EPR-based Oximetric Imaging: A Combination of Single Point-based Spatial Encoding and T_1 Weighting

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Abstract

Purpose: Spin-lattice relaxation time (T_1)-weighted time-domain EPR oximetry is reported for in vivo applications using a paramagnetic probe, a trityl-based Oxo71.

Methods: The R_1 dependence of the trityl probe Oxo71 on pO_2 was assessed using single point imaging (SPI) mode of spatial encoding combined with rapid repetition, similar to T_1-weighted MRI, where R_1 was determined from 22 repetition times ranging from 2.1–40.0 μs at 300 MHz. The pO_2 maps of a phantom with three tubes containing 2 mM Oxo71 solutions equilibrated at 0%, 2%, and 5% oxygen were determined by R_1 and apparent spin-spin relaxation rate (R_2*) simultaneously.

Results: The pO_2 maps derived from R_1 and R_2* agreed with the known pO_2 levels in the tubes of Oxo71. However, the histograms of pO_2 revealed that R_1 offers better pO_2 resolution than R_2* in low pO_2 regions. The standard deviations of pixels at 2% pO_2 (15.2 mmHg) were about 5 times lower in R_1-based estimation than R_2*-based estimation (mean ± SD: 13.9 ± 1.77 mmHg and 18.3 ± 8.70 mmHg, respectively). The in vivo pO_2 map obtained from R_1-based assessment displayed a homogeneous profile in low pO_2 regions in tumor xenografts, consistent with previous reports on R_2*-based oximetric imaging. The scan time to obtain the R_1 map can be significantly reduced using three repetition times ranging from 4.0–12.0 μs.

Conclusion: Using the SPI modality, R_1-based oximetry imaging with useful spatial and oxygen resolutions for small animals was demonstrated.

Keywords: non-invasive in vivo oximetry, tumor tissue oxygen concentration, EPR single point imaging, partial oxygen pressure, spin-lattice relaxation time, triarylmethyl paramagnetic oxygen probe

Introduction

The importance of EPR imaging (EPRI) stems from the fact that it can be used to quantitatively determine tissue oxygenation, as both the spin-lattice relaxation time (T_1) and spin-spin relaxation time (T_2) of paramagnetic electrons are shortened in the presence of oxygen. EPRI can be performed in vivo either in the continuous wave mode or in the time domain using pulsed Fourier transform techniques with exogenous paramagnetic probes (1–4). The observed EPR spectral line widths of many paramagnetic systems, such as lithium phthalocyanine (5,6) and trityl radicals (7–9), linearly depend on the partial oxygen pressure (pO_2). In fact, the time domain approach to EPR imaging became feasible for in vivo applications after the availability of narrow-line spin probes based on the triphenylmethyl (trityl) radical (7–9).

Recently developed trityl derivatives (8,9) with T_1 and T_2 of several microseconds are non-toxic at the concentrations required for imaging and have pharmacologic half-lives sufficient for 3-dimensional imaging. Using excitation pulses ~50 ns and low Q resonators, in vivo EPR images were generated using a strategy known as single point imaging (SPI) involving pure phase encoding in the presence of static gradients, and subsequent Fourier transformation (10–20). In order to perform EPR oximetry, the apparent spin-spin relaxation time (T_2*-), T_2-, or T_1-weighted maps can be obtained as in MRI to generate oxygen-dependent quantitative contrast in the images (11-16).

EPR oximetry based on measuring the spectral line width (LW), where $LW = R_2/\gamma_e$ and γ_e is the gyromagnetic ratio of the electron, needs correction for self-broadening of the probe due to its own spin-spin interactions, which manifest at concentrations greater than 5 mM (18,21). This self-broadening of the probe cannot be easily deconvolved from the oxygen-dependent broadening in vivo, as it requires quantitative estimation of the spin probe's accumulation in specific tissues, e.g., in kidneys and tumors. Halpern and coworkers reported R_1-based EPR oximetry by an inversion recovery electron spin echo (IRESE) sequence and discovered negligible dependence of self-broadening of the spin probe Oxo63 on its concentration (21), providing a means to map in vivo pO_2 at greater accuracy for the first time. An analogous technique in MRI using ^{19}F-labeled tracers has been developed and validated in studying tumor oxygenation (22). In this study, we present another strategy for R_1-based EPR oximetry that combines the high resolution capability of SPI by a single pulse sequence (18), and the effect of interpulse delays and R_1 on the steady state

magnetization for rapid signal averaging (23–28). This approach is applicable to a wide range of R_1 values, is independent of differences in the concentration of the contrast agent as originally demonstrated by Epel and Halpern, and has a lower specific absorption rate (SAR). Oxo71, a deuterated Oxo63 probe, was assessed for: 1) the linear relationship between R_1 and pO_2, 2) R_1 mapping of a phantom by EPR imaging, and 3) in vivo oximetry of a mouse tumor based on a R_1 map.

