Qualification of a Noninvasive Magnetic Resonance Imaging Biomarker to Assess Tumor Oxygenation

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Abstract

Purpose: Although hypoxia has been long recognized as a crucial factor impairing tumor response in many therapeutic schemes, atraumatic and reliable methods of individually quantifying tumor oxygenation are still lacking in day-to-day clinical practice. The aim of this work was to investigate the potentially quantitative properties of our recently described noninvasive magnetic resonance (MR) technique "MOBILE" (mapping of oxygen by imaging lipids relaxation enhancement) and to qualify this endogenous contrast as a tumor hypoxia marker.

Experimental Design: The "MOBILE" technique, which assesses the longitudinal MR relaxation rate, $R_1$, of lipid protons, was benchmarked with the parent technique which assesses the global (or water) $R_1$, in response to a hyperoxic challenge (carbogen breathing) and to a hypoxic challenge (combretastatin A4) in MDA-MB-231 xenografts and in NT2 mammary tumors. Electron paramagnetic resonance (EPR) oximetry was used to quantitatively assess the tumor $pO_2$ in matching tumors longitudinally.

Results and Conclusion: Our study evidenced that (i) positive and negative changes in tumor oxygenation can be detected using MOBILE; (ii) a change in the $R_1$ of lipids is positively correlated with a change in the tumor $pO_2$ ($P = 0.0217, r = 0.5097$); (iii) measured lipid $R_1$ values are positively correlated with absolute $pO_2$ values in both tumor models ($P = 0.0275, r = 0.3726$); and (iv) changes in the $R_1$ of lipids are more sensitive than changes in the global $R_1$. As this technique presents unique translational properties, it seems promising for the individual longitudinal monitoring of tumor oxygenation in a clinical setting.

Introduction

Tumor hemodynamics has become a key target in preclinical and translational cancer research (1), involving both negative and positive modulations of tumor oxygenation/perfusion. On the one hand, attempts are made to target the established tumor vasculature or neovasculature by the use of antivascular or antiangiogenic agents (2–6), whose effects are traditionally assessed noninvasively not only using dynamic contrast MRI (DCE-MRI) after administration of an exogenous paramagnetic contrast agent (7–9), but also possibly using endogenous hemodynamic markers, including BOLD-MRI (blood oxygen level dependent MRI; ref. 10). On the other hand, positive modulations of tumor hemodynamics, tumor hypoxia, or environmental pH, for example, (11), are being considered in the field of tumor radiosensitization (12–14).

Most solid tumors contain regions of acute and chronic hypoxia that indicate a negative clinical outcome after radiotherapy (15). To bridge the gap between the occurrence of tumor hypoxia and clinical radiation practice, there is an essential need to predict the presence of hypoxic regions in tumors individually. On the basis of the individual tumor characteristics and/or the ability to alleviate the tumor hypoxia, it will become possible to adapt the individual treatment either by boosting optimal radiation doses in the resistant areas, by adapting the radiotherapy to each tumor throughout the course of treatment, or by administering an associated treatment to potentiate the effectiveness of the radiation treatment.

There is therefore a critical need to develop accurate, noninvasive and quantitative in vivo imaging methods of mapping tumor oxygenation in cancer management, both for the purpose of targeting tumor blood vessels and for the oxygen-induced resistance to radiation of tumors. Noninvasive, safe, and repeatable techniques to map tumor hypoxia are therefore required.

Direct quantitative methods, including Eppendorf microelectrodes (16), electron paramagnetic resonance (EPR) oximetry (17), $^{19}$F relaxometry (18), or Overhauser enhanced MRI (19), are either invasive or require the injection of a reporter probe, and are currently not clinically...
The MOBILE technique presents unique translational properties and seems promising for further clinical monitoring of individual tumor oxygenation with potential applications for the planning of radiotherapy or for assessing the response to anti-angiogenic or anti-vascular treatments.

Translational Relevance
This study describes MOBILE (mapping of oxygen by imaging lipids relaxation enhancement) as a sensitive, noninvasive endogenous marker of tumor oxygenation, as the technique is sensitive to both positive and negative modulations of tumor oxygenation and, more importantly, significantly correlated to actual pO2 values in matching tumors for both tumor models being studied. The MOBILE technique presents unique translational properties and seems promising for further clinical monitoring of individual tumor oxygenation with potential applications for the planning of radiotherapy or for assessing the response to anti-angiogenic or anti-vascular treatments.

