Free Radical Research, August 2012; 46(8): 1004–1010

informa
healthcare

REVIEW ARTICLE

Imaging *in vivo* redox status in high spatial resolution with OMRI

KAZUHIRO ICHIKAWA[1] & KEIJI YASUKAWA[2]

[1]*Innovation Center for Medical Redox Navigation, Kyushu University, Fukuoka, Japan, and* [2]*Laboratory of Bio-function Science, Faculty of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan*

(Received date: 30 December 2011; Accepted date: 25 February 2012)

Abstract
Redox-reactions are playing a significant role in regulation of homeostasis of organism. Disorder of the redox-status is related with the onset and/or propagation of oxidative diseases such as lifestyle-related diseases, including cancers and cardiac diseases, etc. *In vivo* imaging of redox-status is thereby important in the analysis of mechanisms of oxidative diseases and developments of new medicines for the diseases. Aminoxyl radicals are redox-sensitive reporter molecules, which lose their paramagnetic moiety by reactions of free radicals or reducing compounds. Electron spin resonance (ESR) technique has been used to measure the molecules *in vivo*. *In vivo* spatial resolution in ESR imaging is in the range of a few millimeters and is not sufficient for the detailed diagnosis of disease models. Overhauser enhanced MRI (OMRI) is an emerging free radical imaging technique, which utilised electron–proton coupling to image the distribution of free radicals. *In vivo* imaging of redox-status is applicable with OMRI/aminoxyl radical technique. The detailed imaging analysis was demonstrated in oxidative diseases, such as tumour-bearing, neurodegeneration or gastric ulcer models. The OMRI/aminoxyl radical technique has a large potential as a diagnostic system for biomedical applications in the future.

Keywords: In vivo *imaging, electron spin resonance, Overhauser effect, aminoxyl radical, redox-status*

Abbreviations: *TEMPO, piperidine-N-oxyl; PROXYL, pyrrolidine-1-oxyl; carbamoyl-PROXYL, 3-carbamoyl-2,2,5,5-tetramethylpyrrolidine-1-oxyl; carboxy-PROXYL, 3-carboxy-2,2,5,5,-tetramethylpyrrolidine-1-oxyl; MC-PROXYL, 3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine-1-oxyl; TEMPOL, 4-hydroxy-2,2,6,6-tetra-methylpiperidine-N-oxyl; ESR, electron spin resonance; NMR, nuclear magnetic resonance; MRI, magnetic resonance imaging; OMRI, Overhauser enhanced MRI; NSAIDs, nonsteroidal antiinflammatory drugs; ROS, reactive oxygen species; 6-OHDA, 6-hydroxydopamine; FOV, field of view; ROI, region of interest; TR, repetition time; TE, echo time; TESR, ESR excitation time*

Introduction

Changes in *in vivo* redox-status are involved in processes of oxidative diseases. Excess production of reactive oxygen species (ROS) and decrease in endogenous antioxidants level are related to causes and development of diseases. The increase of oxidative marker such as lipid peroxidation and 8-hydroxydeoxyguanosine levels has been reported in oxidative diseases. ROS formation is related with the gastric ulcers induced by non-steroidal antiinflammatory drugs (NSAIDs) including indomethacin and aspirin [44]. The redox-status monitoring is thus important to diagnose redox-related diseases and to assess cure effects of medicines. *In vivo* imaging especially molecular imaging in animal is regarded as a promising methodology for investigation of the mechanisms of diseases, since non-invasive assessment of genetic expression is advantageous than *ex vivo* or *in vitro* examination such as measurement of oxidative end products or histological examination. In biological samples, specific free radicals would be measured directly, such as superoxide ions [34] or nitric oxide [39,50] by using spin trapping technique. However, the ROS level in cells is low and the technique is available for imaging to a limited degree.

Correspondence: Kazuhiro Ichikawa, Innovation Center for Medical Redox Navigation, Kyushu University, 3-1-1 Maidashi, Fukuoka 812-8582, Japan. Tel: +81-92-642-6134. Fax: +81-92-642-6024. E-mail: ichikawa.kazuhiro.684@m.phar.kyushu-u.ac.jp

ISSN 1071-5762 print/ISSN 1029-2470 online © 2012 Informa UK, Ltd.
DOI: 10.3109/10715762.2012.670874

