The aim of this study was to develop an MRI fluorocarbon oximetry technique using snapshot inversion recovery and compare it with fluorescence quenching fiber-optic probe oximetry (OxyLite) performed simultaneously in experimental mouse tumors. The oxygen reporter probe hexafluorobenzene (HFB) was injected directly into the tumors, along with the insertion of the OxyLite probe. Tumor oxygenation (pO2) was modified using carbogen or lethal doses of the anesthetic gas. MRI pO2 maps were generated in 1.5 min with an in-plane spatial resolution of 1.88 mm. MRI and OxyLite showed consistent baseline and postmortem pO2 values. Increases in tumor pO2 during carbogen breathing showed similar kinetics for the two methods. The pO2 values observed using the OxyLite corresponded with relatively hypoxic values observed by MRI. The apparent discrepancy between mean values might be due to the difference in sampling volumes of the techniques and the observation of multiple locations using 19F MRI versus a single location using the large optical fiber. Overall, the present method provides a rapid way to map the tumor oxygenation and is particularly suitable to monitor acute changes of pO2 in tumors. Magn Reson Med 61:634–638, 2009. © 2008 Wiley-Liss, Inc.

Key words: hypoxia; OxyLite; 19F MRI; fluorescence quenching

Hypoxia is a characteristic feature of locally advanced solid tumors resulting from an imbalance between oxygen supply and consumption (1). The role of hypoxia in increasing tumor resistance to radiation therapy has been well established (2). More recently, tumor hypoxia has been shown to decrease the efficacy of certain cytotoxic drugs (3,4). Moreover, investigations conducted over the past two decades have demonstrated that tumor hypoxia, in addition to diminishing therapeutic efficacy, plays a pivotal role in malignant progression. Methods to determine tumor oxygenation are therefore of crucial importance for the prediction of therapeutic outcome. Among the numerous techniques considered to increase temporarily intratumoral oxygenation for therapeutic advantage in experimental tumors (5,6), few have been tested in the clinical setting. This might be due to the lack of methods available to monitor dynamically and noninvasively tumor oxygenation in humans. Many current methods, such as Eppendorf electrodes, near infrared spectroscopy, hypoxia reporter agents, and susceptibility NMR-based methods are either highly invasive, nonquantitative, or lack spatial resolution. An emerging method to dynamically map tumor oxygenation is electron paramagnetic resonance (EPR) imaging. MRI methods, however, are still more widely available and 19F MRI oximetry methods have shown their potential utility. Mason and colleagues (7) have been successfully developing FREDOM MRI (fluorocarbon relaxometry using echo planar imaging for dynamic oxygen mapping) following direct intratumoral injection of the oxygen reporter molecule hexafluorobenzene (HFB). The technique was used to study tumor oxygenation after chemotherapy and radiotherapy and was compared with near-infrared spectroscopy (NIRS), fiber optic probes, oxygen needle electrodes, and the hypoxia marker pimonidazole (8–11). The aim of the current study was to develop an MRI fluorocarbon relaxometry technique (similar to Mason’s FREDOM) to measure tumor oxygenation and compare it with simultaneous measurements from an MR-compatible fiber-optic probe.

MATERIALS AND METHODS

Tumor Model and Experimental Protocol

A transplanted live tumor model (TLT) (12) was implanted in the leg of NMRI mice. For inoculation, ~106 cells in 0.1 mL of media were injected intramuscularly into the right leg. Mice developed palpable tumors within a week of inoculation. Tumors were allowed to grow to 8 mm in diameter prior to experimentation. Animals were anesthetized by inhalation of isoflurane mixed with 21% oxygen (air) in a continuous flow (1.5 L/h), delivered by a nose cone. Anesthesia was induced using 3% isoflurane. It was then stabilized at 1.8% for a minimum of 15 min before any measurement. Warm air was flushed into the magnet in order to maintain normothermia. All experiments were conducted according to national animal care regulations. After induction and stabilization of anesthesia, baseline measurements were acquired under air-breathing conditions for 10 min. Subsequently, air was switched to carbogen (95% O2, 5% CO2) for 50 min. Finally, the mouse was sacrificed using 5% isoflurane in air for 20 min.

pO2 Measurements

19F MRI Measurements

MRI was performed with a 4.7T (200 MHz, 1H), 40 cm inner diameter bore system (Bruker Biospec, Ettlingen, Germany).
Comparing $^{19}$F MRI and OxyLite

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FIG. 1. Schematic description of the SNAP-IR pulse sequence.

