Published in final edited form as:

Magn Reson Med. 2018 February ; 79(2): 1212–1218. doi:10.1002/mrm.27008.

Effect of Body Temperature on the Pharmacokinetics of a Triarylmethyl-Type Paramagnetic Contrast Agent Used in EPR Oximetry

Ken-ichiro Matsumoto¹, Fuminori Hyodo³, James B. Mitchell², and Murali C. Krishna^{2,*}

¹Quantitative RedOx Sensing Team, Department of Basic Medical Sciences for Radiation Damages, National Institute of Radiological Sciences, National Institutes for Quantum and Radiological Science and Technology, Chiba-shi, Chiba 263-8555, Japan

²Radiation Biology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892-1002, USA

³Department of Frontier Science for Imaging, School of Medicine, Gifu University, Japan

Abstract

Purpose—Pharmacokinetics of tri[8-carboxy-2,2,6,6-tetrakis(2-hydroxymethyl)benzo[1,2-d:4,5-d']bis(1,3)dithio-4-yl]methyl radical (Oxo63) after a single bolus and/or continuous intravenous infusion was investigated in tumor bearing C3H mice with or without body temperature control while under anesthesia.

Meshod—The *in vivo* time course of Oxo63 in blood was measured using X-band electron paramagnetic resonance (EPR) spectroscopy. Distribution of Oxo63 in normal muscle and tumor tissues was obtained using a surface coil resonator and a 700 MHz EPR spectrometer. The whole body distribution of Oxo63 was obtained by 300 MHz continuous wave (CW) EPR imaging. The high resolution 300 MHz time-domain EPR imaging was also carried out to probe the distribution of Oxo63.

Results—Urination of mice was retarded at low body temperature making the concentration of Oxo63 in blood attain high levels. However, the concentration of Oxo63 in tumor tissue was lower with no control of body temperature than active body temperature control. The non-systemized blood flow in the tumor tissues may pool Oxo63 at lower body temperature.

Conclusions—Pharmacokinetics of the contrast agent was found to be significantly affected by body temperature of the experimental animal and can influence the probe distribution and the image patterns.

Keywords

functional imaging; anesthesia; body temperature; urinary excretion; trityl free radical; *in vivo* pO₂ imaging

*Correspondence to: Murali C. Krishna, Building 10, Room B3B69, NIH, Bethesda, MD 20892-1002, Tel: 301-496-7511. Fax: 301-480-2238. cherukum@mail.nih.gov.

INTRODUCTION

A variety of molecular imaging techniques have been developed in pre-clinical and clinical research such as PET, MRI, EPRI, ultrasound, luminescence imaging to obtain imaging biomarkers related to physiology and metabolism. Recently, combination of multi-modal imaging methods is used to investigate detailed pathophysiological information (1, 2). Several noninvasive and quantitative *in vivo* oxymetric imaging methods based on electron paramagnetic resonance (EPR) techniques have been developed recently (3–7). EPR oximetry involves direct and/or indirect measurement of linewidth of EPR resonance lines, which depend on the interaction between the paramagnetic probe and molecular oxygen which is also paramagnetic. Time-domain (TD) EPR, continuous wave (CW) EPR and Overhauser enhanced MRI (OMRI) based oxymetric imaging are modalities which can obtain tissue oxygen levels by monitoring the EPR linewidth. For these techniques to provide reliable and valid maps of tissue pO₂, understanding the pharmacokinetic behavior of the paramagnetic agent is necessary.

The trityl free radical, tri[8-carboxy-2,2,6,6-tetrakis(2-hydroxymethyl)benzo[1,2-*d*:4,5-*d'*]bis(1,3)dithio-4-yl)methyl radical (Oxo63), is commonly used as a paramagnetic probe for EPR oxymetric imaging. The concentration of the Oxo63 should be sufficient to provide adequate signal-to-noise ratio (SNR). Simultaneously, the plasma concentration of Oxo63 should be kept below 2 mM to eliminate over-estimation of line width as a measure of pO₂, which can occur by self-broadening at higher concentrations of Oxo63. In prior studies optimal and stable blood concentration of Oxo63 was achieved using combination of bolus and continuous *i.v.* infusion of the spin probe solution using normal healthy female C3H mice (8).

