy image of the brain seen in Fig 1.A. (tumor is delineated by white arrows). The rind and inner part exhibit intense fluorescent signal coming from the $\alpha_v\beta_3$-targeted nanoparticles. Note that the enhancement pattern observed in the inner part of the tumor by ^{19}F MRI can be recognized.*

Conclusion

Using a PFOB-optimized high sensitivity MSE sequence, we were able to detect picomolar concentrations of $\alpha_v\beta_3$-targeted PFOB emulsion *in vivo* in a U87 human glioblastoma mouse model. To our knowledge, this study constitutes the first proof of concept of *in vivo* brain tumor detection using ^{19}F MRI of a functionalized perfluorinated emulsion. This work demonstrates that ^{19}F MRI may be an alternative to existing techniques (such as [^{18}F]Galacto-RGD PET imaging [4]) to reveal brain tumor angiogenesis.

References

[1] Waters et al., MRM 2008; [2] Giraudeau et al., MRM 2010; [3] Hsu et al. Mol Imaging Biol, 2006; [4] Schnell et al., Neuro Onco 2009