FIG. 2. Calibration ($R_1$) of HFB with respect to $pO_2$ (mmHg).

PARAMETRIC IMAGES OF THE SPIN-LATTICE RELAXATION TIME ($T_1$) WERE ESTIMATED USING A SNAPSHOT INVERSION RECOVERY (SNAP-IR) PULSE SEQUENCE (13–15). THE PULSE SEQUENCE CONSISTED OF A NONSELECTIVE HYPERBOLIC SECANT INVERSION PULSE (10 ms LENGTH), FOLLOWED BY ACQUISITION OF A SERIES OF 512 RAPID GRADIENT ECHO IMAGES (REPETITION TIME = 10.9 ms, ECHO TIME = 4.2 ms, FLIP ANGLE = 1°, MATRIX = 32 × 16, FIELD OF VIEW = 60 × 30 mm, BANDWIDTH = 12.5 kHz, SINGLE THICK SLICE [PROJECTION], TOTAL ACQUISITION TIME = 1.5 min) (Fig. 1).

RAW (K-SPACE) DATA WERE ZERO-FILLED TO A MATRIX SIZE OF 64 × 64, RESULTING IN INTERPOLATED 64 × 64 IMAGES. FOR EACH IMAGE PIXEL $T_1$ WAS ESTIMATED BY FITTING THE STANDARD 3-PARAMETER MONOEXponential RECOVERY EQUATION (MAGNITUDE DATA ONLY) DESCRIBING THE SIGNAL AS A FUNCTION OF INVERSION TIME (13–15). WE DENOTE THIS COMPUTED $T_1$ AS $T_1'$ BECAUSE THE RECOVERY CURVE IS SOMEWHAT PERTURBED BY THE RF PULSES (16). WE THEN COMPUTED $R_1' = 1/T_1'$. CALIBRATION OF HFB ($R_1'$ WITH RESPECT TO $pO_2$) WAS PERFORMED BY MEASURING $R_1'$ IN THREE DIFFERENT SEALED TUBES CONTAINING 300 μL HFB RESPECTIVELY BUBBLED WITH NITROGEN (0% $O_2 = 0$ mmHg), AIR (21% $O_2 = 160$ mmHg), AND CARBON (95% $O_2 = 722$ mmHg) FOR 20 MIN IN A 37°C WATER BATH BEFORE MEASUREMENT.

FOR IN VIVO STUDIES, HFB WAS INJECTED INTO THE TUMOR VIA AN INSULIN SYRINGE (29G) AND DEPOSITED ALONG THREE TRACKS (3 × 30 μL) ENCOMPASSING BOTH CENTRAL AND PERIPHERAL REGIONS IN A CORONAL PLANE. HFB WAS DEOXYGENATED BY BUBBLING NITROGEN FOR 5 MIN BEFORE USE (10). MRI SCOUT IMAGES WERE OBTAINED FOR BOTH $^1H$ (200.1 MHz) AND $^{19}$F (188.3 MHz) TO REVEAL HFB DISTRIBUTION WITHIN THE TUMOR. SNAP-IR IMAGES WERE USED TO MEASURE P02 WHILE MICE BREATHED AIR, THEN CARBON, THEN ISOFLURANE 5% IN AIR.

Fluorescence Quenching Measurements

We used the OxyLite (Oxford Optronix, Oxford, UK) system for continuously monitoring tumor oxygenation and temperature simultaneously with $^{19}$F MRI. An MR-compatible fiber-optic microprobe was inserted into the tumor (under the MR surface coil). The probe was inserted longitudinally into the middle of the tumor, parallel to the plane of the surface coil. This microprobe consisted of a thermocouple and an oxygen sensor based on the fluorescence quenching of a platinum-based fluorophore. Probes (tip diameter: 350 μm) were inserted using a 26G needle to make a track inside the tissue. Data were collected continuously at a sampling frequency of 0.1 Hz before, during, and after carbogen breathing. No change in temperature was observed during $^{19}$F MR pulsing. OxyLite pO2 measurements are single-point measurements; the volume sampled is confined to the sensor tip.