In our previous study (8) we investigated the pharmacokinetics of Oxo63 in healthy normal mice to regulate the blood concentration of Oxo63 in mice. At that time, the predominant rapid urinary excretion of Oxo63 and its saturation by the high blood concentration of Oxo63 were noticed. Based on the results of the previous study (8), a combination of bolus and continuous injection of Oxo63 was employed to obtain a steady blood concentration of Oxo63 and used for oxymetric imaging studies (7, 9). However, in this study, the influence of anesthesia-induced lower body temperature in tumor-bearing mice, used for EPR oximetry imaging was investigated, and we found that the urinary excretion of Oxo63 was retarded at low body temperature.

Anesthetics used in small animal imaging experiments, in part to reduce movement of the animal, usually cause a decrease in core body temperature and low blood flow (10) which in turn can impact tissue pO₂ and/or oxygenation levels (11, 12). To maintain normal core body temperature of the animal, it is necessary to maintain normal blood flow to permit assessment of tissue pO₂ reliably. However, variation of the tissue blood flow may affect the pharmacokinetics of the paramagnetic probe. Therefore, the core body temperature of the animal can also influence the pharmacokinetics of the contrast agent. The intrinsic but unknown pharmacokinetics of the contrast agents in pathophysiological conditions can introduce artifacts in the image contrast. Previous investigations of the pharmacokinetics of

the paramagnetic probes underscore its importance to understand image contrast observed reliably (13–17).

During a past couple of decades, autoradiography using an RI-labeled compound was the main approach to investigate the pharmacokinetics of the test compound in a whole animal. In such studies a time course study was challenging. Using a non-invasive molecular imaging techniques, currently we can visualize the pharmacokinetic and also pharmacodynamics of a paramagnetic contrast agent in a living whole intact animal. Therefore, this study demonstrated the possibility of magnetic resonance imaging techniques on the whole body pharmacokinetics/pharmacodynamics studies instead of the conventional RI-tracer techniques. Oxo63 has now become the paramagnetic probe of choice for *in vivo* pO₂ imaging applications. Its pharmacokinetic behavior in mice under anesthesia is therefore important. In this study, effect of body temperature to the pharmacokinetics of a triarylmethyl-type paramagnetic probe after bolus and/or continuous intravenous infusion was investigated in tumor bearing C3H mouse using EPR based non-invasive methods.

MATERIALS AND METHODS

Chemicals

Oxo63 was obtained from GE Health Care (Milwaukee, WI). Figure 1 shows the structure of the Oxo63. Deionized water (deionization by the Milli-Q system) was used for preparing injectable isotonic (75 mM) Oxo63 solution.

Animals

Female C3H Hen MTV mice were supplied by the Frederick Cancer Research Center, Animal Production (Frederick, MD). Animals, received at six weeks of age, were housed five per cage in climate controlled, circadian rhythm-adjusted rooms and were allowed food and water ad libitum. Squamous cell carcinoma (SCC) was implanted in the biceps femoris muscle of right thigh two weeks before the experiments. The approximate size of the tumor in mice used in imaging experiments was 1.5–2.0 cm (largest diameter). The control group had no tumors implanted. Experiments were carried out in compliance with the Guide for the Care and Use of Laboratory Animal Resources (1996), National Research Council, and approved by the National Cancer Institute Animal Care and Use Committee. Experiments were performed within 2–4 weeks of their arrival at the facility. Their body weight measured before the experiments was in the range 22–27 g.

Time Course of Blood Concentration of Oxo63

Time course of blood concentration of Oxo63 in an anesthetized mouse was measured by EPR spectroscopy at X-band frequency using the *Dashitari-Iretari* (DI) method (8, 18). Figure 2A shows a schematic of the experimental setup for the X-band EPR version of the DI method. Each mouse was anesthetized by isoflurane (1.5% in medical air, 700 mL/min). The jugular vein was cannulated by PE-10 tubing. The jugular vein cannula was allowed to traverse through the X-band EPR cavity and the end of the cannulation line was connected to a 1 mL syringe. The cannulation line and the syringe were filled with heparinized saline previously. The core body temperature (sampled from the rectum) was monitored by a

