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Heterogeneity of Regional Redox Status and Relation of the Redox Status to Oxygenation in a Tumor Model, Evaluated Using Electron Paramagnetic Resonance Imaging

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Abstract

It is widely accepted that redox status, along with the partial pressure of oxygen (pO_2), determines the efficacy of some therapeutic methods applied to treat tumors, including radiation. Redox status, evaluated by the reduction of a nitroxyl probe, was reportedly heterogeneous in a mouse tumor model. However, neither variation of heterogeneity of the redox status among mice nor the relation of the redox status to pO_2 in tumors has been characterized sufficiently. In this study, the regional reduction status in a mouse radiation-induced fibrosarcoma tumor model was evaluated using sequential three-dimensional electron paramagnetic resonance (EPR) imaging after i.v. injection of a tissue-permeable nitroxyl probe, HM-PROXYL. The regional decay of HM-PROXYL signal obeyed first-order kinetics, and the amplitude of the reduction rate and extent of its heterogeneity in a tumor varied among six mice. The tissue pO_2 was measured using EPR oximetry with lithium phthalocyanine (LiPc) microcrystals implanted within the tumor. The location of LiPc was determined with EPR imaging. A sequential image was obtained following the injection of HM-PROXYL, even after LiPc implantation, by choosing an HM-PROXYL signal peak which does not overlap with the signal of LiPc. The relationship between pO_2 and the reduction rate at the region of pO_2 measurement was found to be low ($r = 0.357$) in 13 tumor-bearing mice, indicating that the extent of oxygenation does not necessarily affect the redox status

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under air-breathing conditions. The results strongly indicate the necessity of measurements of both redox status and oxygenation in every tumor to characterize tumor physiology.

Introduction

Radiation therapy, photodynamic therapy, and chemotherapy of tumors do not require surgical operations, and they should be ideal methods if they gave good result by themselves. Some part of the mechanisms of these therapeutic methods relate to the generation of free radicals and reactive oxygen species in a tumor (1–3). The biological effects of free radicals and reactive oxygen species are enhanced by dissolved oxygen and are inhibited by reducing molecules such as glutathione (GSH). Thus, obtaining knowledge of redox status and the partial pressure of oxygen (pO_2) in each tumor should be useful for accurate prognosis of the therapeutic efficacy of these methods. However, only a few studies have reported on the relation of redox status and pO_2 in a tumor because of a lack of evaluation methods for these variables in the same tumor.

Electron paramagnetic resonance (EPR) spectroscopy/imaging operating at low microwave frequencies (L-band or lower frequencies) is a noninvasive technique to measure durable paramagnetic species in an animal body (4, 5). Low molecular weight cyclic nitroxyl radicals have been used as a probe to measure biological redox (4–8). The nitroxyl radicals are reduced to their corresponding hydroxylamine in an animal body. Numerous *in vivo* and *in vitro* studies have clarified that small-molecule antioxidants such as ascorbic acid and GSH, as well as enzymatic systems, contribute to the reduction (6, 9–12). This reduction is reversible (13) and enhanced under hypoxic conditions *in vivo*, as well as *in vitro* (14, 15). On the other hand, pO_2 in tumors has also been measured by EPR spectroscopy/imaging (EPR oximetry) using water-soluble or water-insoluble paramagnetic probes (7, 16). The technique is based on line-broadening of the EPR probe signal, which is caused by Heisenberg spin-exchange interaction with the oxygen molecule, which is also a paramagnetic species. The heterogeneous oxygen map of a tumor has been revealed using a water-soluble triaryl methyl radical probe (17). Because of rapid renal clearance of this probe, continuous infusion is necessary for pO_2 measurements. Furthermore, to obtain the oxygen map, spectral-spatial imaging techniques are used, which requires specialized apparatus to generate a high magnetic field gradient in addition to the development of software to reconstruct the oxygen map. In contrast, a water-insoluble probe such as paramagnetic lithium phthalocyanine (LiPc) could provide regional pO_2 by implanting the probe in a tumor (18, 19). The method is somewhat invasive, but the probe is stable for a long period of time, and regional pO_2 could be obtained easily and repeatedly using EPR spectroscopy without special apparatus and software for spectral-spatial imaging.