Aminoxyl radical as redox reporter molecule

Group of aminoxyl radicals is reported to be sensitive to redox status of redox-related compounds. Most frequently used aminoxyl radicals *in vivo* are piperidine (TEMPO) or pyrrolidine (PROXYL) class. These reporter molecules are stable and less-toxic radicals, and thereby preferable for *in vivo* application [8]. The redox potentials of aminoxyl radicals are similar to that of redox enzymes [13]. The aminoxyl radicals are easily reduced to the corresponding hydroxylamine and are metabolised in the reaction with ROS [17]. In redox-related disease model, the metabolism rates of the molecule to non-paramagnetic forms are modified compared with that in control animals. Mechanisms of redox-related diseases were investigated *in vivo*, including cancer [30], diabetes [36], ischemia reperfusion injuries [47] and gastric ulcer [42] utilising the aminoxyl radicals as redox-status reporter molecules.

Structure of aminoxyl radicals used for measurement of *in vivo* redox status

Aminoxyl radicals can be modified at the substituents to have specific tissue localising capabilities or high reactivities to specific radicals, and kinds of aminoxyl radical have been synthesized. Typical aminoxyl radicals used in *in vivo* redox researches are shown in Table I. Most of TEMPO and PROXYL derivatives do not pass through the blood–brain barrier. Methoxycarbonyl-PROXYL (MC-PROXYL) is one of the aminoxyl radicals, which distribute to brain region for investigation of redox-status and free radical reactions in brain diseases [35]. MC-PROXYL is not hydrolysed with any esterase, which may enhance its retention in brain, and is gradually lost from the brain tissue. Series of tetraethyl-substituted aminoxyl radicals have high stability towards reduction by ascorbic acid, since Gibbs energy is positive between them [15]. Further, the tetraethyl-substituted aminoxyl radicals could inhibit lipid peroxidation.

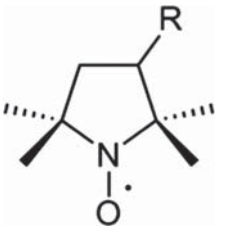
ESR technique for detection of *in vivo* redox-status

Since the aminoxyl reporter molecules lose its paramagnetic moiety during the redox-reaction process, the process can be monitored with magnetic resonance technique. Electron spin resonance (ESR) is a direct analytical technique for measurement of free radicals. ESR spectroscopy has been employed in *in vitro* redox researches [3,7]. For the detection of the aminoxyl radicals in animals, low field ESR technique was developed for *in vivo* application. Low frequency ESR technique combined with aminoxyl spin probe (ESR/spin probe technique) has been applied to measure redox status [8,12,20–22,40] or free radical generation in *in vivo* disease models [42,49]. Paramagnetic reporter molecules for other biomedical application, such as triarylmethyl radicals used for tumour oxymetry, have narrow ESR linewidth in the range of hundreds milligauss, and both continuous wave and pulsed approaches have been applied for the measurements [5,19,45]. Since the linewidths of aminoxyl radicals are typically 1 gauss, which correspond to electron relaxation times less than micro seconds, ESR pulsed experiments for the aminoxyl radical are quite difficult in terms of its instrumentation for redox imaging research in animals. Continuous wave measurement was thereby frequently used in redox-status experiments and the spatial resolution of ESRI is usually in the range of millimeter level [9]. Changes in redox-status using ESR are detected from successive monitoring of the ESR signal of the aminoxyl radical *in vivo* and calculating the decreasing rate of this signal due to the metabolism of the paramagnetic molecule to non-paramagnetic species. Enhancements of signal decays of aminoxyl radicals at regions of the diseases were significantly related with degrees of oxidative stress.

Application of ESR/aminoxyl probe technique in disease models

Redox-related compounds including ascorbate, glutathione and superoxide participate in redox

Table I. Structure of aminoxyl radicals used for measurement of *in vivo* redox-state.

Basic structure	R	Abbreviation	Po/w
	—$CONH_2$	Carbamoyl PROXYL	0.68
	—COOH	Carboxy PROXYL	0.01
	—$COOCH_3$	MC PROXYL	8.7
	—$COOCH_2OCOCH_3$	AMC PROXYL	4.1

reactions [17,38,43,46]. Aminoxyl radical can be an acceptor or source of electron and receives an electron to form corresponding hydroxylamine and gives an electron to form corresponding oxo-ammonium ion [16]. In biological conditions, ESR signal of aminoxyl radical decays mainly by reduction to hydroxylamine [2,6,38]. This aminoxyl metabolism is attenuated by redox-status, ROS generations, amounts of antioxidants, oxygen concentration, etc. ROS generation enhanced aminoxyl radical metabolism, whereas administration of antioxidants caused slower rate of aminoxyl metabolism. The redox-status or free radical dependent metabolism of aminoxyl radical has been reported in disease models.