nonmagnetic probe (FISO technologies Inc., Quebec, Canada) during EPR measurements. The experiment was started after the mouse body temperature reached 36.5°C, then the body temperature was kept at $37 \pm 1^\circ\text{C}$ during the EPR measurement using an air heater. Isotonic Oxo63 aqueous solution was injected through a cannula inserted in the tail vein with a bolus 0.75 $\mu\text{mol/g}$ b.w. dose or a continuous 0.06 $\mu\text{mol/g}$ b.w./min dose following a bolus 0.75 $\mu\text{mol/g}$ b.w. dose. Immediately after the bolus injection, blood was drawn up to the X-band EPR cavity from the jugular vein cannula, and EPR signal was measured. The sampling volume in the EPR cavity was approximately 2 μL . The blood was pumped back into the jugular vein with additional 10–30 μL of heparinized saline flush immediately after the measurement. The X-band EPR measurements were repeated by several draw-measure-pump back sequences until 60 min after the initial injection of Oxo63 solution. The EPR conditions were follows; microwave frequency was 9.4 GHz, microwave power was 0.5 mW, magnetic field modulation frequency was 100 kHz, magnetic field modulation amplitude was 0.1 Gauss, time constant was 0.064 sec, magnetic field scan rate was 2 Gauss/min. The experiment was performed under controlling body temperature $37 \pm 1^\circ\text{C}$ (TC group) or under no special control of the body temperature (NC group).

Time Course of Tissue Oxo63 Level (by 700 MHz CW EPR Surface Coil)

Each mouse was anesthetized and restrained on a special mouse holder. A single loop surface coil (7.3 mm i.d.) was placed on the thigh. The mouse body temperature was kept at $37 \pm 1^\circ\text{C}$ during the experiment using an air heater. Isotonic Oxo63 aqueous solution was injected through a cannula inserted in the tail vein with a bolus 0.75 $\mu\text{mol/g}$ b.w. dose. EPR signal was repeatedly measured by a 700 MHz CW EPR spectrometer (11). EPR conditions were as follows: microwave frequency = 700 MHz, microwave power = 10 mW, field modulation frequency = 13.5 kHz, field modulation amplitude = 0.1 Gauss, time constant = 0.03 s, sweep width = 10 Gauss, scan time = 16 s. The EPR signal height of Oxo63 was repeatedly monitored every 30 s for 60 min. EPR signals of Oxo63 in tumor bearing thigh and normal thigh were measured separately using different mice. The experiment was performed under controlling body temperature $37 \pm 1^\circ\text{C}$ (TC group) or under no special control of the body temperature (NC group).

Whole Body Pharmacokinetics of Oxo63 by 300 MHz CW EPR imaging

An anesthetized mouse was placed on special mouse holder and restrained. The urethra was cannulated previously using a 10-cm segment of PE-10 tubing to drain the urine. The tail vein was cannulated to inject Oxo63 solution. The mouse was set in a resonator tuned to 300 MHz (30 mm diameter, 50 mm length) (19). The mouse body temperature was kept at $37 \pm 1^\circ\text{C}$ during the EPR measurement using an air heater. A single bolus dose of Oxo63 (0.75 $\mu\text{mol/g}$ b.w.) was injected through the tail vein. The 2D CW EPR imaging data acquisition was started immediately after injecting Oxo63 and images were repeatedly collected for 20 min. Details of the 300 MHz CW EPR imaging scanner were reported elsewhere (20). EPR imaging conditions were as follows: microwave frequency = 300 MHz, microwave power = 10 mW, field modulation frequency = 13.5 kHz, field modulation amplitude = 0.1 Gauss, time constant = 0.03 s, sweep width = 10 Gauss, scan time = 16 s, projection number = 18, field gradient = 2.5 Gauss/cm. EPR images were reconstructed by filtered back-projection with a standard Shepp-Logan filter. The experiment was performed under controlling body

temperature $37 \pm 1^\circ\text{C}$ (TC group) or under no special control of the body temperature (NC group).