Materials and Methods
Tumor models
A total of 7 × 10⁶ NT2 cells (provided by Elizabeth M. Jaffee, M.D., The Sidney Kimmel Cancer Center at Johns Hopkins, Baltimore, MD) or 10 × 10⁶ MDA-MB-231 (LGC Promochem), amplified in vitro, were collected by trypsinization, washed three times with Hanks Balanced Salt Solution (HBSS), and resuspended in 100 μL HBSS. These mammary tumor cells were injected subcutaneously into the right upper mammary fat pad of 6-week-old FVB/N or nude NMRI female mice (Janvier). The tumors were analyzed when they reached 5 mm in diameter. The animals were anesthetized by inhaling isoflurane (Forene) mixed with 21% oxygen (air) in a continuous flow (1.5 L/hour). This anesthetic has been shown not to interfere with tissue hemodynamics (41). The respiratory rate and body temperature (37.0°C ± 1.0°C) were monitored and maintained with a circulating water blanket. Studies were undertaken in accordance with the national and local regulations of the ethical committee (agreement number UCL/2010/MD/001).
Hypoxic and hyperoxic challenges
To negatively modulate tumor oxygenation, CA4 was administered at a dose of 100 mg/kg and MR measurements were taken before injection and repeated 3 hours after the administration of CA4. EPR oximetry was performed on the same tumors, in a similar way, before injection and directly after the second MRI session. To positively modulate tumor oxygenation, carbogen breathing (95% O2, 5% CO2) was used. For that purpose, three MR measurements of each type (global R1 and R1 of lipids) were taken sequentially and repeated three times while the subject was breathing air. The gas was then switched to carbogen, and MR measurements were repeated at 10, 15, and 20 minutes after the switch, as it is known that oxygenation is significantly increased after 10 minutes’ breathing (42). EPR experiments were subsequently performed on the same tumors. We considered five hyperoxic challenges and five hypoxic challenges in each tumor model. Both challenges could have been applied on the same tumor for three NT2 tumors and two MDA-MB-231 tumors. The remaining experiment was performed on different tumors.

MR experiments
T1 measurements.
A segmented IR FISP (Inversion-Recovery Fast Imaging with Steady state Precession) sequence (SSFP FID mode) was used to acquire parametric images of T1 relaxation time, as described previously (33). Briefly, the acquisition parameters were TR/TE/FA/BW/matrix = 4 ms/1.2 ms/5°/100 kHz/64 x 64, four segments, and a total acquisition time of 1 minute 20 seconds. For the global proton experiment (global R1, essentially reflecting the water peak), a series of 100 images were taken, with a slice thickness of 1 mm. For the lipid experiments (MOBILE), the offset between water and lipid peaks was assessed experimentally with a single pulse sequence (the lipid peak of interest was ~4.0 ppm) and then used as an imaging frequency offset in the same IR FISP protocol. We added a saturation pulse to spoil the water signal. A series of 40 images (spaced by scan repetition time, TR = 100 ms) with a slice thickness of 2 mm were acquired with a spatial resolution of 0.344 x 0.344 mm. The images were then fitted using a home-made program written in Matlab (The MathWorks, Inc.) to determine the T1 relaxation time in the regions of interest (ROI), as described previously (33).

EPR oximetry.
The in vivo tumor pO2 was monitored using EPR oximetry, with charcoal as the oxygen-sensitive probe (17). EPR spectra were recorded using a 1.1 GHz EPR spectrometer (Magneettech). Calibrations curves were made by measuring the EPR line width as a function of the pO2. Mice were injected in the center of the tumor using the suspension of charcoal (100 mg/mL, 40 µL injected) 24 hours before the experiment. The tumor being studied was placed in the center of the extended loop resonator whose sensitive volume extends 1 cm into the tumor mass. The pO2 measurements correspond to an average of pO2 values in the volume. MOBILE and EPR measurements were taken the same day as we know from unpublished data that a carbogen challenge on one tumor is reproducible on the same day within a 3-hour interval.