OMRI technique for detection of free radical

Overhauser enhanced MRI (OMRI, Figure 1) is a technique for imaging free radicals based on the Overhauser effect [24]. The detailed physics of OMRI was described elsewhere [1,32]. In brief, in the presence of free radicals, proton and unpaired electron in the free radical molecules coupled to each other to form multiple spin states of both spins. By excitation of the electron spin, the large magnetic moment of electron is utilised to enhance the nuclear magnetic resonance (NMR) signal of proton by affecting the relative populations of the spin states. Thus, the free radical information can be monitored through observation of changes in proton NMR signal intensity. The spatial resolution in OMRI imaging is in principle as same as that in MRI.

Advantage of OMRI technique in redox-status imaging

Continuous wave ESRI can image unique spectroscopic information of the aminoxyl radical, which reflects molecular circumstances including viscosity, the spatial resolution of *in vivo* ESRI for redox-status is less than that of OMRI due to the large ESR signal linewidth [9]. Figure 2 represents the differences in spatial resolution of aminoxyl radicals in mouse lung with ESRI and OMRI. After intratrachea administration of carboxy PROXYL to two mice, either ESR or OMR image was obtained. Note that in case of the ESR experiment, the aminoxyl radical was put into the right lung lobe in this specific mouse due to the experimental procedure and that the image was blurred due to the absence of high frequency information in continuous wave in ESR image [27]. MRI image of the mouse was obtained independently with 0.2-T MRI and superimposed onto the ESR image. In both cases, aminoxyl radical localisation was clearly imaged in mouse lung region. However, the spatial resolution of the images was different between ESRI and OMRI and was estimated 2 and 0.3 mm for ESRI and OMRI, respectively. OMRI technique has, thereby, advantages over ESR approaches in terms of *in vivo* redox-status imaging in high spatial resolution. We have also developed molecular imaging and simultaneous assessment of redox processes by using OMRI with ^{14}N- and ^{15}N-labelled nitroxyl probes with different distribution properties [41]. This OMRI technique with dual probes may become a powerful tool to clarify mechanisms of disease and to monitor pharmaceutical therapy [10].

Moreover, the OMRI technique is applicable for various functional imaging *in vivo*. Since during electron–proton coupling process, the information of electron absorption is transferred to proton as the changes in the proton NMR intensity, electron absorption spectra are reconstructed in multiple OMRI images. Reporter radical molecules are synthesized for functional imaging such as redox status

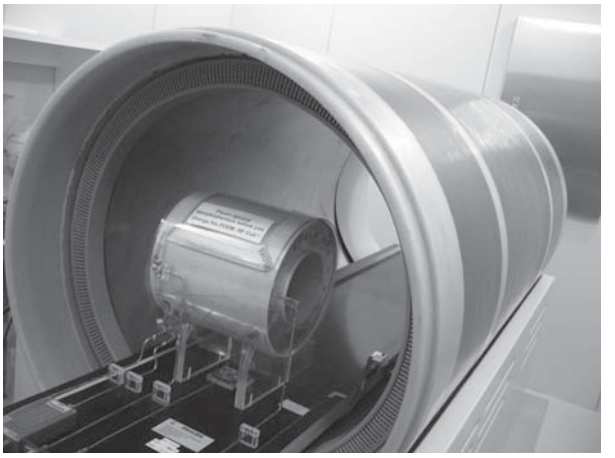


Figure 1. Photograph of an OMRI scanner including the main magnet and OMRI composite-resonator.

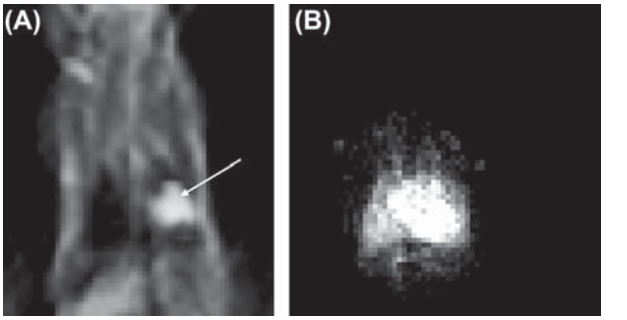


Figure 2. Image comparison between *in vivo* ESRI and OMRI in mouse lung. (A) ESR image superimposed on MRI image. The arrow indicated the location of aminoxyl probe (B) OMR image in the lung region. After intratrachea administration of 100 μL of 2 mM carboxy PROXYL solution to mice either ESR or OMR image were obtained. ESR scan parameters: microwave frequency, 1.01 GHz; microwave power 1 mW; Projections, 18; scan width, 5 Gauss. OMRI scan parameters: FOV, 48 × 48; matrix, 128 × 128; slice thickness, 30 mm; TR/TE/TESR, 1200/25/600 ms.