Theory

Methods for R_1 mapping, such as inversion recovery (29), saturation recovery (30) and variable nutation angle (31), are unsuitable for EPR in vivo studies due to high SARs and long scan times. Approaches to the estimation of R_1 by the steady-state free precession sequences at short repetition times, and their relative merits and shortcomings, are well documented in the NMR literature (23,24,26). R_1-dependent magnetization recovery, i.e., T_1-weighted signal intensity, is defined as a function of the TR to estimate R_1 by these sequences.

The saturation by fast repetition (SFR) sequence (28) for R_1-weighted in vivo imaging offers a high scan speed at low SAR. The SFR sequence consists of a train of phase-coherent RF pulses of a specific flip angle. In this scheme, when TR is small ($TR < 3T_2$), the signal behavior becomes complex due to the refocusing of the residual transverse magnetization M_{xy} from one pulse by its succeeding pulses, resulting in spin and stimulated echoes (26,27). MRI sequences such as steady-state free precession and gradient recalled echo use this scheme, interleaved with spoiler gradient pulses to destroy the residual transverse magnetization before the next pulse is applied. Such spoiler gradient sequences are not practical for EPRI because of the microsecond time scales required for the gradient settling times, which are in the same time range as the FIDs. In this study, we examined the signal intensities using an SFR sequence of $\pi/2$ pulses by varying TR values from 2.1 to 40 μs. The time-domain signal intensity $S(t_p)$ at a delay time t_p from the $\pi/2$ pulse is approximated to:

$$S(t_p) = M_0 [1-\exp(-R_1 TR)] \exp(-t_p R_2^*) \quad [1]$$

where M_0 is equilibrium magnetization. In SPI, image reconstruction is done by monitoring the phase of a single point in the FID at a chosen delay time (t_p) following the excitation pulse. The image pixel intensities reflect both R_1 and R_2^* effects. Hence it is possible to obtain pO_2 maps

based on both R_1 and R_2* effects from the same data set.

Methods

Chemicals

The trityl probe Oxo71, which is the deuterated form of Oxo63 at the methylene moieties indicated by asterisks in Figure 1A, was obtained from GE Health Care (Milwaukee, WI). Gas tanks of argon, air, and mixtures of 2%, 5%, 10% oxygen with nitrogen were procured from local suppliers (Roberts Oxygen, Rockville, MD). Argon and medical air were used for 0% oxygen and 21% oxygen, respectively. The pressure of 21% O_2 after equilibration is 760 mmHg × 0.21 = 160 mmHg, which is approximately 0.25 mM O_2 at 25 °C.

Animals

Female C3H Hen MTV mice, supplied by the Frederick Cancer Research Center's Animal Production unit (Frederick, MD, USA), were housed in a climate-controlled and circadian rhythm-adjusted room and allowed food and water *ad libitum*. Squamous cell carcinoma (SCCVII) cells were implanted in the femoral muscle of the right thigh. Approximate tumor size during experimentation was about 10 mm. Experiments were conducted in compliance with the Guide for the Care and Use of Laboratory Animal Resources (National Research Council, 1996) and were approved by the National Cancer Institute's Animal Care and Use Committee.

Acquisition of Calibration Data

The spectral and imaging data were scanned on a home built time-domain EPR imager described previously (32) and operating at 300 MHz. The power amplifier used in this study (BT0250-EF-RBB, Tomco) had a rise time and fall time of < 20 ns with a duty cycle of 5%. This allowed a repetition time TR of ~ 2 microseconds using pulse widths of 110 ns for a $\pi/2$ pulse. The pO_2 calibration experiments were performed using aqueous solutions of 2 mM Oxo71 equilibrated at five oxygen levels (0%, 2%, 5%, 10% and 21%) and 1, 5, and 10 mM Oxo71 solutions equilibrated at 0% oxygen in separate glass tubes. Oxygen levels were achieved by bubbling the appropriate gas into the sample for about 45 min and maintained by sealing with epoxy. Time-domain EPR signals were recorded by a series of $\pi/2$ pulses separated by TR ranging

from 2.1 to 40 μs.