Statistical analysis
ROIs were delineated by hand to select the tumor tissue without charcoal, although it has been previously shown that charcoal does not interfere with T1 measurements (33). As we recorded three parametric maps of the global R1, and three parametric maps of the R1 of lipids, as well as three EPR measurements in each condition (at baseline, during carbogen breathing and 3 hours after CA4 administration), from each set of three measurements, we calculated the mean as well as the SD used for the correlations. Pearson correlations between the relaxation rates R1 (or ΔR1) and the actual pO2 values (or ΔpO2) were performed using the GraphPad software. Deming regression was performed to compare the sensitivities of the techniques.

Results
MOBILE enables the follow-up of positive and negative variations in tumor oxygenation
Tumor oxygenation was modulated in two distinct tumor models, one syngenic (NT2 mammary tumors) and one xenograft (MDA-MB-231 xenografts), using a hypoxic challenge (i.e., administration of CA4 3 hours before measurement) or a hyperoxic challenge (i.e., carbogen breathing). Two MDA-MB-231 tumors underwent both challenges, whereas six other tumors underwent hypoxic (n = 3) or hypoxic challenges (n = 3) only. Three NT2 tumors were then submitted to both challenges and four other tumors were submitted to carbogen breathing (n = 2) or were targeted by CA4 (n = 2). Typical maps of the R1 of lipids at baseline, and both after a hypoxic and a hyperoxic challenge are shown in Fig. 1, together with similar challenges assessed using global R1 mapping.

A change in the R1 of lipids is related to a change in the tumor pO2
While pooling data from 20 different tumors (10 of each tumor model), we can observe a significant correlation between the ΔR1 of lipids (MOBILE) and the ΔpO2 in the same tumors (P = 0.0059, r = 0.5097; Fig. 2A), whereas similar analysis using the global R1 does not give a significant correlation (Fig. 2B). Individual changes in the R1 corresponding to an increase in absolute pO2 values, as assessed by EPR oximetry, evidence that (i) the R1 of lipids increased concomitantly (by 2%–17% of relative increase) when the pO2 increased, except for one tumor (2.7% decrease; Fig. 2A) and (ii) the global R1 increased from 0% to 6% with respect to the same pO2 increase, except for two tumors which showed decreases of 0.5% and 7% (Fig. 2B). As regards changes in the R1 with respect to a decline in the pO2, the graphs in Fig. 2 show that (i) the R1 of lipids falls systematically (by 0.1%–13.6%; Fig. 2A) and (ii) the global R1 shows mixed results with both negative (down 4.4%) and positive (up 5.6%) changes in matching tumors (Fig. 2B).

www.aacrjournals.org Clin Cancer Res; 20(21) November 1, 2014 OF3

Published OnlineFirst September 10, 2014; DOI: 10.1158/1078-0432.CCR-13-3434
Individual R1 values of lipids are related to absolute pO2 values

To investigate the potentially quantitative properties of MOBILE, mean individual R1 values of lipids were compared with individual pO2 values within each tumor model. For this purpose, all pre- and postchallenge mean values of the R1 of lipids (MOBILE) were plotted together with respect to all corresponding pre- and postchallenge pO2 values (Fig. 3A and B).

Since some tumors underwent both challenges, only one basal value of each parameter (pO2, global R1 and R1 of lipids) was assessed for these tumors, resulting in a number of points that differ from 20: we considered 17 points and 18 points for the NT2 and MDA-MB-231 models, respectively. We were able to observe a positive correlation between the R1 values of lipids and absolute pO2 values in both models [(P<0.0001, r = 0.8164 and P = 0.0378, r = 0.5069) for MDA-MB-231 and NT2 tumor models, respectively], and between global R1 and pO2 values in the MDA-MB-231 tumor model (P = 0.0025, r = 0.6673).

When all the data were pooled, a significant correlation was established between the R1 values of lipids and the pO2 values (P = 0.0275, r = 0.3726; Fig. 4). We could not pool the global R1 data from both tumor models because there is no correlation in the NT2 tumor model between pO2 values and global R1 values. All these data argue in favor of qualifying MOBILE as a sensitive method of assessing tumor oxygenation.

Furthermore, according to Fig. 4, the relaxation rates of lipids calculated within the MDA-MB-231 tumor model seem to be superior to those obtained in the NT2 tumor model. This observation goes hand in hand with the difference in oxygenation levels that was evidenced by EPR oximetry: MDA-MB-231 tumors are well oxygenated at baseline (10.5 ± 6.5 mm Hg) compared with NT2 tumors (4.8± 3.1 mm Hg).