[4,23,28,37,48], partial oxygen pressure [18,19], pH [14], etc., based on the spectroscopic changes due to the corresponding physiological changes. By choosing appropriate free radical molecule, the OMRI technique can be applied to various functional imaging.

OMRI instrumentation for better image quality

The electron excitation should be carried out at low magnetic field, that is, 5–20 mT to ensure good microwave penetration into animal. Field cycling technique is used in OMRI to enhance NMR sensitivity [25,33]. In field cycling technique, the magnetic field strength is dynamically switched between low and high magnetic field for ESR irradiation and MR detection, respectively. MR detection field of up to 0.45 T was reported for field cycling OMRI [26], though huge electric current deposition was needed to achieve this high MR detection field strength.

To improve the OMRI enhancement at the low ESR field, we have developed a surface-coil type ESR irradiation coil [29], which is a device widely used in MR research to enhance sensitivity in limited field of view (FOV). The introduction of ESR surface-coil enhanced B1 generation capability in the region of surface-coil compared with volume-coil type ESR irradiation coil and thereby improved the signal to noise ratio of OMRI images at the region 5–10 times higher.

Gastric ulcer examined using OMRI/aminoxyl probe technique

NSAIDs are a widely used in rheumatoid arthritis to reduce pain and swelling of arthritis. However, it has been reported NSAID-treated rheumatoid arthritis patients suffered from gastrointestinal injuries. Animal experiments supported the clinical data. *In vivo* redox-status was imaged for adjuvant arthritis animal, using aminoxyl radicals [4]. The gastric lesion formation with indomethacin administration was severer in adjuvant arthritis rats than that in control rats as reported previously. Administration of the aminoxyl radicals suppressed the gastric mucosal damages induced by indomethacin administration in adjuvant arthritis rats in a dose-dependent manner. OMRI images demonstrated that aminoxyl radical disappeared faster in the indomethacin-treated adjuvant arthritis rats than in the indomethacin-treated control rats (Figure 3). The aminoxyl radical metabolism agreed with the degrees of suppression of gastric injuries by indomethacin treatment, supporting that enhanced aminoxyl metabolism monitored with OMRI represents the key events in gastric injuries in indomethacin-treated adjuvant arthritis rats.

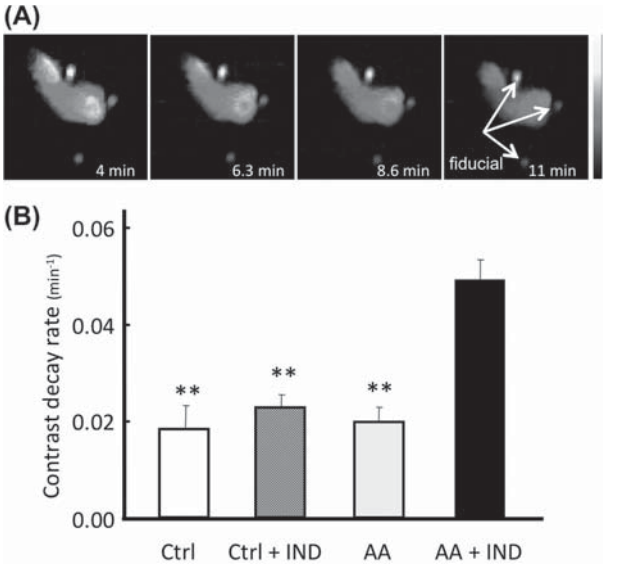


Figure 3. Redox-status changes in rat gastric injury model. (A) Sequential OMR images obtained after intragastrical administration of aminoxyl radical to rats. (B) The contrast decay rate at the ROI. After administration of 2 mM TEMPOL, OMRI measurement was carried out. OMRI scan parameters: FOV, 64×64; matrix, 128×128; slice thickness, 100 mm; TR/TE/TESR, 1000/70/400 ms. Each value represents the mean $\pm$ SE of 3–6 rats. $^{**}p < 0.01$ vs. control group. Ctrl: Control, IND: Indomethacin, AA: Adjuvant Arthritis.