Using a sequential EPR imaging technique, heterogeneous distribution of nitroxyl clearance has been reported in a radiation-induced fibrosarcoma (RIF-1) tumor model (20). The clearance was related to GSH content in a tumor (20, 21). In combination with EPR oximetry using LiPc, it has also been observed that carbogen-breathing increased oxygenation and suppressed nitroxyl clearance in the tumor model (19). However, the extent

of heterogeneity in redox status among tumors and the relationship between the redox status and pO₂ in each tumor are still unclear.

In this study, we obtained the rate of nitroxyl decay at four regions in RIF-1 tumors using sequential three-dimensional EPR-computed tomography (CT) imaging with a tissue-permeable nitroxyl probe, and examined the difference in the heterogeneity of the decay rate among tumors. Then, we correlated the pO₂ and nitroxyl decay at the same region within the same tumor. For this purpose, two different EPR-CT images were successfully obtained from the same tumor, one for the determination of LiPc position and another for the measurement of decay rate of nitroxyl probe at the region of pO₂ measurement.

Materials and Methods

Chemicals

3-Carboxy-PROXYL was purchased from Sigma. 3-Hydroxymethyl-2,2,5,5-tetramethylpyrrolidine-N-oxyl (HM-PROXYL) was synthesized from 3-carboxy-PROXYL following the methods of Yokoyama and colleagues (22). Paramagnetic LiPc was synthesized electrochemically as previously reported (23, 24). Dulbecco's PBS without Mg²⁺ and Ca²⁺ (PBS) was purchased from Life Technologies (Invitrogen). All other reagents were of the highest purity commercially available.

Animals and animal care

Male C3H/He mice (5 weeks old) were supplied by Japan SLC Co. (Hamamatsu, Japan). The mice were housed five per cage and given a commercial diet (MB-1, Funabashi Nojo) and acidified water *ad libitum*. The animal room was maintained on a 12-hour light/dark cycle at a temperature of 23°C and a humidity of 55%. All animals used in the present study were treated and handled according to recommendations for the handling of laboratory animals for biomedical research, which complied with the Committee on the Safety and Handling Regulations for Laboratory Animal Experiments in our institutes.

RIF-1 tumor-bearing mice

RIF-1 cells were kindly provided by Dr. James Mitchell (Radiation Branch, National Cancer Institute) on November 15, 2002. The cells were stocked in liquid nitrogen until use. The cells were grown in RPMI 1640 (Life Technologies) containing 5% fetal bovine serum. Solid tumors were produced by s.c. inoculation of 10⁵ cells suspended in 100 µL of PBS into the right hind leg of C3H/He mice. The tumor was maintained via serial passage in mice. A tumor was removed aseptically, minced, and dissociated in 20 mL of digest buffer [0.2% collagenase (Sigma), 0.02% DNase (Sigma) in PBS containing 0.10 g/L CaCl₂, and 0.10 g/L MgCl₂ • 6H₂O] for 15 minutes at 37°C. The suspension was mixed with 20 mL of RPMI 1640 and left for 1 minute. The supernatant was collected and centrifuged at 1,000 r.p.m. for 5 minutes. The pellet of cells was suspended in 1 mL of RBC-lysing buffer (150 mmol/L NH₄Cl, 10 mmol/L KHCO₃, 0.01 mmol/L EDTA) for 2 to 3 minutes. PBS (30 mL) was added to the suspension, and the suspension was centrifuged at 1,000 r.p.m. for 5 minutes. The pellet was resuspended in PBS at 2.5 × 10⁶ cells/mL. The tumor cell suspension (100 µL) was injected s.c. into the right hind leg of C3H/He mice (3542 days old, males). The

size (diameter) of the tumor was measured with a caliper. The tumor volume was calculated according to the formula: tumor volume = $(\pi/6) (d_1 \cdot d_2 \cdot d_3)$, where d_1 , d_2 , and d_3 are the three orthogonal diameters of the tumor.