Comparison of pharmacokinetics of Oxo63 in tumor and normal tissues by 300 MHz TD EPR imaging

To obtain better spatial resolution, EPR imaging experiment was performed by single-point imaging (SPI) modality (21) using 300 MHz TD EPR imager. The experimental conditions for *in vivo* experiments were the same as in the case of CW EPR experiments except only the thigh of the mouse was set in the resonator (25 mm diameter, 25 mm length) (20, 22). Images were obtained every 5 min after infusing a single bolus dose of Oxo63 (0.75 $\mu\text{mol/g}$ b.w.). The 2D TD EPR SPI data were acquired with following conditions; number of field gradient steps was 21×21 , number of FIDs averaged was 2000, and maximum gradient was 1.0 Gauss/cm. Details of the 300 MHz TD EPR imager were reported elsewhere (20). The experiment was performed with controlled body temperature $37 \pm 1^\circ\text{C}$ (TC group) or under no active control of the body temperature (NC group).

Statistical Analyses

The statistical differences were estimated with TTEST function in the Microsoft Excel XP. The suitable 'type' and 'tail' for the test was selected according to the correspondence and variance of the data. Significances were estimated when p value was less than 0.05.

RESULTS

The core body temperature of the mouse was around 30°C without active temperature control, at the start of the experiment and gradually decreased to almost room temperature ($22\text{--}24^\circ\text{C}$) during the 60 min measurement time window (data not shown). Figure 2B shows the time course of blood concentration of Oxo63 of 3 mice after a bolus injection (0.75 $\mu\text{mol/g}$ b.w.) with or without body temperature control. The vertical axis indicates logarithmic value of the blood concentration of Oxo63 to facilitate visualizing differences in decay curves of the three groups. The blood concentration of Oxo63 displayed a biphasic pattern after a bolus injection of Oxo63 solution in both cases. The decay constant of the slow-phase (after 10 min) in a tumor bearing mouse (open squares), which was calculated as $0.035 \pm 0.001 \text{ min}^{-1}$, was significantly ($p < 0.05$) slower compared with that for a healthy mouse (non-tumor bearing, open circles), which was $0.049 \pm 0.003 \text{ min}^{-1}$, even when the mouse body temperature was maintained at 37°C . The blood concentration of Oxo63 at the same time point was higher in tumor bearing mice compared to that of healthy mice. When the body temperature was not maintained at 37°C , the decay constant of the slow-phase significantly ($p < 0.001$) decreased to be $0.002 \pm 0.001 \text{ min}^{-1}$ and the concentration of Oxo63 in blood became stable. The decay constant of the slow-phase in healthy control mice without temperature control was reduced to $0.011 \pm 0.006 \text{ min}^{-1}$ ($n = 5$, data not shown), which was significantly ($p < 0.05$) faster than that of tumor bearing mice group.

Some EPR studies need long imaging times requiring the maintenance of stable levels of Oxo63 which is achieved by a bolus dose followed by continuous infusion during the study. Figure 2C shows a comparison of the time course of blood concentration of Oxo63 during a

continuous injection (0.06 $\mu\text{mol/g b.w./min}$ was followed a 0.75 $\mu\text{mol/g b.w.}$ bolus injection) with or without body temperature control. The vertical axis indicates the blood concentration of Oxo63. The combination of a bolus and continuous injection of Oxo63 solution achieved a stable blood concentration around 2 mM when the body temperature was regulated (open symbols). In this case, concentration of Oxo63 in blood of tumor bearing mice displayed a slightly higher concentration compared to that of healthy mice, even when the body temperature was regulated. When the mouse body temperature was not controlled, the blood concentration of Oxo63 increased during continuous injection and finally reached up to a level of 7 mM.