Neurodegeneration model examined using OMRI/aminoxyl probe technique

Oxidative stress was involved in the dopaminergic neurodegeneration models [31,48]. After treatment with 6-hydroxydopamine (6-OHDA), redox-status was imaged with OMRI using an aminoxyl radical in rat brain using a blood–brain-barrier permeable aminoxyl radical [48]. The *in vivo* aminoxyl metabolism rate decreased significantly in lesioned hemispheres compared to their corresponding contralateral hemispheres of the rat brain. To elucidate the source of the decreased metabolism, we fractionated lesioned and corresponding contralateral hemispheres of the rat brain to obtain mitochondrial and cytoplasmic fractions separately. When the aminoxyl molecule was mixed with the mitochondrial fraction, the metabolism of the aminoxyl molecule was slower with the sample from the lesioned hemispheres than that of the corresponding contralateral hemispheres. There was no difference in the metabolism of the molecule after mixing with the cytoplasm fractions from lesioned or corresponding hemispheres. The data was

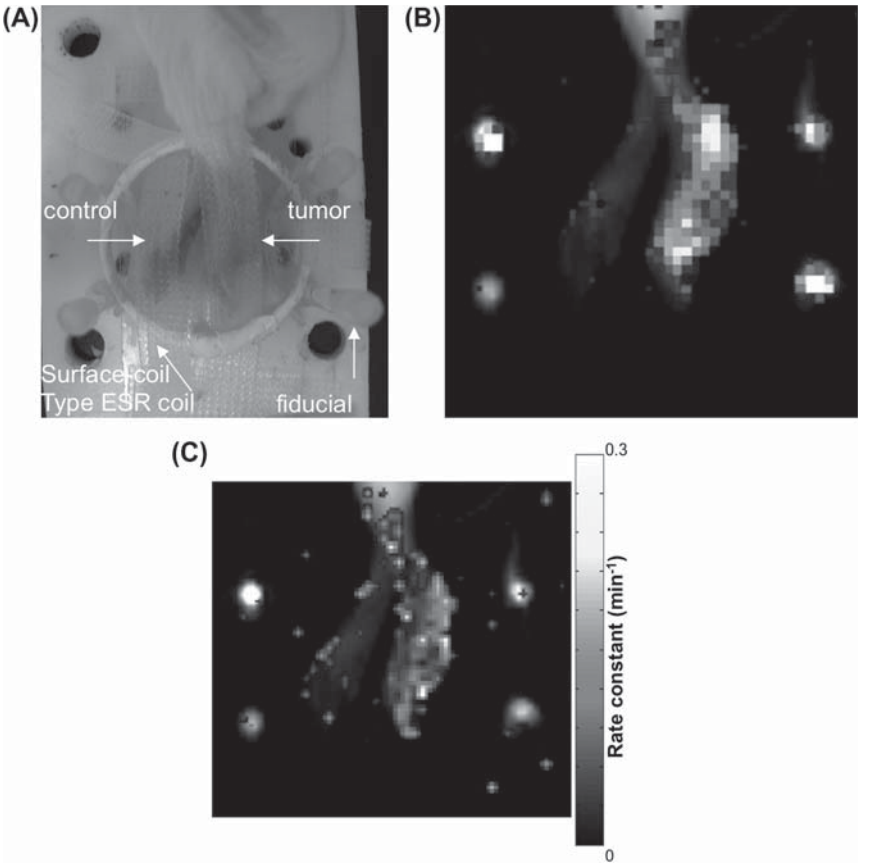


Figure 4. OMRI image obtained using carbamoyl- PROXYL as redox-molecular probe. (A) A photograph of tumour region of the animal 6 days after inoculation of colon carcinoma in hind footpad. Surface-coil type ESR coil was used to enhance OMRI sensitivity in the region. (B) Aminoxyl radical distribution in the region. (C) Aminoxyl radical metabolism rate. For this specific mouse, the metabolic rates were 0.057 and 0.14 for control and tumour regions respectively.

supported by the decreased activity of mitochondrial complex I in lesioned hemispheres.

Tumour model examined using OMRI/ aminoxyl probe technique

Tumour tissue is reported to be in reducing circumstances [11,21] and the imaging of *in vivo* redox-status in tumour is considered as a potential marker for tumour diagnosis. Tumour bearing mouse model was prepared by inoculation of NL-17 colon carcinoma into the hind footpad of Balb/c female mouse. An aminoxyl radical was administered intravenously to the tumour-bearing mouse and OMRI scan was carried out. Anatomical information was also obtained with MRI to superimpose the OMRI and MRI images. The image intensities of OMR images increased according to tumour growth in the footpad of the mouse, due to the increased distribution volume of the aminoxyl molecule in the tumour tissue (Figure 4). The redox-status image in the tumour showed the aminoxyl metabolism was faster in the tumour area in the footpad. Histological observation of the tissue supported the faster metabolism of aminoxyl molecule in the tumour region.