Phantom 2D Imaging

Three tubes containing 2 mM Oxo71 solution equilibrated at 0%, 2%, and 5% oxygen were mounted parallel to the magnetic field Y-axis in an equilateral triangular geometry in a plastic holder placed in a resonator of 25 mm diameter (Fig. 1B). The phantom 2D data were acquired in the XZ-plane (cross-section of the tubes) using a 31 × 31 Cartesian grid, G_{max} = 10 mT/m, 5000 signal averages, a sampling dwell time of 5 ns, a flip angle of 90°, TR = 3 - 40 μs, and dead time of 0.25 μs. Calculations and image reconstruction were done by MATLAB (Mathworks Inc., Natick, MA, USA) scripts developed in house.

In Vivo Imaging

A mouse was anesthetized by 2% isoflurane in medical air, mounted prone on a custom designed holder, maintained at a breathing rate of 60 per min and a core body temperature of 37 ± 1°C by a flow of warm air. The urethra was cannulated using a PE-10 tube. The urine was drained during the experiment. A 30 G needle extended using polyethylene tubing (PE-10) was cannulated into the tail vein. The mouse thigh having the SCCVII tumor was positioned in a 19 mm diameter resonator. Oxo71 solution at 75 mM concentration was administered through tail vein cannulation by giving an initial bolus of 1.125 μmol/g body weight and continuous administration of 0.06 μmol/g/min subsequently to maintain a steady signal level. Images were acquired in the YZ-plane, which corresponds to the sagittal plane on the mouse leg, at 21 × 21 phase encodings in the Cartesian grid, by varying the TR from 2.3 to 40 μs.

Scan Times

The data acquisition time for a single scan = $TR \times N_{av} \times (N_k + 1) + dT$, where N_{av} is the number of averages, N_k is number of all phase encodings, and dT is about 1 sec, which is sum of the instrument delays before acquisition after changing the configuration or gradient. Background signals were acquired under identical conditions before the data acquisition. For phantom imaging, N_{av} = 5000, $N_k=31^2$, and scan times at TR = 3.4, 8, 12, and 40 μs were 18, 40, 59 and 194 s,

respectively. Approximate R_2* may be assessed by a single scan at a specific TR, while three scans at different gradient maxima are necessary to minimize edge artifacts. The scan time for R_2* mapping at TR = 40 μs was 194 s and at TR = 12 μs was 59 s. The scan time for three gradient scans performed at TR = 8 μs was 120 s. The scan time for the estimation of R_1 from 22 TR values was 24 min and from three TR values at 5, 8, and 12 μs was 125 s. For mice, $N_k = 21^2$ and single scan times at TR = 3.4, 8, 12, and 40 μs were 9, 19, 28, and 90 sec, respectively. The R_1 estimation from 23 TR values was 11 min.

Results

Calibration Parameters

The relationships (i) LW versus pO_2 where LW (μT) = R_2*/(28025π), (ii) LW versus [Oxo71], and (iii) pO_2 versus longitudinal relaxation rate R_1 (= $1/T_1$) were calculated from Oxo71 calibration spectral data. The apparent transverse relaxation rate R_2* (= $1/T_2$*) was considered as the asymptotic R_2* value at a long TR (Fig. 2A) to avoid echo contributions observed at short TR (Fig. 2B). The linear fit of R_2*-based LW to pO_2 in the range of 0–21% (Fig. 2C) indicated the following relationship:

$$LW\ (\mu T) = 0.1257\ pO_2\ (mmHg) + 8.786. \quad [2]$$

Eq. [2] was used directly to calculate a pO_2 map of the phantom from the LW. However, in vivo studies require correction for the self-broadening of [Oxo71]. The linear fit of LW to [Oxo71] in the range of 1–10 mM at 0% pO_2 indicated that:

$$LW\ (\mu T) = 0.5745\ [Oxo71]\ (mM) + 6.733. \quad [3]$$

Therefore, the following equation was used for pO_2 estimations from LW in vivo.

$$LW\ (\mu T) = 0.1257\ pO_2\ (mmHg) + 0.5745\ [Oxo71]\ (mM) + 7.637 \quad [4]$$

Next, the R_1 value at each pO_2 was calculated by fitting the observed spectral peak height S_p as a function of TR (Fig. 2D) according to Eq. [5] below.