The levels of Oxo63 were monitored in several regions of the mouse including muscle and tumor. Figure 3 shows the time course of EPR signal intensity of Oxo63 in the femoral muscle tissue of healthy mice (no tumor), normal femoral muscle tissue of tumor bearing mice (normal tissue), and the tumor bearing thigh (tumor tissue) measured using a 700 MHz EPR spectrometer equipped with a surface coil resonator. The EPR signal decay profile of Oxo63 in tissues showed a linear decay after reaching a maximum value. The maximum EPR signal intensity in decay profile of Oxo63 in TC experiments was highest in tumor tissue, while that in NC experiments was lowest in tumor tissue. The time to reach maximum EPR intensity after the injection of probe and the sequential EPR signal decay rate in healthy muscle, normal tissue, and tumor tissue measured with or without body temperature control is summarized in Table 1. The time to reach maximum intensity became longer and the signal decay rates became smaller when the body temperature was not controlled (NC treatment). When TC and NC mice administered with Oxo63 were compared, significant differences were noticed in the times taken to reach maximum intensity in normal tissue, decay rate in normal tissue, and decay rate in tumor tissue. However, no significant differences among tissues were noticed when the body temperature was controlled. Significant differences were observed between normal muscle tissue of tumor bearing mice and healthy muscle tissue of non-tumor bearing mice for the times taken to reach maximum intensity when body temperature was not controlled. Significant differences were also observed between normal tissue and tumor tissue for the decay rate when body temperature was not controlled.

Imaging experiments to monitor the spatial distribution of Oxo63 were carried out in mice with and without body temperature control. Figure 4 shows a comparison of whole body distributions of Oxo63 in tumor bearing mice measured by 300 MHz CW EPRI with or without the body temperature control. When the body temperature was maintained at 37°C, where the urinary excretion profiles were normal, the Oxo63 localization in the kidneys and the bladder can see clearly (Fig. 4, left column). However, the distribution of Oxo63 was more global and stable when body temperature was not controlled (Fig. 4, right column).

TD EPR SPI modality gives higher image resolution compared to that of CW EPR imaging and shapes. Figure 5 shows results of comparison of pharmacokinetics of Oxo63 in tumor tissue implanted in right thigh of a mouse and the contralateral normal thigh and of both right and left legs are clearly observed (Fig. 5A). Higher image intensity in the bladder, i.e. fast urinary excretion of probe, can be observed 5 min after injection of Oxo63 when the mouse body temperature was controlled and kept at 37°C during the experiment (Fig. 5A,

upper left panel). TD EPR image intensity decreased and shapes of both right and left legs became hazy with a concomitant strong signal from the bladder 50 min after injection of Oxo63 under active body temperature control (Fig. 5A, upper right panel). When body temperature was not controlled, signal from the bladder was weak i.e. indicating a lower urinary excretion than expected (Fig. 5A, lower panels). The shapes of both right and left legs can be clearly distinguished 50 min after injection of Oxo63 without temperature control (Fig. 5A, lower right panels). Figure 5B and C shows time courses of TD EPR image intensity in SCC tumor or normal muscle regions obtained in TC and NC experiments, respectively.

DISCUSSION

Body temperature of small animals, such as mice, is known to drop rapidly under anesthesia, because of the larger surface-area/volume ratio compared to larger species. Lower body temperature produced by anesthesia can influence several physiological conditions, such as blood pressure, blood flow, and urinary excretion. Additionally non-physiological conditions such as contrast agents or tracers used in experimentations can modify the pharmacokinetic effect of administered drugs. Therefore, strict control of body temperature of the small experimental animal under anesthesia is necessary to carry out molecular imaging experiments to obtain reliable information.

Oxo63 after bolus administration was mainly excreted in urine in a dose dependent manner (8). The amount of Oxo63 excreted in urine during the 60-min experiment corresponds to approximately 50–60% of the total injected dose (8); however, urination of mice was significantly reduced when the body temperature was not controlled and the core body temperature was $\sim 30^{\circ}\text{C}$ (data not shown). Retardation of urinary excretion at such low body temperature occurred both tumor bearing and healthy (non-tumor bearing) mice.

In general, renal function can decrease under hypothermic conditions (23). Even in the NC experiment in this study, the right and left kidneys were recognizable following administration of Oxo63, while bladder was not (Fig. 4). This result suggests that the inhibition of urination at NC experiment may be due to impaired function of the kidney. The urinary excretion of Oxo63 in this study, can be aided by the normal renal function at normal body temperature but not in disturbed renal function at hypothermia. This result opens a possibility of using these organic paramagnetic species as diagnostic agents for renal functions used in MRI.