Conclusion

The OMRI technique can be applied to *in vivo* experiments to investigate the redox-status and mechanism of oxidative injury in high spatial resolution. This method has a large potential as a new diagnostic system for applications from biomedical to clinical applications in the future.

Declaration of interest

The data in this manuscript was obtained in part supported by Development of Systems and Technology for Advanced Measurement and Analysis of the Japan Science and Technology Agency; and a Grant-in-Aid for Scientific Research (B) from the Japan Society for the Promotion of Science (JSPS). Support was also given by the JSPS Core-to-Core Program and "Formulation of Advanced Collaborative Medical Innovation Center" as Special Coordination Funds for Promoting Science and Technology,

Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

[1] Ardenkjaer-Larsen JH, Laursen I, Leunbach I, Ehnholm G, Wistrand LG, Petersson JS, et al. EPR and DNP properties of certain novel single electron contrast agents intended for oximetric imaging. J Magn Reson 1998;133:1–12.

[2] Belkin S, Mehlhorn RJ, Hideg K, Hankovsky O, Packer L. Reduction and destruction rates of nitroxide spin probes. Arch Biochem Biophys 1987;256:232–243.

[3] Chen KY, McLaughlin MG. Differences in the reduction kinetics of incorporated spin labels in undifferentiated and differentiated mouse neuroblastoma cells. Biochim Biophys Acta 1985;845:189–195.

[4] Deguchi H, Yasukawa K, Yamasaki T, Mito F, Kinoshita Y, Naganuma T, et al. Nitroxides prevent exacerbation of indomethacin-induced gastric damage in adjuvant arthritis rats. Free Radic Biol Med 2011;51:1799–1805.

[5] Elas M, Williams BB, Parasca A, Mailer C, Pelizzari CA, Lewis MA, et al. Quantitative tumor oxymetric images from 4D electron paramagnetic resonance imaging (EPRI): methodology and comparison with blood oxygen level-dependent (BOLD) MRI. Magn Reson Med 2003;49: 682–691.

[6] Gallez B, Bacic G, Goda F, Jiang J, O'Hara JA, Dunn JF, et al. Use of nitroxides for assessing perfusion, oxygenation, and viability of tissues: in vivo EPR and MRI studies. Magn Reson Med 1996;35:97–106.

[7] Halpern HJ, Jaffe DR, Nguyen TD, Haraf DJ, Spencer DP, Bowman MK, et al. Measurement of bioreduction rates of cells with distinct responses to ionizing radiation and cisplatin. Biochim Biophys Acta 1991;1093:121–124.

[8] Halpern HJ, Peric M, Yu C, Barth ED, Chandramouli GV, Makinen MW, et al. In vivo spin-label murine pharmacodynamics using low-frequency electron paramagnetic resonance imaging. Biophys J 1996;71:403–409.

[9] Hyodo F, Yasukawa K, Yamada K, Utsumi H. Spatially resolved time-course studies of free radical reactions with an EPRI/MRI fusion technique. Magn Reson Med 2006; 56:938–943.

[10] Hyodo F, Murugesan R, Matsumoto K, Hyodo E, Subramanian S, Mitchell JB, et al. Monitoring redox-sensitive paramagnetic contrast agent by EPRI, OMRI and MRI. J Magn Reson 2008;190:105–112.

[11] Ichikawa K, Sakabe E, Kuninobu K, Yamori T, Tsuruo T, Yao T, et al. Application of in vivo ESR/spin-probe technique to monitor tumor in vivo in mouse footpad. Antioxid Redox Signal 2007;9:1699–1707.

[12] Ilangovan G, Li H, Zweier JL, Krishna MC, Mitchell JB, Kuppusamy P, et al. In vivo measurement of regional oxygenation and imaging of redox status in RIF-1 murine tumor: effect of carbogen-breathing. Magn Reson Med 2002;48:723–730.

[13] Kato Y, Shimizu Y, Lin YJ, Unoura K, Utsumi H, Ogata T, et al. Reversible half-wave potentials of reduction processes on nitroxide radicals. Electrochim Acta 1995;40:2799–2802.