$$S_p = M_0\ [1-\exp(-R_1 TR)] \quad [5]$$

S_p depends on the FID profile covered. The peak heights of the observed spectra (S_p^{obs}) were obtained by fast Fourier transform of the FID within a constant t_p range beginning right after the dead time and ending just before the intensity rise due to echo. The calculated peak heights (S_p^{calc}) were obtained by fast Fourier transform of simulated time-domain signals according to Eq. [1] for

given R_1, R_2*, TR values, and a scaling factor to account for M_0 within the same t_p range. The Nelder-Mead simplex direct search algorithm (33) was used by varying R_1 and the scaling factor. The R_1 values resulting in the smallest sum of squares of errors between S_p^{obs} and S_p^{calc} were determined. The S_p values shown in Figure 2D were scaled to a maximum of ~1 for each pO_2 separately, as the peak heights decrease with increasing pO_2 levels. This fitting indicated larger deviations at low TR values (<3 μs) owing to echo contributions. Furthermore, the intensity variation of 21% pO_2 was too small to determine R_1 accurately with a 300 MHz EPRI scanner. Therefore, we explored the peak height calculation including the spin and stimulated echo contributions according to the procedure described by Gyngell (26). The fitting improved at low TR values as expected (Fig. 2E), but the R_1 value at 21% pO_2 remained approximately the same as previously estimated by Eq. [1]. It is likely a limitation of the SFR sequence for R_1 estimation that a significant fraction of the the signal is lost in dead time. The relationship between pO_2 and R_1 was found to be linear for $pO_2 \leq 10\%$ (Fig. 2F) according to Eq. [1], as follows.

$$pO_2(\text{mmHg}) = 122.22\ R_1(\text{MHz}) - 20.36 \qquad [6]$$

The advantage of Eq. [1] over the procedure described by Gyngell (26) is its ability to implement the SPI method when the interference from echo signals is negligible.

Phantom T_1-Weighted Imaging

Two-dimensional images of the phantom were scanned by an SFR sequence in the TR range of 3.0–40.0 μs. At low TR values, the echoes led to ghosts at different scales and orientations, superimposed on the original image depicted in Figure 3A. The FOV, orientation, and intensity of a ghost depend on its phase and the time passed since its originating pulse at the current delay time (Figure 3A-3F). For example, at TR = 3 μs and t_p = 1.5, 1.75, and 2.25 μs, the image of original three-tube phantom appears four times, twice in normal orientation and twice in inverted orientation (Fig. 3B–3D). However, only one of those follows the expected intensity decay and phase evolution profile after a π/2 pulse, and the others are removed by fitting. Specific delay times where the interference distorts the image as shown in Figure 3C are to be avoided by suitable choice of delay times (t_p). The early delay times were found to have fewer echo signals.

Initially, the R_2* map was calculated from the pixel intensities of images at 5 delay times equally spaced from 1.0 to 1.4 μs, since R_2* is required to estimate R_1 according to Eq. [1]. The

k-space data were preprocessed prior to image reconstruction to correct for any zero shifts and weighted by a tapered cosine window to minimize ringing artifact from the tubes. All the images were reconstructed by scaling to the smallest FOV via chirp-z transformation. Any marginal FOV mismatches arising from inaccurate estimate of dead time were adjusted by recalculating the scaling factors to minimize the errors. Further reduction of ringing artifacts was accomplished by re-gridding of k-space as previously described (17). The pixel intensities $S(t_p)$ were normalized to account for the change in FOV and the spin density (S_0, pixel intensity at $t_p = 0$), and R_2* maps were calculated by fitting to Eq. [7] as described previously (18).

$$S(t_p) = S_0 \exp(-t_p R_2^*) \qquad [7]$$

The R_1 estimation, however, needs only one delay point at different TR values. If the intensities at a specific delay are normalized to the longest delay by which the intensities reach a constant value asymptotically, then the graphs of intensity as a function of TR coincide for all the images at different single point time delays, and the decay due to R_2* gets eliminated.

The intensities at the first delay time ($t_p = 1.0$ μs) as a function of TR were chosen to estimate R_1 to take advantage of the higher signal to noise ratio. R_1 and M_0 values were calculated for each pixel separately by fitting to Eq. [1], since $S(t_p)$ and R_2* are known. This M_0 map is merely a map of scaling factors obtained from the fitting that may have a complex relationship to the equilibrium magnetization, depending on the pO_2 map. The background regions containing essentially noise were masked prior to fitting to optimize the computation. The pixels at <5% of the maximum intensity were included in the mask.

The pO_2 estimation from LW and R_1 using Eqs. [6] and [2] is shown in Figure 4. The M_0 and R_1 maps were calculated including 22 TR values ranging from 3.4 to 40.0 μs at $t_p = 1.0$ μs, and S_0 and R_2* were calculated at TR = 40 μs from five t_p values equally spaced from 1.0 to 1.4 μs. In the M_0 map (Fig. 4A), the intensities of 0% and 5% pO_2 tubes appear to be distinctly different while this difference is smaller in the S_0 (TR = 40 μs) map (Fig. 4D). The contrast between the three tubes appears relatively more distinct in the R_1 map than in R_2* (Fig. 4B and 4E). However, the pO_2 maps obtained from both R_1 and R_2* are in agreement (Fig. 4C and 4F), despite a better SNR of the pO_2 map derived from R_1. The calculation of the M_0 and R_1 maps was repeated including only three TR values, 5, 8, and 12 μs (Fig. 5), in order to check the feasibility of increasing the scan speed. These images are in good agreement with those calculated from 22 TR

values (with a mean difference of 0.65 mmHg and 95% pixels within ±5.7 mmHg from the mean, Supplementary Figure 3), indicating the possibility of fast R_1 scans using three low TR values approximately in the range of 5-12 μs.