At these lower core body temperatures, the blood flow will be reduced and the distribution of the Oxo63 exhibited a delay in time to reach maximum EPR signal intensity level as shown in Table 1. Therefore, blood, i.e. oxygen, supply to the tissue may be reduced resulting in a lower tissue pO_2 at low body temperature. This interpretation is consistent with EPR oximetry studies in healthy mouse using LiPc crystal as an oxygen probe placed at femoral muscle or mammary pad (11). Therefore, maintaining natural body temperature can keep the blood flow natural even under isoflurane anesthesia.

Since Oxo63 is widely used for *in vivo* pO₂ measurement, its ADME (absorption, distribution, metabolism, and excretion) in mice maintained under anesthesia is important. Low body temperature induced by the anesthesia results in abnormal pharmacokinetics of injected contrast agents or tracers. Since the maintaining body temperature using warming/heating instruments can enable clearance of the contrast agent, slow clearance was not direct effect of anesthesia on the cardiovascular system. Maintaining normal body temperature of the anesthetized animal is necessary to obtain a quantitatively accurate data in tumor oxygen mapping and/or other functional imaging experiment where fluctuations in the administered contrast media should be minimized. In conclusion, results from this study provide useful pharmacokinetic information of Oxo63, which now has widespread applications in *in vivo* oxymetric imaging in pre-clinical mouse models of human cancer.

Acknowledgments

The authors grateful to the experimental helps of Mrs. Atsuko Matsumoto and Mrs. Emi Hyodo.

References

1. Sowers MA, McCombs JR, Wang Y, Paletta JT, Morton SW, Dreaden EC, Boska MD, Ottaviani MF, Hammond PT, Rajca A, Johnson JA. Redox-responsive branched-bottlebrush polymers for *in vivo* MRI and fluorescence imaging. *Nat Commun.* 2014; 5:5460. [PubMed: 25403521]
2. He J, Bonnet CS, Eliseeva SV, Lacerda S, Chauvin T, Retailleau P, Szeremeta F, Badet B, Petoud S, Tóth É, Durand P. Prototypes of Lanthanide(III) Agents Responsive to Enzymatic Activities in Three Complementary Imaging Modalities: Visible/Near-Infrared Luminescence, PARACEST-, and T1-MRI. *J Am Chem Soc.* 2016; 138:2913–2916. [PubMed: 26727374]
3. Weaver J, Burks SR, Liu KJ, Kao JP, Rosen GM. *In vivo* EPR oximetry using an isotopically-substituted nitroxide: Potential for quantitative measurement of tissue oxygen. *J Magn Reson.* 2016; 271:68–74. [PubMed: 27567323]
4. Epel B, Halpern HJ. *In Vivo* pO₂ Imaging of Tumors: Oxymetry with Very Low-Frequency Electron Paramagnetic Resonance. *Methods Enzymol.* 2015; 564:501–527. [PubMed: 26477263]
5. Epel B, Bowman MK, Mailer C, Halpern HJ. Absolute oxygen R_{1ρ} imaging *in vivo* with pulse electron paramagnetic resonance. *Magn Reson Med.* 2014; 72:362–368. [PubMed: 24006331]
6. Subramanian S, Devasahayam N, Matsumoto S, Saito K, Mitchell JB, Krishna MC. Echo-based Single Point Imaging (ESPI): a novel pulsed EPR imaging modality for high spatial resolution and quantitative oximetry. *J Magn Reson.* 2012; 218:105–114. [PubMed: 22578561]
7. Matsumoto K, Subramanian S, Devasahayam N, Aravalluvan T, Murugesan R, Cook JA, Mitchell JB, Krishna MC. Electron paramagnetic resonance imaging of tumor hypoxia: enhanced spatial and temporal resolution for *in vivo* pO₂ determination. *Magn Reson Med.* 2006; 55:1157–1163. [PubMed: 16596636]
8. Matsumoto K, English S, Yoo J, Yamada K, Devasahayam N, Cook JA, Mitchell JB, Subramanian S, Krishna MC. Pharmacokinetics of a triarylmethyl-type paramagnetic spin probe used in EPR oximetry. *Magn Reson Med.* 2004; 52:885–892. [PubMed: 15389949]
9. Ueno M, Matsumoto S, Matsumoto A, Manda S, Nakanishi I, Matsumoto K, Mitchell JB, Krishna MC, Anzai K. Effect of amifostine, a radiation-protecting drug, on oxygen concentration in tissue measured by EPR oximetry and imaging. *J Clin Biochem Nutr.* 2017; 60:151–155. [PubMed: 28584395]
10. Baudelet C, Gallez B. Effect of anesthesia on the signal intensity in tumors using BOLD-MRI: Comparison with flow measurements by Laser Doppler flowmetry and oxygen measurements by luminescence-based probes. *Magn Reson Imaging.* 2004; 22:905–912. [PubMed: 15288130]
11. Matsumoto A, Matsumoto S, Sowers AL, Koscielniak JW, Trigg NJ, Kuppusamy P, Mitchell JB, Subramanian S, Krishna MC, Matsumoto K. Absolute oxygen tension (pO₂) in murine fatty and