Implantation of the LiPc probe and EPR oximetry

Mice were anesthetized with 1.5% isoflurane-air, and microcrystalline powder of LiPc (~0.5 mg) was implanted into the tumor using a 19-gauge needle loaded with LiPc in the tip, and a wire stylus as described in previous literature (19). The depth of implantation was ~3 mm below the skin surface. More than 24 hours after implantation, the mouse was anesthetized with a 1.5% isoflurane-air mixture at a flow rate of 0.7 L/min and placed on a hand-made surface type resonator (single-gap, loop-gap resonator, 15 mm i.d., 5 mm long) embedded in a Plexiglass block. A Teflon plate (1 mm thick) with a circular window (15 mm diameter) was used to position a tumor on the resonator. The EPR spectrum was recorded with an L-band EPR system composed of a JEOL JES RE-1X EPR spectrometer, a JEOL L-band microwave unit, the surface type resonator mentioned above, and a hand-made 100 kHz modulation coil that was attached to the surface of the EPR magnet. The microwave frequency was 1.2 GHz and the microwave power was 1.0 mW. The amplitude of the 100 kHz field modulation was 0.0040 to 0.0063 mT. Calibration data were used to obtain pO_2 from the peak-to-peak line width of the EPR signal. To obtain the calibration line, LiPc powder was suspended in PBS, the suspension was taken in a gaspermeable TPX tube, and inserted into a quartz tube. L-band EPR spectra were recorded under streams of standard gas containing known concentrations of O_2 .

EPR-CT images

The EPR-CT image was reconstructed on the basis of Lauterbur's method (25) as reported previously (26). An in vivo EPR imaging system (JEOL), equipped with a wide gap (250 mm) electromagnet, a four-gap, bridged, loop-gap resonator (38 mm i.d., 28 mm long), an L-band microwave unit, and four sets of coils (three for the magnetic field gradient to three orthogonal directions and one for rapid scanning; ref. 7), were used to obtain three-dimensional EPR images of the infused nitroxyl probe and LiPc. The amplitude of a 100 kHz field modulation was 0.032 mT for LiPc or 0.16 mT for the nitroxyl probe. The field gradient was 0.2 mT/cm for LiPc and 0.5 mT/cm for nitroxyl probe. The projection spectra (1,024 points/projection for LiPc, 512 points/projection for nitroxyl probe) were acquired every 15 degrees as single scans (1.5 s/scan). The projections were deconvoluted with the corresponding spectrum recorded without field gradient and subsampled to 64 points. The three-dimensional EPR images were reconstructed with a filtered back projection method, and then tomography images were obtained as slices of it. Spectral data acquisition, image reconstruction, and data analysis were performed with JEOL ESR-CT software (ver. 1.187).

Calculation of clearance rate of nitroxyl probe

Mice were anesthetized with 1.5% isoflurane-air, and placed in a resonator of the EPR imaging system. An aqueous solution of HM-PROXYL (140 mmol/L, 100 μ L) was injected i.v. EPR-CT images were obtained as described above. To calculate the regional decay rate of the nitroxyl probe, three-dimensional images were obtained in a time course. The data acquisition for images was started at 3 minutes after HM-PROXYL injection, and three

images were obtained at 10-minute intervals. Each image within the series was normalized with respect to the maximum intensity obtained within the series. The logarithm of sum intensity in the region of interest (ROI) on a slice image was plotted against time, and decay rate was obtained as a first-order rate constant.

Results

Differences in regional decay rates of nitroxyl probe in tumors

HM-PROXYL, a nitroxyl probe, was injected i.v. into a RIF-1 tumor-bearing mouse, and EPR-CT images were obtained. To compare the rates of decay of nitroxyl signal at several regions in a tumor, EPR slice images were obtained sequentially at 3, 13, and 23 minutes after probe injection. Figure 1 shows the time course images of axial slices, including tumor position. The volume of the tumor in this image was 525 mm³. The image showed that HM-PROXYL distributes into the tumor of the right hind leg as well as other tissues. The signal intensity of the probe decreased with time. ROIs a, b, c, and d were selected in the tumor as indicated in Fig. 1. The theoretical size of each ROI was $2.34 \times 3.12 \times 0.78$ mm³. The sum intensity in each region was plotted against time. As shown in Fig. 2, the decrease in the intensity was fairly linear on the semilogarithmic plot, indicating that the decay obeys first-order kinetics. A small difference in the rate of the decay was observed among the regions, although the rate was quite similar at a few regions.

To examine the differences in regional decay among mice, six mice carrying RIF-1 tumor were used. The average tumor size was 614 ± 74 mm³ (mean $\pm$ SD). Four ROIs were selected on axial slice images at the positions defined in Fig. 1. The rates of intensity decay at the ROIs of the tumors are summarized in Table 1. The decay rate ranged from 0.0232 to 0.0633 (per minute). The values differed among both tumors and regions. The difference among regional decay rates within a single tumor was large in two mice (nos. 2 and 3; CV = 24.8% and 33.2%, respectively), whereas the difference was <10% in the remaining tumors, as indicated in Table 1. ROIs a, b, and c are located at the edge of the tumor, and ROI d is located in the center. No tendency was observed between the decay rate and the position. The observations indicate that the amplitude of reducing status and the extent of heterogeneity of the status are quite different among tumors, and that the status does not depend on the position in the tumor.