[14] Khramtsov VV, Caia GL, Shet K, Kesselring E, Petryakov S, Zweier JL, et al. Variable field proton–electron double-resonance imaging: application to pH mapping of aqueous samples. J Magn Reson 2010;202:267–273.

[15] Kinoshita Y, Yamada K, Yamasaki T, Mito F, Yamato M, Kosem N, et al. In vivo evaluation of novel nitroxyl radicals with reduction stability. Free Radic Biol Med 2010;49: 1703–1709.

[16] Krishna MC, Grahame DA, Samuni A, Mitchell JB, Russo A. Oxoammonium cation intermediate in the nitroxide-catalyzed dismutation of superoxide. Proc Natl Acad Sci USA 1992;89:5537–5541.

[17] Krishna MC, Russo A, Mitchell JB, Goldstein S, Dafni H, Samuni A, et al. Do nitroxide antioxidants act as scavengers of O_2^-. or as SOD mimics? J Biol Chem 1996;271: 26026–26031.

[18] Krishna MC, Subramanian S, Kuppusamy P, Mitchell JB. Magnetic resonance imaging for in vivo assessment of tissue oxygen concentration. Semin Radiat Oncol 2001; 11:58–69.

[19] Krishna MC, English S, Yamada K, Yoo J, Murugesan R, Devasahayam N, et al. Overhauser enhanced magnetic resonance imaging for tumor oximetry: coregistration of tumor anatomy and tissue oxygen concentration. Proc Natl Acad Sci USA 2002;99:2216–2221.

[20] Kuppusamy P, Afeworki M, Shankar RA, Coffin D, Krishna MC, Hahn SM, et al. In vivo electron paramagnetic resonance imaging of tumor heterogeneity and oxygenation in a murine model. Cancer Res 1998;58:1562–1568.

[21] Kuppusamy P, Li H, Ilangovan G, Cardounel AJ, Zweier JL, Yamada K, et al. Noninvasive imaging of tumor redox status and its modification by tissue glutathione levels. Cancer Res 2002;62:307–312.

[22] Leonard SS, Mowrey K, Pack D, Shi X, Castranova V, Kuppusamy P, et al. In vivo bioassays of acute asbestosis and its correlation with ESR spectroscopy and imaging in redox status. Mol Cell Biochem 2002;234–235:369–377.

[23] Li H, He G, Deng Y, Kuppusamy P, Zweier JL. In vivo proton electron double resonance imaging of the distribution and clearance of nitroxide radicals in mice. Magn Reson Med 2006;55:669–675.

[24] Lurie DJ, Bussell DM, Bell LH, Mallard JR. Proton–electron double magnetic resonance imaging of free radical solutions. J Magn Reson 1988;76:366–370.

[25] Lurie DJ, Li H, Petryakov S, Zweier JL. Development of a PEDRI free-radical imager using a 0.38 T clinical MRI system. Magn Reson Med 2002;47:181–186.

[26] Lurie DJ, Davies GR, Foster MA, Hutchison JM. Field-cycled PEDRI imaging of free radicals with detection at 450 mT. Magn Reson Imaging 2005;23:175–181.

[27] Matsumoto K, Subramanian S, Murugesan R, Mitchell JB, Krishna MC. Spatially resolved biologic information from in vivo EPRI, OMRI, and MRI. Antioxid Redox Signal 2007;9:1125–1141.

[28] Matsumoto K, Hyodo F, Anzai K, Utsumi H, Mitchell JB, Krishna MC, et al. Brain redox imaging. Methods Mol Biol 2011;711:397–419.

[29] Matsumoto S, Yamada K, Hirata H, Yasukawa K, Hyodo F, Ichikawa K, et al. Advantageous application of a surface coil to EPR irradiation in overhauser-enhanced MRI. Magn Reson Med 2007;57:806–811.

[30] Mikuni T, He G, Petryakov S, Fallouh MM, Deng Y, Ishihara R, et al. In vivo detection of gastric cancer in rats by electron paramagnetic resonance imaging. Cancer Res 2004;64:6495–6502.

[31] Nakamura K, Bindokas VP, Kowlessur D, Elas M, Milstien S, Marks JD, et al. Tetrahydrobiopterin scavenges superoxide in dopaminergic neurons. J Biol Chem 2001;276: 34402–34407.

[32] Overhauser AW. Polarization of nuclei in metals. Phys Rev 1953;92:411–415.

[33] Overweg J. A prototype field-cycling Overhauser MRI scanner. ISMRM 1999;8:2212.