- muscle tissue as determined by EPR. *Magn Reson Med*. 2005; 54:1530–1535. [PubMed: 16276490]
12. Reijnders K, English SJ, Krishna MC, Cook JA, Sowers AL, Mitchell JB, Zhang Y. Influence of body temperature on the BOLD effect in murine SCC tumors. *Magn Reson Med*. 2004; 51:389–393. [PubMed: 14755665]
 13. Beckett KR, Moriarty AK, Langer JM. Safe Use of Contrast Media: What the Radiologist Needs to Know. *Radiographics*. 2015; 35:1738–1750. [PubMed: 26466182]
 14. Trembath L, Newell M, Devous MD Sr. Technical Considerations in Brain Amyloid PET Imaging with 18F-Florbetapir. *J Nucl Med Technol*. 2015; 43:175–84. [PubMed: 26271806]
 15. Arami H, Khandhar A, Liggitt D, Krishnan KM. *In vivo* delivery, pharmacokinetics, biodistribution and toxicity of iron oxide nanoparticles. *Chem Soc Rev*. 2015; 44:8576–8607. [PubMed: 26390044]
 16. Fueger BJ, Czernin J, Hildebrandt I, Tran C, Halpern BS, Stout D, Phelps ME, Weber WA. Impact of animal handling on the results of ¹⁸F-FDG PET studies in mice. *J Nucl Med*. 2006; 47:999–1006. [PubMed: 16741310]
 17. Miyake M, Shen J, Liu S, Shi H, Liu W, Yuan Z, Pritchard A, Kao JP, Liu KJ, Rosen GM. Acetoxymethoxycarbonyl nitroxides as electron paramagnetic resonance proimaging agents to measure O₂ levels in mouse brain: a pharmacokinetic and pharmacodynamic study. *J Pharmacol Exp Ther*. 2006; 318:1187–1193. [PubMed: 16757536]
 18. Matsumoto K, Krishna MC, Mitchell JB. Novel pharmacokinetic measurement using electron paramagnetic resonance spectroscopy and simulation of in vivo decay of various nitroxyl spin probes in mouse blood. *J Pharmacol Exp Ther*. 2004; 310:1076–1083. [PubMed: 15105413]
 19. Hyodo F, Subramanian S, Devasahayam N, Murugesan R, Matsumoto K, Mitchell JB, Krishna MC. Evaluation of sub-microsecond recovery resonators for in vivo electron paramagnetic resonance imaging. *J Magn Reson*. 2008; 190:248–254. [PubMed: 18042414]
 20. Yamada K, Murugesan R, Devasahayam N, Cook JA, Mitchell JB, Subramanian S, Krishna MC. Evaluation and comparison of pulsed and continuous wave radiofrequency electron paramagnetic resonance techniques for in vivo detection and imaging of free radicals. *J Magn Reson*. 2002; 154:287–297. [PubMed: 11846586]
 21. Subramanian S, Devasahayam N, Murugesan R, Yamada K, Cook J, Taube A, Mitchell JB, Lohman JA, Krishna MC. Single-point (constant-time) imaging in radiofrequency Fourier transform electron paramagnetic resonance. *Magn Reson Med*. 2002; 48:370–379. [PubMed: 12210946]
 22. Matsumoto K, Chandrika B, Lohman JA, Mitchell JB, Krishna MC, Subramanian S. Application of continuous-wave EPR spectral-spatial image reconstruction techniques for in vivo oxymetry: comparison of projection reconstruction and constant-time modalities. *Magn Reson Med*. 2003; 50:865–874. [PubMed: 14523974]
 23. Moyer JH, Morris G, DeBaakey ME. Hypothermia: I. Effect on renal hemodynamics and on excretion of water and electrolytes in dog and man. *Ann Surg*. 1957; 145:26–40. [PubMed: 13395281]

FIG. 1.
 Structural formula of the Oxo63, tris[8-carboxy-2,2,6,6-tetrakis(2-hydroxymethyl)benzo[1,2-*d*4,5-*d'*]bis(1,3)dithio-4-yl)methyl radical.