The pixel histograms of 0%, 2%, and 5% oxygenated Oxo71 solutions (Fig. 6) indicate better resolution of pO_2 in the maps derived from R_1 than from R_2*. The pO_2 values calculated from the R_1 map are more uniform whether 3 or 22 repetition times were used (Fig. 6A and 6B). pO_2 values of pixels (mean ± SD) in the regions of 0%, 2%, and 5% pO_2 tubes were 0.68 ± 0.20, 1.83 ± 0.23, and 4.95 ± 0.5%, respectively. In contrast, the R_2* map gave broader ranges of pO_2 values in all three regions, leading to higher overlap of histograms (Fig. 6C and 6D). The mean ± SD of pO_2 values for pixels in the regions of 0%, 2%, and 5% pO_2 were 1.58 ± 1.02, 2.41 ± 1.14 and 4.72 ± 0.54%, respectively. The phantom study suggests that oximetry by R_1 yields not only more reliable pO_2 estimates but also better pO_2 resolution than the R_2* method, despite the linearity of R_2* with pO_2 over a wider range.

In Vivo Imaging of Mouse Tumor

The mouse 2D image data were acquired by phase encoding in 21 × 21 Cartesian grid and the images were reconstructed at a 256 × 256 matrix size. A steady infusion of Oxo71 was maintained to provide an adequate signal throughout the data acquisition. The R_2* and S_0 maps were calculated from five equally spaced delay times from 0.85 to 1.25 μs for 23 TR values ranging from 2.3 to 40 μs. Initially the M_0, R_1, and pO_2 maps were calculated at t_p = 0.85 μs from these data without correction for the variations in the infused Oxo71 probe. Comparison of the pixel intensity versus TR profiles of the phantom with those of the mice revealed differences. In the case of the phantom experiment, smooth profiles asymptotically reached a maximum as expected (Supporting Figure S1A). In the case of the mouse experiment, the profiles were noisy and the pixel intensity continued to increase up to the longest TR, due to continuous infusion of the spin probe (Supporting Figure S1B). The intensity change due to the infused probe appeared to be linearly correlated with TR, since the scan time is directly proportional to the local amount of probe in this case. The rate of increase in the signal due to probe infusion was estimated from TR = 16–40 μs where the signal is expected to reach an asymptotic maximum level (99.8% for R_1 = 2.5 μs). Each profile was corrected for this variation and M_0, R_1, and pO_2 maps were recalculated (Fig. 7). Despite the

correction, no appreciable improvement was observed in the R_1 maps, indicating the robustness of this method for a large number of TR values. A calculation using only three TR values (4.2, 6, and 12 μs) without applying this correction produced almost identical M_0 maps (Fig. 7A and 7D), but the R_1 map differed in some regions (Fig. 7B and 7E). However, the pO_2 maps obtained from R_1 measurement were homogeneous.

Discussion

This study provides an approach to calculate pO_2 from both R_1 and R_2* methods for the deuterated trityl probe Oxo71, which has a longer T_2* than Oxo63. The calibration graph shown in Fig. 2F indicates a linear relationship between pO_2 and R_1 for pO_2 ≤10%. Since the linearity below 10% pO_2 is adequate for in vivo studies, extension to higher pO_2 levels is not addressed in this study.

Equilibration of 10% oxygen in water yields approximately 0.12 mM oxygen dissolved at 25 °C. In vivo pO_2 values in muscle tissues are reported to be around 40 mmHg (34), corresponding to 0.05 mM oxygen at 37 °C. Most tissues have lower pO_2 than muscle, except lung and arterial blood. Therefore, the linear range of pO_2 versus R_1 in this phantom study is adequate for most in vivo studies measuring pathophysiological tissue pO_2. The data for SPI-based R_1 estimation inherently contain data for R_2*-based pO_2 mapping, which can estimate higher pO_2 ranges if desired.

