In vivo intracellular pO_2 measurements of perfluorocarbon labeled 9L glioma cells

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Introduction

Tumor oxygenation is strongly associated with the therapeutic efficacy of chemotherapy and radiotherapy against cancers. Non-invasive, *in vivo* oximetry of tumor tissues by MRI has enormous potential for preclinical evaluation of new therapeutics. Prior studies have established that the partial pressure of oxygen (pO_2) can be measured using T_1 relaxation time measurements of perfluoro-15-crown-5 ether (CE) [1]. In these existing methods, CE nanoparticles are injected directly into the blood stream, and tumor cell oximetry is sensed indirectly by the CE sequestered in the interstitial space [2]; however, it has not been possible to measure the intracellular oxygenation status of tumor cells directly. Recent studies have shown that it is possible to track transferred cells *in vivo* using ^{19}F MRI following *ex vivo* labeling with perfluorocarbon nanoemulsions [3, 4, 5]. In this study we apply *ex vivo* labeling with CE nanoemulsion to 9L gliosarcoma cells prior to implantation into the rat brain. We demonstrate the feasibility of measuring the direct intracellular pO_2 of 9L gliosarcoma cells in the brain. Following anatomical imaging of ^1H and ^{19}F showing the labeled 9L cells in their anatomical context, we measured the ^{19}F MRI T_1 and were able to estimate the intracellular pO_2.

Methods

Cultured 9L glioma cells (ATCC, Manassas, VA) were incubated with CE nanoemulsions using similar protocols as described elsewhere [3, 5]. The cell viability and proliferation was assessed using the Cell-Titer-Glo assay (Promega, Madison, WI) and cellular uptake of CE was quantified by ^{19}F NMR of cell pellets. Six week old male Fisher rats (Jackson laboratories, Bar Harbor, ME) were used. $2x10^6$ labeled 9L cells were injected in the right striatum stereotaxically. As a negative control, unlabeled 9L cells were injected into the left striatum. Brain imaging was performed using a Bruker 7 T horizontal-bore imaging system. A birdcage volume resonator was used that could be tuned to either ^{19}F or ^1H. The rats were imaged two days after tumor implantation. Respiration, ECG, temperature and arterial blood oxygen were monitored and maintained at 37 ºC and 180 mm Hg, respectively, throughout the experiment. High-resolution proton images were obtained using a standard spin echo sequence (TR/TE=1000/15 ms, 256x256 matrix size, FOV=4x4 cm, slice thickness=3 mm). ^{19}F images were obtained by rapid acquisition and relaxation enhancement (RARE, rare factor=8, TR/TE=1500/10 ms, 64x32 matrix size, FOV=4x4 cm, slice thickness=3 mm). The ^{19}F image was rendered in pseudo-color and superimposed onto the ^1H image. T_1 measurements were performed by point resolved spectroscopy (PRESS) with a single voxel encompassing the entire tumor and using seven different TR-values ranging from 400 to 8000 ms. A pO_2 versus T_1 calibration curve for CE was also constructed at 7 T and 37 ºC. Animals were cardio-perfused after the experiment and tumor status was assessed by histology.

Results

Our results show that ^{19}F MRI can be used to visualize labeled 9L glioma cells in the brain (Fig. 1A) and that ^{19}F MRS can be used to measure the intracellular pO_2 of the tumor cells. For the 9L cells, we measured a ^{19}F T_1=1.0±0.2 s (Fig. 1B), corresponding to an intracellular pO_2 of ~19.4 %. As a control, pO_2 was measured in a rat culled 1 hr prior; we measured T_1=1.4±0.2 s and ~8.6 % pO_2, which, as expected, is significantly lower.

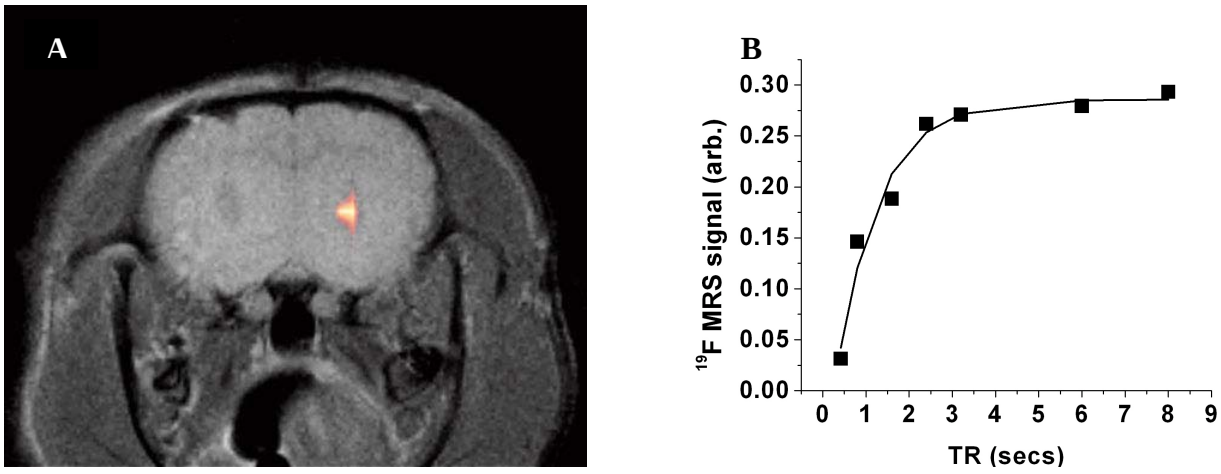

Fig 1. In vivo MRI and ^{19}F T_1 in an anesthetized rat showing labeled 9L glioma cells in the brain. **(A)** A composite $^{19}F/^1H$ image of the labeled 9L glioma cells stereotaxically injected into the right striatum two days prior. The ^{19}F is rendered in a hot-iron intensity scale. As a control, unlabeled 9L cells were injected into the contralateral striatum in the same imaging plane, but are not visible using these methods. Panel **(B)** shows the representative ^{19}F T_1 recovery curve obtained by PRESS of the implanted glioma cells. The solid curve is a two- parameter, single-exponential fit yielding T_1=1.0 ± 0.2 s which corresponds to a pO_2~19.4 %.

Conclusion

We have demonstrated the feasibility of ^{19}F MRI/MRS to localize tumor cells *in vivo* and measure their direct intracellular pO_2. The intracellular pO_2 measurement using ^{19}F MRS of 9L glioma cells has potential to probe the real-time dynamics of tumor cell metabolism before and after the delivery of therapeutics.

References

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