The decay rate was also measured in the tumor whose average size was 152 ± 57 mm³ (mean $\pm$ SD), the size smaller than that of tumors in Table 1. The positions selected are defined in Supplementary Fig. S1, and the rates of the decay of image intensity are presented in Supplementary Table S1. Although some differences were observed among the regions/tumors, the range of the decay rates was almost the same as that of the rate observed in the larger tumors.

Relationship between the decay rate and pO₂

The results obtained above indicate that the reducing status more or less varied at every region in a tumor. Therefore, measurement of regional pO₂ is necessary to examine the relationship between reducing status and oxygenation. The regional pO₂ was measured with

LiPc probe implanted in a tumor, based on the broadening of its EPR signal measured using EPR spectroscopy. The position of the LiPc implant was determined by EPR-CT imaging (Fig. 3A, top row). Then, EIM-PROXYL was injected i.v. into the same mouse to obtain the rate of nitroxyl signal decay at the region. Elsewhere, the EPR signal of LiPc overlapped with the triplet-signal of EIM-PROXYL (Fig. 4). This might interfere with the reconstruction of the spatial image of HM-PROXYL. However, this problem was overcome through the use of the HM-PROXYL signal peak at a lower magnetic field, because the lower field peak of triplet signal did not overlap with the LiPc signal (Fig. 4) under these conditions. Because HM-PROXYL was distributed throughout the tumor tissue, its image should delineate the tumor. The position of LiPc superimposed on the image of HM-PROXYL (Fig. 3A, white square in the bottom row) coincided exactly with the position of LiPc (Fig. 3B, arrowhead). The theoretical size of ROI was $3.12 \times 3.12 \times 0.78 \text{ mm}^3$. The rate of decay of nitroxyl signal at the region of LiPc was obtained using sequential EPR-CT images of HM-PROXYL as described above.

Thus, both the pO_2 and the decay rate of HM-PROXYL were obtained at the same region in a tumor. Figure 5 shows the relationship between pO_2 and the decay rate measured with 13 mice bearing RIF-1 tumors with variations in size (59–730 mm^3). The relationship between pO_2 and the decay rate was low ($r = 0.357$), although the decay rate had a weak tendency to decrease with an increase in pO_2 . This indicates that the factor affecting the reducing status is not simply the extent of oxygenation in RIF-1 tumor tissue under air-breathing conditions.

Discussion

In the present study, a variation of heterogeneity in the reducing status among tumors/mice and low correlation between the reducing status and pO_2 were shown. Sequential EPR-CT with a nitroxyl probe in combination with EPR oximetry with LiPc probe was a powerful tool to reveal these results in live animals. The separate imaging of two different probes enabled the determination of the position of LiPc on images obtained using the nitroxyl probe. The images were successfully obtained by the choice of signal peaks in different magnetic fields.

Decay of the EPR signal of the nitroxyl probe in a tumor might be caused by one-electron reduction of the probe to corresponding hydroxylamine and/or elimination of the probe itself from the tumor by blood perfusion. However, it has been observed that the rate of decay of nitroxyl probes, such as 3-carbamoyl-PROXYL, varied depending on the position measured (7), suggesting that a reduction in parenchymal cells is a dominant factor for the *in vivo* decay of the nitroxyl signal. If perfusion was the dominant factor, the rate of signal decay should be identical in any part. In the present study, HM-PROXYL was used as a probe. The value of the *n*-octanol/PBS partition coefficient was 1.2 for HM-PROXYL and 0.45 for 3-carbamoyl-PROXYL.⁸ The decay of image intensity in a tumor was monophasic for HM-PROXYL, as shown in Fig. 2, although the decay of the probe was biphasic in the blood circulation (Supplementary Fig. S2). In contrast, the decay of image intensity for 3-carbamoyl-PROXYL was biphasic, which is similar to its decay in the blood circulation

⁸K. Takeshita and S. Okazaki, unpublished results.

(Supplementary Figs. S2 and S3). These basic observations indicate that, in the case of 3-carbamoyl-PROXYL, probes in the blood circulation contribute, to some extent, to the change in the image intensity, although the contribution for HM-PROXYL is smaller than that for 3-carbamoyl-PROXYL. Therefore, HM-PROXYL should be more suitable to probe the reduction status in tumor tissues than 3-carbamoyl-PROXYL.