Phantom imaging experiments suggest that the pixel intensities follow Eq. [5] for a wide range of TR values fairly well at a constant delay time (t_p) (Supporting Figure S1A). Three TR values defining the intensity curve were found to be sufficient to produce a reliable pO_2 map in vitro, where probe concentrations remain constant with time. Interestingly, the quality of both phantom and mouse images was unaffected by the echoes when suitable delay times and TR values were chosen. The choices of TR and t_p are not critical except for avoiding the overlap of ghosts as shown in Fig. 3C, where the FOVs and phases of the original and ghost images are almost equal (which happens in special cases of TR <5 μs for Oxo71).

The estimation of R_1 in vivo by EPRI was previously reported by Halpern and coworkers using the IRESE sequence π-T-π/2-τ-π-τ-echo (21). Subsequently a comparison of various pulse schemes to measure R_1 by EPRI was reported by these authors (28) where an inversion recovery

sequence was recommended for reliable spin probe concentration-independent pO_2 mapping. An SFR sequence can provide T_1-weighted maps from which pO_2 can be determined. Though the R_1 is not as accurate as the IRESE sequence, it offers faster scan speeds and high spatial resolution at low SAR. This single-pulse experiment is widely used in MRI to estimate R_1 (23,24,26). We chose rectangular hard pulses at a flip angle of 90^o for this study, where transverse magnetization did not completely decay at TR <3 μs. Optimization of this method using fewer TR values will reduce the scan times to acceptable levels for in vivo imaging, similar to R_2*-based SPI oximetry. The phantom studies revealed that the ratio of SDs in R_2* versus R_1 methods for pO_2 <2% is about 5, indicating the possibility of higher resolution by R_1 at pO_2 values below 2%. In SPI, the one-dimensional spatial resolution Δ is given by $FOV/N = 2\pi/(\gamma_e G^{max} t_p)$ where N is the number of phase encodings, γ_e is the gyromagnetic ratio of the electron, G^{max} is the maximum gradient, and t_p is the delay time (20). The R_2* computation involves FOV scaling as the t_p value is varied. The spatial resolution is 2 mm for the mouse data at t_p = 0.85 μs (1.7 mm for the phantom at t_p=1 μs). However, a spatial resolution of 1.4 mm (1.25 mm for phantom) was realized in the pO_2 maps due to extension of k-space dimensions by the regridding necessary for R_2* estimation.

Experiments in this study were done with a single maximum-field-gradient configuration, although the multiple maximum-field-gradient configuration was proposed in a previous study (18) to avoid the edge artifacts. The single maximum-field-gradient configuration causes alteration of SPI resolution along with t_p (17). Due to edge artifacts in R_2* and S_0, some artificially high values unnaturally distributed on the edge of theobject can be obtained. On the other hand, the R_1 and the M_0 estimations, along with TR, did not give such edge artifacts, since a set of SPIs obtained at the identical t_p have the same resolution. The R_1 map was computed at shortest t_p value but at the same resolution applied to R_2*. The images were reconstructed at a single t_p value with the best S/N ratio, reducing the uncertainties.

The mouse and phantom images at 5 delay times (t_p = 0.85–1.25 μs) and 3 TR values (TR = 4.2, 6, and 12 μs) are shown respectively in Fig. 8 and Supporting Figure S2 in matrix form for better insights into the data. The M_0 and R_1 maps calculated at early t_p (0.85 μs in Figure 9 and 1.0 μs in Supporting Figure S2) are shown on the right, and the S_0 and R_2* calculated for each column are shown in the top two rows. As the FOV changes with t_p but not by TR, the M_0 map resembles the images at shortest t_p. In contrast, the S_0 map depends on the intensity variations with t_p at each

TR. The FID signal in some regions, e.g., those having high pO_2 or low intensities, decays to noise faster than the other regions, leading to stronger contrasts in S_0 and R_2* maps. Further, the relaxation of transverse magnetization varies with TR, leading to differences in the S_0 and R_2* maps with TR. As a result, the SDs observed in pO_2 values based on R_2* are inherently higher. In addition, the accuracy of pO_2 estimates from R_2* depend on the ability to correct for self-broadening accurately. While this is not obvious in the phantom where the concentrations are uniform, it is apparent in mouse imaging when a highly concentrated bolus of the spin probe distributes inhomogeneously in the body via systemic circulation, yielding a wide range of concentrations. This suggests that the use of R_1 is superior to R_2* for in vivo pO_2 studies. The advantage of SPI using an SFP sequence is fast data acquisition at the equivalent scan time of the multigradient approach previously reported for R_2*-based oximetry (34).