[34] Rosen GM, Finkelstein E, Rauckman EJ. A method for the detection of superoxide in biological systems. Arch Biochem Biophys 1982;215:367–378.

[35] Sano H, Naruse M, Matsumoto K, Oi T, Utsumi H. A new nitroxyl-probe with high retention in the brain and its application for brain imaging. Free Radic Biol Med 2000; 28:959–969.

[36] Sano T, Umeda F, Hashimoto T, Nawata H, Utsumi H. Oxidative stress measurement by in vivo electron spin resonance spectroscopy in rats with streptozotocin-induced diabetes. Diabetologia 1998;41:1355–1360.

[37] Shiba T, Yamato M, Kudo W, Watanabe T, Utsumi H, Yamada K, et al. In vivo imaging of mitochondrial function in methamphetamine-treated rats. NeuroImage 2011;57: 866–872.

[38] Swartz HM, Sentjurc M, Morse PD 2nd. Cellular metabolism of water-soluble nitroxides: effect on rate of reduction of cell/nitroxide ratio, oxygen concentrations and permeability of nitroxides. Biochim Biophys Acta 1986;888: 82–90.

[39] Tsai P, Porasuphatana S, Halpern HJ, Barth ED, Rosen GM. In vivo in situ detection of nitric oxide using low-frequency EPR spectroscopy. Methods Mol Biol 2002;196: 227–237.

[40] Ueda A, Nagase S, Yokoyama H, Tada M, Ohya H, Kamada H, et al. Identification by an EPR technique of decreased mitochondrial reducing activity in puromycin aminonucleoside-induced nephrosis. Free Radic Biol Med 2002;33:1082–1088.

[41] Utsumi H, Yamada K, Ichikawa K, Sakai K, Kinoshita Y, Matsumoto S, et al. Simultaneous molecular imaging of redox reactions monitored by Overhauser-enhanced MRI with ^{14}N- and ^{15}N-labeled nitroxyl radicals. Proc Natl Acad Sci USA 2006;103:1463–1468.

[42] Utsumi H, Yasukawa K, Soeda T, Yamada K, Shigemi R, Yao T, et al. Noninvasive mapping of reactive oxygen species by in vivo electron spin resonance spectroscopy in indomethacin-induced gastric ulcers in rats. J Pharmacol Exp Ther 2006; 317:228–235.

This paper was first published online on Early Online on 21 March 2012.

[43] Voest EE, van Faassen E, Marx JJ. An electron paramagnetic resonance study of the antioxidant properties of the nitroxide free radical TEMPO. Free Radic Biol Med 1993;15: 589–595.

[44] Wallace JL, Arfors KE, McKnight GW. A monoclonal antibody against the CD18 leukocyte adhesion molecule prevents indomethacin-induced gastric damage in the rabbit. Gastroenterology 1991;100:878–883.

[45] Williams BB, al Hallaq H, Chandramouli GV, Barth ED, Rivers JN, Lewis M, et al. Imaging spin probe distribution in the tumor of a living mouse with 250 MHz EPR: correlation with BOLD MRI. Magn Reson Med 2002;47:634–638.

[46] Xavier S, Yamada K, Samuni AM, Samuni A, DeGraff W, Krishna MC, et al. Differential protection by nitroxides and hydroxylamines to radiation-induced and metal ion-catalyzed oxidative damage. Biochim Biophys Acta 2002; 1573:109–120.

[47] Yamato M, Shiba T, Yamada K, Watanabe T, Utsumi H. Noninvasive assessment of the brain redox status after transient middle cerebral artery occlusion using Overhauser-enhanced magnetic resonance imaging. J Cereb Blood Flow Metab 2009;29:1655–1664.

[48] Yamato M, Shiba T, Naganuma T, Ichikawa K, Utsumi H, Yamada K, et al. Overhauser-enhanced magnetic resonance imaging characterization of mitochondria functional changes in the 6-hydroxydopamine rat model. Neurochem Int 2011; 59:804–811.

[49] Yokoyama H, Itoh O, Aoyama M, Obara H, Ohya H, Kamada H, et al. In vivo EPR imaging by using an acyl-protected hydroxylamine to analyze intracerebral oxidative stress in rats after epileptic seizures. Magn Reson Imaging 2000;18:875–879.

[50] Yoshimura T, Yokoyama H, Fujii S, Takayama F, Oikawa K, Kamada H, et al. In vivo EPR detection and imaging of endogenous nitric oxide in lipopolysaccharide-treated mice. Nat Biotechnol 1996;14(8):992–994.