It has been reported that microsomes (11), mitochondria (12), and sulfhydryl-related systems (6) contribute to nitroxyl reduction in a body. Additionally, inhibition experiments with ascorbate oxidase suggested that ascorbic acid is a main cytosolic reducing agent for pyrrolidine nitroxyl radicals in biological systems (10, 27, 28). Therefore, mechanisms related to the reduction of nitroxyl radicals should be complicated in biological systems. In contrast, the previous articles (20, 21) showed that the decay rate of nitroxyl probe was correlated with GSH content in a RIF-1 tumor, although GSH reduced the nitroxyl radical very slowly (data not shown). The literature shows that GSH supports the activity of ascorbic acid by recycling ascorbic acid from its oxidized form in the normal tissues (29, 30). Therefore, it is likely that the nitroxyl radical is reduced by ascorbic acid-GSH cooperating systems in a tumor. It has been reported that GSH and ascorbic acid contents are related to the sensitivity of experimental tumors to ionizing radiation or some anticancer drugs (31–34). Thus, reduction status measured with nitroxyl probe might reflect tumor physiologic condition related to resistance to ionizing radiation and some anticancer drugs.

The size of the ROI for measurement of nitroxyl decay at the position of the LiPc probe was $3.12 \times 3.12 \times 0.78 \text{ mm}^3$. The LiPc powder was dispersed spherically with a diameter of $\sim 1.5 \text{ mm}$ as observed in the incised tumor after measurement. The spatial resolution of EPR imaging has been determined to be 1 to 1.5 mm (20, 26). Considering this resolution, the size of ROI was determined. Therefore, the regional signal decay observed in this study was not accurately microscopic. However, the regional rates obtained at several parts differed in a single tumor, indicating that the regional rate obtained in this study was sufficient to evaluate the heterogeneity of redox status, although the spatial resolution was somewhat low.

An oxygenated environment might consume endogenous antioxidants, such as ascorbic acid and GSH, and bring redox status to an oxidative condition. It has been reported that carbogen-breathing fairly suppressed the decay of nitroxyl probes in a RIF-1 tumor (19, 20), indicating that the nitroxyl clearance reflects not only reducing capacity, but also oxygenation within the tumor. Therefore, some relation may be expected between the nitroxyl signal decay rate and pO_2 . However, the relationship was low in this study, although the decay rate had a weak tendency to decrease with an increase in pO_2 . The range of pO_2 variation was 2.8 to 15.7 mm Hg in tumors under air-breathing conditions. This range is much smaller than the alteration of pO_2 by carbogen-breathing in which the pO_2 increased from 2 to 3 mm Hg to 10 to 40 mm Hg (19). Thus, it is likely that the variation of nitroxyl decay might depend primarily on reducing capacity, and that the effect of pO_2 variation on the nitroxyl decay might be very small under air-breathing conditions.

There are some differences in the decay rate of nitroxyl radicals among the parts in a single tumor and tumors in different mice (Table 1). In particular, the difference among tumors/mice was larger than the difference among the parts in a single tumor. The relationship

between nitroxyl decay and vascularization is interesting. The value of pO_2 should be an index of vascularization as previous articles (35, 36) have shown a good correlation between vascular perfusion and pO_2 in tumor models. The low correlation between the decay rate of nitroxyl radicals and pO_2 (Fig. 5) indicates that the relationship between the nitroxyl decay and vascularization is low. This contention is supported by the observation that the decay rate at the center of the tumors was not necessarily higher than the rate at the edge of the tumors. It is generally accepted that the core region of a large tumor should be hypoxic because of the lack of a vascular system. The activity of reduction systems, including GSH, might be controlled by many physiologic factors such as the supply of nutrients by blood circulation, expression of enzyme proteins, viability of the tissue cells, and pH.

Recently, Liu and colleagues (37) reported water-soluble trityl-nitroxyl biradicals to probe redox status and pO_2 simultaneously. In their technique, both redox status and pO_2 were given from spectral information such as ratio of signal intensities within a spectrum and line-width of signal. Therefore, spectral-spatial analysis is necessary to obtain information related to redox status and pO_2 at a selected region, although the spatial resolution would be higher than in the present technique if it had been done. In the technique used in the present study, a small amount of water-insoluble oxygen probe was implanted in a tumor to determine the regional pO_2 , and redox information was obtained from the decay of intensity at the same region on an intensity map of water-soluble nitroxyl radicals. LiPc was used in this study, but any water-insoluble oxygen probes whose EPR signals are narrow and separable from at least one line of the nitroxyl signal could be used instead of LiPc. The probes applicable to this technique include lithium naphthalocyanine (38), synthetic chars (39), and India ink (40), etc.