It has been shown by inversion recovery methods that R_1 is linearly proportional to pO_2 in the range of 0-21% for Oxo63 (21). In this work, although the range of pO_2 assessed by R_2* includes 0-21%, the R_1-based method limits the pO_2 range to 0-10% (Fig. 2). This limitation arises from the availability of the FID signal after the dead time at a flip angle of $\pi/2$. Recall that the smaller flip angle limits the change in z-magnetization (Mz) and hence the accuracy of R_1 assessment. The best R_1 assessment is accomplished when pO_2 is close to 0% and scans include both low and high TR values to define the intensity versus TR profile adequately. About 12 TR values from 4.2 to 40 μs were found to be adequate for accurate pO_2 estimation by the R_1 method. Calibration experiments on individual Oxo71 solutions suggest that it is possible to attain a linear relationship between pO_2 and R_1 for pO_2 <10%. The slope and intercept of eq. [6] are 122.22 and -20.35, with standard errors of ±1.96, and ±0.96, respectively. These values indicate that pO_2 can be determined at a resolution of 0.6 mmHg at 0% and ~3 mmHg at 10%. The sensitivities of both R_1 and R_2* methods were compared with the pO_2 levels estimated from phantom images. The R_1 and R_2* methods estimated 0% pO_2 as 5 and 12 mmHg respectively. At 2% and 5%, the pO_2 estimations are closer to the expected values. The slope of observed versus expected pO_2 in the 0-5% range is closer to 1 for R_1 (slope = 0.88) than R_2* (slope = 0.71), indicating that pO_2 estimated by R_1 is more specific and more sensitive than the estimate by R_2*. R_1 values estimated by three TR points indicated a lower slope of 0.84, pointing to a loss of sensitivity. Although SDs of histograms are expected to decrease with increasing the number of TR points, no apparant improvement was

observed by increasing to 5 TR points. About 12 TR points (phantom scan time = 14 min, supplementary Figure 5) were found to be adequate to obtain SDs similar to those of Fig. 6A. In order to perform 3D imaging in vivo, the scan time is to be reduced to under 10 minutes by constraining severall parameters such as N_{av}, matrix size, partial k-space acquisition and leaving out long TR values.

Conclusion

This study demonstrated the feasibility of mapping spin-lattice relaxation times based on SPI data collection and their use to determine spatially resolved oxygen levels in vivo. The scan time can be reduced to match conventional R_2* measurements by using a combination of relatively short TR data sets and further optimization. The single pulse sequence with variable TR is an attractive approach to evaluate R_1 for in vivo oximetry due its low SAR. The R_1 estimation by SPI simultaneously determines R_2*. The fact that the R_1 method offers a more accurate pO_2 estimation with very little dependence on spin probe concentration, coupled with the intrinsic line width-independent high resolution and the relatively large effective uniform excitation band width, makes this a highly practical approach for small animal EPR oximetric imaging.

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Figure Legends

Figure 1. A. The structure of triarylmethyl probe Oxo71. The asterisks indicate deuterated methylene moieties. B. Schematic (left) and cross-section (right) of the three-tube phantom showing the tubes filled with spin probe solution as filled circles.

Figure 2. Dependence of R_1 on pO_2 for Oxo71. A. Phantom imaging FID signals acquired at TR = 12 μs. B. Steady state free precession FID profiles observed in phantom imaging at TR = 2.1 μs. Note the beginning of echoes at the short TR. The echo is truncated by the TR. C. Linear dependence of line width calculated from R_2* on pO_2. D. The spectral peak heights normalized to $M_0 = 1$ as a function of TR in the range 2.1–40 μs at 0%, 2%, 5%, and 10% pO_2 of a 2mM Oxo71 solution. Observed data are shown by the symbol × and continuous lines indicate the fit to Eq. [5]. Note the larger deviations at low TR values. E. The deviations are reduced at low TR if the echo contributions are included in the fit. F. Linear dependence of pO_2 on R_1.

Figure 3. The effect of spin and stimulated echoes at short repetition times for a phantom containing three tubes. A-C. TR = 3 μs and delay times of 1.0, 1.5, and 2.25 μs, respectively. D-E. Delay time = 1.75 μs and TR values of 3, 5, and 12 μs, respectively. The crosshairs are not part of the map. Notice multiple ghost 3-tube shapes of different sizes and orientations at TR = 3μs in C and D where two overlapping images are in normal orientation and two more are in inverted orientation at lower scales. These begin to disappear at TR = 5 μs and are fully absent at TR = 12 μs.

Figure 4. Phantom images calculated using data acquired at 22 TR values (3.4, 3.8, 4.2, 4.6, 5, 5.5, 6, 6.5, 7, 7.5, 8, 9, 10, 11, 12, 14, 16, 20, 25, 30, 35, and 40 μs). Scan time = 23 min. A-C. M_0, R_1, and pO_2 maps, respectively. D-F. Spin density, R_2*, and pO_2 maps calculated from 5 delay times of 1.0–1.4 μs at TR = 40 μs. Scan time = 3.2 min.