Relative changes in the R1 of lipids are more sensitive than relative changes in the global R1

Sensitivities were assessed by comparing the slopes of the linear regression graphs for traditional oxygen-enhanced MRI and MOBILE data. This comparison can only be assessed within the MDA-MB-231 model because both
techniques show a significant correlation in this tumor model, which is not the case for the NT2 model. When comparing the slopes providing by the Deming regressions \( Y = 0.01486 X + 0.9143 \) for Lipids R1 and \( Y = 0.01486 X + 0.9143 \) for global R1, we observe that MOBILE is approximately 1.5 times more sensitive than global R1 measurement.

The magnitude of response to CA4 is related to basal pO2

Figure 5 presents the magnitude of response in terms of the global R1, the R1 of lipids, and the pO2, in relation to basal means of these three parameters. Although the response to a carbogen breathing challenge does not depend on basal pO2 (Fig. 5A), as already published (42), the fall in pO2 induced by CA4 is greater when the basal oxygenation level is higher. This is also assessed using the R1 of lipids (Fig. 5C). However, neither basal values of the R1 of lipids nor the global R1 can help predict the extent of the response to carbogen or CA4 (Fig. 5D–I).

Discussion

This study demonstrates the ability of MOBILE to follow positive and negative changes in tumor oxygenation further to hypoxic or hyperoxic challenges, suggesting that the endogenous source of contrast relying on the R1 of lipids in MRI can constitute a sensitive noninvasive marker of tumor hypoxia. The MOBILE technique enables the assessment of the relaxation parameter "R1 of lipids" and is consecutive to a parent emerging technique assessing the "global R1," which is prominently influenced by the R1 of water, but lacks good sensitivity to changes in oxygenation. As R1 techniques are sensitive to tissue oxygenation, they appear to be complementary to the routinely used
functional imaging or BOLD-MRI technique, assessing changes in the $R_2$ relaxation parameter (29), which is sensitive to changes in oxygenation in the vascular compartment, yet with significant limitations in terms of quantitative aspects and in sensitivity.

Further validation of the hypoxia marker requires a correlation of the new method with a quantitative method in the preclinical setting. This was assessed using EPR oximetry, an invasive but quantitative and highly sensitive method able to assess tissue oxygenation in vivo (17). Our study evidenced that (i) positive and negative changes in tumor oxygenation can be detected using MOBILE; (ii) a $\Delta R_1$ of lipids is positively correlated with a $\Delta pO_2$ in vivo; (iii) individual $R_1$ values of lipids are positively correlated to absolute $pO_2$ values; and (iv) changes in the $R_1$ of lipids are more sensitive than changes in the global $R_1$. This makes MOBILE a sensitive method to assess changes in tumor oxygenation. This is not systematically the case for the global $R_1$, showing global $R_1$ changes that are not always correlated to the changes in the $pO_2$ and that are also smaller in magnitude (less sensitive), as observed on individual graphs.

The ability of the MOBILE technique to follow the effect of an antivascular agent (combretastatin A4) longitudinally as well as that of a hypoxic challenge (carbogen breathing) noninvasively could find direct applications in the clinical setting for the individual monitoring of patients treated using anticancer agents. To this end, individual monitoring of the actual effect of a drug on individual tumor hemodynamics could help the clinician in therapeutic decisions. This has also prompted efforts to combine antiangiogenic or antivascular agents together or with other treatment modalities (43–46).

When investigating the potentially quantitative properties of MOBILE (i.e., comparison of individual $R_1$ values of lipids vs. the actual $pO_2$ values), the $R_1$ values of lipids were shown to be correlated in individual models and on pooled data from both tumor models, whereas the global $R_1$ was not able to show such correlation. The significant correlations between $R_1$ of lipids and $pO_2$ argue in favor of a potential quantitative aspect of the MOBILE technique. However, although EPR oximetry measurements have shown that MDA-MB-231 tumors exhibit higher oxygenation level than NT2 tumors, the difference between the $R_1$ values of lipids at baseline between the two tumor models could imply that this parameter is also tissue dependent. Therefore, we cannot exclude that the $R_1$ is also influenced by tissue type and composition.
especially for tumor oxygenation lower than 5 mm Hg. It should be noted that tissue type dependency would also be applicable for the global R₁. Moreover, on the single MDA-MB-231 tumor model, the global R₁ also correlated positively with the absolute pO₂ values. Accordingly, MOBILE seems to be more adapted to assessing tumor oxygenation than the parent technique assessing the global R₁ in the tumor models included in this study, and is systematically more sensitive than the global R₁. Nevertheless, both techniques remain complementary in nature because the origin of the information is different for lipid and global R₁, and because it is not proven, yet that “MOBILE” could be applied on tumor models with a low content of lipids.

