FORUM EDITORIAL

In Vivo Electron Paramagnetic Resonance: Radical Concepts for Translation to the Clinical Setting

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Abstract

Electron paramagnetic resonance (EPR)-based spectroscopic and imaging techniques allow for the study of free radicals—molecules with one or more unpaired electrons. Biological EPR applications include detection of endogenous biologically relevant free radicals as well as use of specially designed exogenous radicals to probe local microenvironments. This Forum focuses on recent advances in the field of *in vivo* EPR applications discussed at the International Conference on Electron Paramagnetic Resonance Spectroscopy and Imaging of Biological Systems (EPR-2017). Although direct EPR detection of endogenous free radicals such as reactive oxygen species (ROS) *in vivo* remains unlikely in most cases, alternative approaches based on applications of advanced spin traps and probes for detection of paramagnetic products of ROS reactions often allow for specific assessment of free radical production in living subjects. In recent decades, significant progress has been achieved in the development and *in vivo* application of specially designed paramagnetic probes as “molecular spies” to assess and map physiologically relevant functional information such as tissue oxygenation, redox status, pH, and concentrations of interstitial inorganic phosphate and intracellular glutathione. Recent progress in clinical EPR instrumentation and development of biocompatible paramagnetic probes for *in vivo* multifunctional tissue profiling will eventually make translation of the EPR techniques into clinical settings possible. *Antioxid. Redox Signal.* 28, 1341–1344.

Keywords: electron paramagnetic resonance, spin trap, spin probe, electron paramagnetic resonance imaging, proton–electron double-resonance imaging, reactive oxygen species

THE INTERNATIONAL CONFERENCE on Electron Paramagnetic Resonance Spectroscopy and Imaging of Biological Systems (EPR-2017) was held from July 16 to July 22, 2017, at Lakeview Resort Conference Center in Morgantown, West Virginia. This was a combined meeting of the “16th *In Vivo* EPR Spectroscopy and Imaging” and the “13th Spin Trapping/Spin Labeling” conferences organized and hosted by the *In vivo* Multifunctional Magnetic Resonance Center, Health Sciences Center, and Department of Biochemistry, School of Medicine, West Virginia University. Nearly 150 participants from 12 countries presented recent innovations, developments, and applications of electron paramagnetic resonance (EPR)-related technologies to study the biological processes related to human health. These included instrumentation, imaging and coimaging techniques, spin trapping and spin labeling, and *in vivo* applications in the

preclinical and clinical settings. This Forum contains seven review articles prepared by conference speakers with a focus on *in vivo* detection of endogenous free radicals and application of specially designed exogenous radical probes. The subjects of these reviews are tightly interconnected and complementary as illustrated in Figure 1.

An EPR-based spectroscopic and imaging technique is a methodology that allows for direct detection of free radicals—molecules with one or more unpaired electrons. Numerous studies have recognized important roles of free radicals both in normal physiology and in pathophysiology of many diseases. Accordingly, by its nature, EPR technology is a primary method of choice for quantitative assessment and mapping of free radicals in biological systems. However, most of biologically relevant free radicals such as superoxide, $O_2^{\bullet -}$, hydroxyl, $\bullet OH$, nitric oxide, $NO^{\bullet}$, alkyl, $R^{\bullet}$, peroxy,

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FIG. 1. *In vivo* EPR and spin trapping in biology and medicine. Biologically relevant endogenous free radicals ($R^\bullet$) are short-lived species and in most cases they are not directly detected. Use of STs (2) or spin probes such as HAs (1) allows for indirect assessment of $R^\bullet$ by EPR detection of their paramagnetic adducts, $ST-R^\bullet$ or nitroxide products, $NR^\bullet$, correspondingly. Understanding free radical generation by redox-active compounds may guide rational design of effective anticancer drugs (7). The spin adducts of the most popular nitron STs further decay into diamagnetic products, $ST-R$ s. In particular case of DMPO spin trap EPR-silent DMPO-R product can be detected by IST MRI, allowing for *in vivo* mapping of the site of free radical generation (8). Stable nitroxyl, $NR^\bullet$, and trityl (Trityl $^\bullet$) radicals are widely used as functional EPR probes to assess and map the physiologically important parameters of the tissue microenvironment in living objects by CW (3) and pulsed EPR techniques (3, 5) as well as using MRI-based PEDRI (4). The numbers inside the arrows indicate the references on the corresponding Forum reviews. CW, continuous wave; DMPO, 5,5-dimethyl-1-pyrroline N-oxide; EPR, electron paramagnetic resonance; HAs, hydroxylamines; IST, immuno-spin trapping; MRI, magnetic resonance imaging; $NR^\bullet$, nitroxide radical; PEDRI, proton–electron double-resonance imaging; STs, spin traps.