FIG. 2.

Effect of body temperature control on pharmacokinetics of Oxo63 in the blood. (A) Schematic drawing of experimental set-up of DI method. (B) A comparison of the time course of blood concentration of the Oxo63 after a bolus injection ($0.75 \mu\text{mol/g b.w.}$) with or without body temperature control. The vertical axis of (B) is natural logarithmic value of μM . (C) A comparison of the time course of blood concentration of the Oxo63 during a continuous injection ($0.06 \mu\text{mol/g b.w./min}$ was followed a $0.75 \mu\text{mol/g b.w.}$ bolus injection) with or without body temperature control. Open and closed squares indicate values obtained from SCC tumor bearing mice with (TC) and without (NC) the body temperature control,

respectively. Gray circles indicate values obtained from normal healthy mice with controlling temperature, which data were referred from the previous report (8).

FIG. 3.

Effect of body temperature control on decay profiles of the EPR signal intensity of Oxo63 in several tissues measured by 700 MHz surface coil resonator. Decay profiles of the EPR signal intensity of Oxo63 in (A and B) the femoral muscle of healthy mice, (C and D) the normal femoral muscle of tumor bearing mice, and (E and F) the tumor tissue were shown. Left (A, C, and E) and right (B, D, and F) column indicate results with (TC) and without (NC) the body temperature control, respectively. The marks and error bars indicate average $\pm$ SD of 3–5 experiments. The numbers of animals used were given in Table 1.

FIG. 4.

Effect of body temperature control on whole body distribution of Oxo63 measured by 2D CW EPRI. Time course of EPR images were measured (A) with or (B) without the body temperature control. The letter H and F indicate head and feet, respectively. The letter R and L mean right and left, respectively. FOV was 6.4 cm. (A) The body temperature control maintained the normal urination, and the Oxo63 localized in the kidneys and the bladder can see clearly. (B) The distribution of Oxo63 became flat and stable when the body temperature was not controlled.

FIG. 5.

Comparison of pharmacokinetics of Oxo63 in tumor tissue planted in right thigh of a mouse and the contrastive normal thigh. Time course of 2D images was measured by 300 MHz TD EPR imager with or without the body temperature control. (A) Comparison of TD EPR images observed in TC and NC experiments. (B) Time course of TD EPR image intensity scanned under temperature control. (C) Time course of TD EPR image intensity scanned without temperature control. Markes in (B) and (C) indicate values from individual animal. TC and NC experiments used diferent animals.

Table 1

Time to reach maximum and the decay rates of EPR signal of Oxo63 in the normal and tumor tissues.

	Time to reach maximum (min)		Decay rate (min ⁻¹)	
	TC	NC	TC	NC
Muscle (Healthy animal)	5.7 ± 1.2 (4)	11.0 ± 5.9 (5)	0.018 ± 0.001	0.010 ± 0.007
Normal muscle (Tumor bearing animal)	4.1 ± 0.1 (3)	25.4 ± 5.1 (4) ^{**} , #	0.027 ± 0.012	0.003 ± 0.003 ^{*'}
Tumor tissue	6.6 ± 2.8 (4)	19.1 ± 11.2 (3)	0.028 ± 0.008	0.010 ± 0.003 ^{*', \$}

Values were indicated as average ± SD of numbers of experiments which was shown in the parenthesis.

^{*'} and ^{**} indicate significance between TC and NC as p < 0.01 and p < 0.001, respectively.

indicates significance as p < 0.05 compared with healthy muscle.

\$ indicates significance as p < 0.01 compared with normal tissue.

The decay rates were estimated from the time period from 35 min to 60 min.