In the field of radiotherapy, individual monitoring and quantification of tumor oxygenation could find application in the identification of a therapeutic window during which oxygenation is effectively modified following treatment aimed at modifying oxygen supply and/or consumption. Overall modification of tumor hypoxia has been shown to significantly improve the efficiency of RT for locoregional control and for overall survival (47). Recent reports have also pointed out that the hypoxia-targeted approach ARCON (accelerated radiotherapy plus carbogen inhalation and nicotinamide) had an impact on the patients’ outcome in hypoxic laryngeal tumors but not in well-oxygenated tumors (48), outlying that segmentation of the patients with respect to their basal oxygenation level is mandatory for the optimization of radiotherapy. The MOBILE technique could therefore help in the proper qualification of drugs targeting hypoxia. In addition, the technique could guide intensity modulated radiotherapy planning (IMRT; refs. 49–51) currently used in the clinical setting, which enables millimetric irradiation of each individual tumor to boost the radiation dose in the most hypoxic regions. Importantly, the time and spatial resolutions of the MOBILE technique are compatible with longitudinal monitoring of tumor oxygenation with sufficient spatial resolution to guide IMRT. IMRT is currently not guided in day-to-day clinical practice using hypoxia tracers (52), because none of them are as yet adapted for the purpose.

As far as we know, there is no direct, quantitative, dynamic, and accurate noninvasive/irradiating method that is part of routine clinical practice to assess tumor oxygenation. That is why, despite the dispersion of our data in correlation graphs, that prevents its use in routine clinical practice for exact pO₂ measurement at this stage, the significant correlation found between the R₁ of lipids and the pO₂ using MOBILE makes this a very interesting tool for assessing tumor oxygenation variations. This will need to be further evaluated in additional preclinical and clinical studies. Experiments showing positive correlations in a wider range of tumor models and on other types of tissues, as well as a cross-validation with an alternative clinically available method able to map tumor hypoxia (i.e., PET imaging with nitroimidazoles), would be required.

In conclusion, the current study qualifies MOBILE as a sensitive, noninvasive, and potentially quantitative endogenous marker of tumor oxygenation, as the technique is sensitive to both positive and negative modulations of tumor oxygenation and, more importantly, significantly correlated to actual pO₂ values in matching tumors for both tumor models being studied. As the MOBILE technique presents unique translational properties and can be successfully implemented in the clinical setting, it is promising for further clinical monitoring of individual tumor oxygenation to assess the response to antiangiogenic or antivascular treatments, for treatment combination and for planning radiotherapy. If further characterization is required for pure quantitative routine clinical applications, the technique can be considered to study modulations of tumor oxygenation in patients and clinical intratumoral heterogeneity.

Disclosure of Potential Conflicts of Interest

B.F. Jordan, J. Magat, and B. Gallez are co-inventors of a patent on in vivo quantification of a variation in a tissue by using a MRI technique. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

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Development of methodology: J. Magat, B. Gallez

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): F. Colliez, M.-A. Neveu, T.T.C. Pham, B. Gallez

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): F. Colliez, B. Gallez

Writing, review, and/or revision of the manuscript: F. Colliez, B. Gallez, B.F. Jordan

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): F. Colliez, M.-A. Neveu

Study supervision: B. Gallez, B.F. Jordan

Acknowledgments

The authors thank Philippe Levêque for assistance with statistical analysis.

Grant Support

This study was supported by grants from the Belgian National Fund for Scientific Research (FNRS), the "Fournier-Majoie Foundation", the Joseph Maisin Foundation, the "Actions de Recherches Concertées-Communauté Française de Belgique, Grant No. ARC.09/14-020," and the "Belgian Cancer Plan."

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Received December 20, 2013; revised August 7, 2014; accepted August 8, 2014; published OnlineFirst September 10, 2014.

References


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Clin Cancer Res; 20(21) November 1, 2014 OF7


Clinical Cancer Research

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Clin Cancer Res  Published OnlineFirst September 10, 2014.

Updated version  Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-13-3434

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