RESULTS

In Vitro Data

The respective $R_1'$ measurements for HFB in 0, 21, and 95% $O_2$ were 0.105 ± 0.006 s⁻¹, 0.407 ± 0.048 s⁻¹, and 1.56 ± 0.08 s⁻¹, providing a calibration curve of $R_1' = 0.1048 (± 0.0060) + [0.002000 (± 0.000005)] pO2$, where $R_1'$ is in units of s⁻¹ and $pO2$ is in units of mmHg (Fig. 2). This is similar to the calibration curve found by Mason and colleagues (7), $R_1 = 0.0835 (± 0.0010) + [0.001876 (± 0.000009)] pO2$. (The better precision reported by the Mason group is probably due to their larger number of calibration samples used.) The relation $pO2 = [R_1' - 0.1048]/0.002$ was used voxel-by-voxel for the remainder of our experiments.

In Vivo Data

Tumor oxygenation was monitored simultaneously with $^{19}$F MRI and OxyLite in TLT tumors before, during, and after a respiratory challenge with carbogen. The temperature was monitored during the experiments. Carbogen breathing is known to improve tumor oxygenation in experimental models. A typical result is shown in Fig. 3, with OxyLite and $^{19}$F MRI measurements recorded for the same mouse. We can see that the dynamics of response are similar between the two techniques, but that the amplitude of response to carbogen inhalation is quite different despite similar baseline and postmortem values. During carbogen breathing the $pO2$ measured by $^{19}$F MRI was higher than that measured by OxyLite for the majority of experiments. This is illustrated in Fig. 4, where all values from both techniques are reported. Color $pO2$ maps were created from the $^{19}$F MRI data to investigate heterogeneity of response (Fig. 5). Histograms of the $^{19}$F MRI data were also generated (Fig. 5). The color maps show that each region of the tumor responds differently to the respiratory challenge, with some groups of pixels having values above 100 mmHg. From the histograms we observe a clear shift to

A tunable $^1H$/ $^{19}$F surface coil was used for RF transmission and reception.

Parametric images of the spin-lattice relaxation time ($T_1$) were estimated using a snapshot inversion recovery (SNAP-IR) pulse sequence (13–15). The pulse sequence consisted of a nonselective hyperbolic secant inversion pulse (10 ms length), followed by acquisition of a series of 512 rapid gradient echo images (repetition time = 10.9 ms, echo time = 4.2 ms, flip angle = 1°, matrix = 32 × 16, field of view = 60 × 30 mm, bandwidth = 12.5 kHz, single thick slice [projection], total acquisition time = 1.5 min) (Fig. 1). Raw (k-space) data were zero-filled to a matrix size of 64 × 64, resulting in interpolated 64 × 64 images. For each image pixel $T_1$ was estimated by fitting the standard 3-parameter monoeXponential recovery equation (magnitude data only) describing the signal as a function of inversion time (13–15). We denote this computed $T_1$ as $T_1'$ because the recovery curve is somewhat perturbed by the RF pulses (16). We then computed $R_1' = 1/T_1'$.

Calibration of HFB ($R_1'$ with respect to $pO_2$) was performed by measuring $R_1'$ in three different sealed tubes containing 300 μL HFB respectively bubbled with nitrogen (0% $O_2 = 0$ mmHg), air (21% $O_2 = 160$ mmHg), and carbon (95% $O_2 = 722$ mmHg) for 20 min in a 37°C water bath before measurement.

For in vivo studies, HFB was injected into the tumor via an insulin syringe (29G) and deposited along three tracks (3 × 30 μL) encompassing both central and peripheral regions in a coronal plane. HFB was deoxygenated by bubbling nitrogen for 5 min before use (10). MRI scout images were obtained for both $^1H$ (200.1 MHz) and $^{19}$F (188.3 MHz) to reveal HFB distribution within the tumor. SNAP-IR images were used to measure $pO2$ while mice breathed air, then carbon, then isoflurane 5% in air.

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the right of the median pO2 value under carbogen-breathing conditions. For most tumors (such as in Fig. 5) a bimodal distribution was observed for the carbogen breathing, reflecting the high heterogeneity of response of TLT tumors.