In conclusion, the amplitude and heterogeneity of redox status varied among tumors, and the extent of oxygenation does not necessarily affect the redox status under air-breathing conditions. The results strongly indicate the necessity of measurements of both redox status and oxygenation in every tumor to characterize tumor physiology. EPR-CT in combination with EPR oximetry should be a powerful technique to obtain these data. ^{15}N -substituted nitroxyl probes produce a doublet signal, and no peak would overlap with the LiPc signal (19). Therefore, if ^{15}N -substituted nitroxyl probe is used instead of ^{14}N -nitroxyl one, pO_2 can be measured even after the injection of a nitroxyl probe without having to change the magnetic field. This could widen the range of applications of this technique.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Time course of EPR slice images of a tumor-bearing mouse injected with HM-PROXYL. HM-PROXYL was i.v. injected into a mouse bearing a RIF-1 tumor, and an axial-slice image (0.78 mm thick) including tumor position (A) was obtained from a three-dimensional image which corresponds to a size of $28.9 \times 28.9 \times 28.9 \text{ mm}^3$. The time course slice images (B) and schematic explanation are indicated (C). The time under each image indicates starting time of EPR imaging after HM-PROXYL injection. ROIs a–d indicate the region of decay rate measurement. Each ROI has a size of $2.34 \times 3.12 \times 0.78 \text{ mm}^3$. The volume of each pixel is calculated to have a dimension of $0.78 \times 0.78 \times 0.78 \text{ mm}^3$. The resolution of EPR imaging has been determined to be 1 to 1.5 mm (20, 26). Thus, the real resolution should be somewhat lower than the pixel-based resolution. The slice image does not include the part of the highest intensity (yellow), which was observed in the other slices.

Figure 2. Comparison of regional rate of decay of nitroxyl signal in a tumor. Sum intensity in each ROI in Fig. 1 was plotted against time after HM-PROXYL injection.

Figure 3.

Determination of position of LiPc implantation on an image of HM-PROXYL probe. A, EPR slice image of LiPc implanted in a tumor was obtained to determine the region of pO_2 measurement (top row). Then, an image of HM-PROXYL was obtained after i.v. injection of HM-PROXYL as described in the text. ROI of LiPc location was superposed on the HM-PROXYL image to obtain the rate of signal decay in this ROI (bottom row). B, a tumor used for images in A was incised after euthanasia of the mouse by excess administration of pentobarbital sodium salt. It was confirmed that the location of ROI in axial-slice image of HM-ROXYL (in A) coincides exactly with the location of LiPc (white arrowhead).

Figure 4. EPR spectra of LiPc (A) and HM-PROXYL (B). L-band EPR spectra of LiPc suspended in PBS and an aqueous solution of HM-PROXYL (2 mmol/L) was recorded under air atmosphere. Modulation amplitude was 0.032 mT for LiPc and 0.16 mT for HM-PROXYL. Relative position of centerfield of sweep and sweep width for imaging was indicated under each spectrum.

Figure 5. Correlation between pO₂ and rate of nitroxyl signal decay in a tumor under air-breathing. The value of pO₂ in a tumor in the leg of mouse was measured with LiPc probe. The decay rate of HM-PROXYL signal at the region of LiPc implantation was obtained as mentioned in the text and plotted against pO₂. Correlation coefficient was 0.357.

Table 1.

Regional rate constant of decay of HM-PROXYL in tumors

Positions	Mouse					
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
a	0.0369	0.0633	0.0512	0.0254	0.0304	0.0459
b	0.0411	0.0514	0.0307	0.0233	0.0259	0.0396
c	0.0466	0.0354	0.0237	0.0232	0.0272	0.0438
d	0.0419	0.0429	0.0351	0.0232	0.0316	0.0458
Mean	0.0416	0.0483	0.0352	0.0238	0.0288	0.0438
SD	0.0040	0.0120	0.0117	0.0011	0.0027	0.0029
CV (%)	9.6	24.8	33.2	4.6	9.3	6.7

NOTE: Rate constants (per minute) of decay of HM-PROXYL in tumors were measured at positions corresponding to ROIs indicated in Fig. 1.