Figure 5. Phantom images calculated from signal intensities at 3 TR values (5, 8, and 12 μs). Scan time = 2 min. A-C. M_0, R_1, and pO_2 maps, respectively. D-F. Spin density, R_2*, and pO_2 maps calculated from 5 delay times of 1.0–1.4 μs at TR = 12 μs. Scan time = 1 min.

Figure 6. Histograms of pO_2 maps in the regions of tubes at pO_2 = 0%, 2%, and 5%. A. Calculated using R_1 derived from 22 TR values. B. Calculated using R_1 derived from 3 TR values. C. Calculated using R_2* derived from 5 points at TR = 40 μs. D. Calculated using R_2* derived from 5 points at TR = 12 μs. Scan time = 27 s.

Figure 7. In vivo R_1-based mapping of a mouse bearing a SCCVII tumor. A-C. M_0, R_1, and pO_2 maps calculated from signal intensities measured at 23 TR values (2.3, 3.2, 3.4, 3.8, 4.2, 5, 5.5, 6, 6.5, 7, 7.5, 8, 9, 10, 11, 12, 14, 16, 20, 25, 30, 35, and 40 μs). Scan time = 10.8 min. D-F. M_0, R_1, and pO_2 maps calculated using three TR values (4.2, 6, and 12 μs). Scan time = 52 s. The background is masked in all maps. A region of interest covering high M_0 is indicated by the dashed contour line in all the maps to allow comparisons.

Figure 8. Overview of R_1 and R_2* determination methods. Single point images $S(t_p)$ of a mouse leg bearing a SCCVII tumor calculated at TR = 4.2, 6, and 12 μs and t_p = 0.85, 0.95, 1.05, 1.15 and 1.25 μs are shown as a matrix. The images in the columns are used to calculate R_2* and S_0 maps. The images in the rows are used to calculate R_1 and M_0 maps. The images at longest TR (column at 12 μs) and shortest t_p (row at 0.85 μs) provide relatively better signal to noise.

Figure 9. *Left*: R_2* and S_0 calculated from single point image intensities $S(t_p)$ at TR = 4.2, 6, and 12 μs and t_p = 0.85, 0.95, 1.05, 1.15 and 1.25 μs of a mouse leg bearing a SCCVII tumor. *Bottom right*: M_0 and R_1 calculated at t_p = 0.85 μs. *Top right*: The pO_2 maps calculated from R_2* at TR= 12 μs and from R_1 calculated at t_p=0.85 μs. The relationship between R_2* and pO_2 is: pO_2 (mmHg) = 7.957 LW (μT) - 4.571 [oxo71] (mM) - 60.765. LW (μT) = 0.1257 pO_2 (mmHg) + 8.786, and LW (μT) = 0.5745 [oxo71] (mM) + 6.733, so the relationship between R_1 and pO_2 is pO_2 (mmHg) = 122.21 R_1(MHz) - 20.36.

Supporting Figure S1

The pixel intensity profiles as a function of TR. A. Phantom data in the 0% pO_2 region. B and C. In vivo data of a mouse tumor for the top 1000 pixels, before and

after probe abundance correction, respectively.

Supporting Figure S2

Overview of R_1 and R_2* determination methods. Single point images of a three-tube phantom calculated at TR = 5, 8, and 12 μs and t_p = 1.0, 1.1, 1.2, 1.3 and 1.4 μs are shown in the black box. The rows above the box are the R_2* and S_0 maps calculated at the corresponding TR. Right, top row: The pO_2 map calculated from R_2* at TR= 12 μs and the pO_2 map calculated from R_1. Right, bottom row: M_0 and R_1 maps calculated at t_p=1.0 μs are indicated by the red arrow.

Supporting Figure S3

Bland-Altman plot comparing pixel to pixel pO_2 values of the phantom calculated using (*a*) 22 TR values and (*b*) 3 TR values. The ordinate is (*a*) – (*b*) and the abscissa is their mean.

Supporting Figure S4

Relationship between R_1 determined by SFR sequence and R_2* of Oxo71 as the pO_2 is varied from 0 – 10%.

Supporting Figure S5

A. Phantom pO_2 map calculated using data acquired at 12 TR values (4.2, 4.6, 5, 6, 7, 8, 10, 14, 20, 25, 30 and 35 μs). B. Histograms of pO_2 in the regions of tubes at pO_2 = 0%, 2%, and 5%. Scan time = 14 min.