**DISCUSSION**

The SNAP-IR pulse sequence allowed us to sample tumor oxygenation with an effective in-plane spatial resolution (1.88 mm) similar to that of FREDOM (1.25 mm) (7). Our acquisition time was 1.5 min, which is shorter than that of FREDOM (6.5 min). For $T_1$ measurements it has been theorized that a Look-Locker (17) data acquisition strategy (e.g., SNAP-IR) provides nearly twice the $T_1$ measurement precision per unit acquisition time compared to a saturation-recovery data acquisition strategy (e.g., FREDOM). However, we did not investigate further this potential efficiency advantage of SNAP-IR over FREDOM. The extra signal-to-noise ratio obtained from the relatively high amount of HFB injected into the tumors ensured that the higher temporal resolution could be obtained without sacrificing precision.

Mean basal pO2 values were quite variable from one tumor to another, ranging between 5 and 20 mmHg with both techniques. However, pO2 maps and histograms obtained with $^{19}$F MRI showed many regions of highly hypoxic pixels, which are the radiobiologically relevant regions. Tumor pO2 measured with both techniques showed a consistent increase during the carbogen intervention. Our in vivo data corroborate with results obtained with previous $^{19}$F MRI and OxyLite studies (18,19) as well as with other techniques, such as EPR oximetry or BOLD MRI (19,20), in terms of pO2 changes under similar respiratory challenges. Indeed, changes in pO2 from 20 to >100 torr in response to carbogen breathing have been reported in this tumor model, entirely in line with $^{19}$F MRI measurements here. There is, however, a huge difference in the magnitude of response between the two techniques. The high heterogeneity of response might explain the lack of direct agreement between $^{19}$F MRI and OxyLite. The sampling volumes of the two techniques are indeed very different. Moreover, for the $^{19}$F MRI data there was always a small region of “high responding” pixels that had a huge influence on the mean and median values. The probability of inserting the probe at that very location was low. Results obtained with the fiber-optic probes were more similar to the “low responding” pixels observed with $^{19}$F MRI, which are more numerous. Indeed, such voxels are apparent in the pO2 maps in Fig. 5.

By combining three OxyLite probes simultaneously in the same tumor, Zhao et al. (18) were able to interrogate different tumor regions so as to examine baseline and dynamic responses of regions, which were hypoxic, of low oxygenation, and with high baseline oxygenation. This information would have been particularly relevant to the present study, although it would have been technically impossible to combine three probes within the sensitive
Finally, it has been described that the relationship of the OxyLite sensor response versus oxygen concentration is nonlinear (21). Therefore, errors in determining fluorescence lifetime are more serious at high oxygen concentrations (where the Stern–Volmer curve is flatter) than at low concentrations. This could explain why we only saw a modest increase in pO₂ in response to carbogen breathing with OxyLite in comparison with 19F MRI.

Intratumoral injection of hexafluorobenzene has been argued to result in 19F MRI oximetry interrogating poorly perfused, chronically hypoxic tumor regions (22). However, considering the distribution of the basal values in the current study, as well as the large response to carbogen breathing observed with 19F MRI, we do believe that we interrogated both poorly perfused (chronically hypoxic) regions and well-perfused regions. Moreover, the role of acute hypoxia is now well recognized and this might explain why regions that seem completely hypoxic at one given time are still able respond to carbogen breathing.

It is possible, in principle, to correct the $R_1$ measurements to obtain $R_1$ values that would be measured from an unperturbed longitudinal relaxation recovery curve (16). This correction is given by $R_1 = R_1' + \ln(\cos(\alpha)) / \text{TR}$, where $\alpha$ is the flip angle and TR is the repetition time (spacing between RF pulses). The only effect that this correction would have on our data is that the intercept of the $R_1$ vs. pO₂ calibration curve would change from $0.1048 \pm 0.0060$ s⁻¹ to $0.0908 \pm 0.0060$ s⁻¹, putting it closer to Mason’s $0.0835 \pm 0.0010$ s⁻¹ (7). The correction would change neither the slope of the calibration curve nor the final pO₂ values estimated in vivo.

In conclusion, we present here a new 19F MRI method for oxygen mapping using a SNAP-IR pulse sequence that is particularly relevant in terms of temporal resolution. We used the method to map tumor oxygenation in experimental tumors and showed the sensitivity of the technique to changes in tumor pO₂ with respect to respiratory challenges. Since the present sequence is more rapid than FREDOM, we suggest that it is particularly suitable to monitor acute changes of pO₂ in tumors.

REFERENCES