$ROO^\bullet$, and thiyl, $RS^\bullet$ radicals represent so-called reactive oxygen species (ROS), reactive nitrogen species, and reactive sulfur species that, with a few exceptions, possess extraordinary low lifetimes ($\leq ns$) having concentrations in living tissues ($\leq nM$) below EPR detection limits. The other obstacle for detection of these radicals is their short relaxation times in body liquids making their line width too broad and EPR signal invisible. EPR spin trapping (ST EPR) overcomes these obstacles in the detection of short-lived radicals by introducing a compound, a spin trap, that captures short-lived radicals and converts them into more stable paramagnetic adducts. ST EPR is considered one of the gold standard methods for detection of various radical species, each producing a spin adduct with very characteristic spectral features. In this Forum, Hardy *et al.* (2) overview recent advances in chemical characterization of the specific adducts formed in the reactions with various physiologically relevant radicals using ST EPR as well as other techniques such as fluorescent probes, high-performance liquid chromatography, and liquid chromatography–mass spectrometry. The establishment of species-specific products can be used for specific detection of free radicals in cell-free systems and cultured cells *in vitro*, and in animals *in vivo*.

Among various spin trap structures, nitron compounds have been shown to be the most efficient for ST EPR detection of short-lived radicals. However, the corresponding nitroxide radical ($NR^\bullet$) adducts being converted into diamagnetic EPR-silent products due to biological reductive and/or oxidative processes are still not sufficiently stable for

in vivo EPR detection. The development of an immuno-spin trapping (IST) assay (6) for macromolecular radical detection using the specific free radical reactivity of nitron spin trap, 5,5-dimethyl-1-pyrroline N-oxide (DMPO), in conjunction with DMPO-antibody sensitivity and specificity, greatly expanded the utility of the spin trapping technique. In this Forum, Towner and Smith (8) describe an extension of the IST for an *in vivo* application that combines IST with magnetic resonance imaging (IST MRI). The advantage of this MRI-based approach is that it allows for *in vivo* mapping of heterogeneous distribution of trapped free radicals to pinpoint where radicals are formed in different tissues. The authors overview IST MRI applications to image free radicals in various animal disease models, including diabetes, amyotrophic lateral sclerosis, gliomas, and septic encephalopathy.

The main bottlenecks for the application of ST EPR using nitron spin traps are the reduction of the corresponding paramagnetic adducts to EPR-silent hydroxylamines (HAs) by endogenous reducing agents, and a low rate constant for the trapping of superoxide radical. Alternative to this approach, cyclic HAs have proven to be extremely effective probes for use in tissues and cultured cells for detection of free radicals: they readily react with short-lived ROS to produce stable NRs, which can be quantitatively measured by EPR. The advantages of HAs application include relatively high rate constants for the oxidation by superoxide (10^3 – $10^4 M^{-1} s^{-1}$) and stability of the nitroxide adducts. In contrast, a major disadvantage is the nonspecific nature of HA oxidation that does not allow for discrimination between oxidizing agents

based on the EPR spectra of the same radical product. In this Forum, Dikalov *et al.* (1) critically review recent applications of various cyclic HA probes as well as acyl-protected cyclic HA probes to study oxidative stress, both *in vitro* and *in vivo*, as well as their advantages and limitations.

The applications of EPR in biological systems are not only limited to the detection of endogenous free radicals but also rely on using exogenous paramagnetic probes as “molecular spies” to obtain physiologically relevant, functional information such as tissue perfusion, oxygenation, redox status, pH, and concentrations of interstitial inorganic phosphate and intracellular glutathione. Specially designed NRs and trityl radicals represent two major classes of soluble paramagnetic probes used for *in vivo* functional EPR measurements. Recent advances in the development of functional paramagnetic probes and their *in vivo* application for molecular EPR-based spectroscopy and imaging of tumor microenvironment and redox are reviewed by Khramtsov (3). In particular, application of recently developed multifunctional nitroxide and trityl probes provides unsurpassed opportunity for *in vivo* concurrent measurements of several tissue microenvironmental parameters for preclinical studies. The measurement of several parameters using a single probe allows for their correlation analyses independent of probe distribution and time of measurements.

Spectral spatial EPR imaging (EPRI) is capable of providing spatially resolved functional information using exogenous paramagnetic probes. Similar to MRI, the spatial distribution of the probe can be measured using magnetic field gradients and, from its spectral features and time evolution, spatial maps of oxygen, pH, and redox can be obtained in live objects (3, 5). Continuous wave EPRI remains the preferred method for signal detection of spin probes with relatively large line widths such as most of the NRs. Recent progress in the synthesis of trityl radical probes with narrow EPR line width and long relaxation time makes pulsed EPRI a useful technique in preclinical research with potential for clinical translation. In this Forum, specific applications of pulsed EPRI oximetry for the study of tumor physiology are discussed by Kishimoto *et al.* (5).

EPRI lacks anatomical localization of different organs on EPR images and has low spatiotemporal resolution compared with MRI. A lack of anatomical resolution can be overcome by combining anatomic MRI scans and functional EPRI of pO_2 (5) or pH (3). Proton–electron double-resonance imaging (PEDRI), also termed Overhauser-enhanced MRI, represents an alternative nuclear magnetic resonance (NMR)-based approach for imaging of paramagnetic probes based on the enhancement of the proton MRI signal after EPR irradiation. It inherently offers high spatial resolution, plane selectivity, and rapid image data collection, directly providing information on the object anatomy and indirectly on EPR signal of the paramagnetic probe, therefore allowing for complementary functional mapping. Also in this Forum, Kishimoto *et al.* (4) review *in vivo* PEDRI applications that provide information about radical probe distribution and the local microenvironment such as oxygen (pO_2), tissue permeability, redox status, and acid–base balance (pH). Functional spatially resolved information obtained from PEDRI provides insight into the pathology and etiology of various human diseases in preclinical studies using animal disease models.

It is well recognized that anticancer effects of a number of chemotherapeutic drugs and photodynamic therapy (PDT)

approaches are based on their redox activity and ROS generation. Among them some of the substituted quinones, in particular anthracycline antibiotics such as doxorubicin, daunomycin, and mitomycin C, are widely used in chemotherapy but possess high cardiotoxicity. Based on detailed EPR and NMR studies of ROS-mediated mechanisms of quinone action, Polyakov *et al.* (7) have proposed a new type of quinone capable of coordinating metal ions. The authors discuss properties and mechanisms of action, cell delivery, and cell toxicity of new metal-chelating quinones as well as future directions in their use as targeted anticancer agents suitable for chemotherapy and PDT.

Figure 1 shows the subjects of the topics discussed in this Forum and relationships between them. The EPR-based techniques offer unique methods of direct, definitive, noninvasive, sensitive, and quantitative determination of free radicals and paramagnetic species in biological samples. As discussed in reviews (1, 2, 8), this provides an indispensable tool in the study of endogenous free radicals and oxidative stress in biology and medicine and may guide rational design of redox-active drugs (7). In addition, combination of EPR-based techniques with exogenous paramagnetic probes allows for functional mapping of physiologically relevant parameters of tissue microenvironment, providing new insights into the biological processes related to normal physiology and pathophysiology of the various diseases in preclinical and, potentially, clinical settings (3–5).

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Author Disclosure Statement

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Abbreviations Used

DMPO = 5,5-dimethyl-1-pyrroline N-oxide
EPR = electron paramagnetic resonance
EPRI = EPR imaging
HAs = hydroxylamines
IST = immuno-spin trapping
MRI = magnetic resonance imaging
NMR = nuclear magnetic resonance
NR = nitroxide radical
PDT = photodynamic therapy
PEDRI = proton–electron double-resonance imaging
ROS = reactive oxygen species
ST EPR = EPR spin trapping