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Editorial
In vivo EPR: when, how and why?

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ABSTRACT: This special issue is aimed at providing the readers of this journal with an indication of the exciting and important areas in which *in vivo* electron paramagnetic resonance (EPR) [or equivalently electron spin resonance (ESR)] is making contributions to experimental progress and to provide perspectives on future developments, including the potential for *in vivo* EPR to be an important new clinical tool. There also are many situations where the combination of *in vivo* EPR with NMR may be very synergistic. EPR (ESR) is a magnetic resonance-based technique that detects species with unpaired electrons. The technique has become a major tool in diverse fields ranging from biology and chemistry to solid-state physics. In the last few years, many publications have demonstrated that EPR measurements in living animals (*in vivo* EPR) can provide very significant new insights to physiology, pathophysiology and pharmacology. The most successful applications of *in vivo* EPR have been non-invasive measurements of oxygen, nitric oxide, bioradicals, pH and redox state, with applications in oncology, cardiology, neuroscience and toxicology. EPR also appears to be the method of choice for measuring radiation dose retrospectively, including the potential to do this *in vivo* in human subjects. While far from comprehensive, the reviews, original contributions and viewpoints provided in this issue by several leaders in the field of *in vivo* EPR should provide the readers with confirmation that *in vivo* EPR is an exciting field that is likely to provide very valuable complementary information for many NMR-based studies in experimental animals and, probably, also for clinical studies.

KEYWORDS: EPR; ESR; spectroscopy; imaging; oximetry; metabolism; free radicals

INTRODUCTION

It is impossible to prepare a special issue '*In vivo EPR*' in *NMR in Biomedicine* without referring to NMR, which is much more widely used and developed by the readers of this journal. NMR and electron paramagnetic resonance (EPR) were discovered about 60 years ago: Zavoisky observed the paramagnetic resonance phenomenon for the first time in 1944, at about the same time as Bloch and Purcell discovered the nuclear magnetic resonance phenomenon in condensed matter. Both magnetic resonance methods have continued to develop, with many applications in chemistry, physics and biology. However, the development of NMR in biomedicine has been by far more impressive than the development of EPR. This year, the whole magnetic resonance community is celebrating the Nobel Prize in physiology and medicine that was won by Paul C. Lauterbur and Sir Peter Mansfield for their discoveries concerning magnetic resonance imaging. In three decades, NMR has become perhaps the most powerful method for the non-invasive investigation of human anatomy, physiology and pathophysiology. Thousands of scientists around the world are contributing each day to the advances of NMR and MRI for the diagnosis and treatment of human diseases. In comparison to this flourishing field, the development of *in vivo* EPR has been rather slow. There are several reasons for that, especially linked to the considerable technical challenges that make *in vivo* EPR potentially difficult. Although there are few fundamental differences between the principles of electron and nuclear magnetic resonance, differences in physical and chemical properties of the resonant species (unpaired electrons vs nuclei with net spin) lead to profound differences in the techniques that are used to record the spectra or to reconstruct an image. Three major differences can be emphasized: the size of the magnet moments, which fixes the ratio between the frequency and the magnetic field; the limited amounts of naturally occurring paramagnetic compounds *in vivo*; and the very short relaxation times of electron spins. As a consequence of these three factors, achieving adequate sensitivity with *in vivo* EPR is very challenging. The usual frequencies used for EPR in physics and chemistry are too high for use in tissues, because of non-resonant absorption by water, and therefore frequencies that are suboptimal for EPR (usually <1200 MHz) need to be used. The lack of high concentrations of naturally occurring paramagnetic

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material often requires that the paramagnetic materials need to be added, leading to the usual challenges involved in the administration of material to human subjects. The short relaxation times make time domain techniques very difficult to implement.

The key technical advances that were necessary to develop *in vivo* applications of EPR were: (1) development of highly sensitive spectrometers operating at low frequency (typically between 200 MHz and 1.5 GHz) suitable for use with animals; (2) development of detectors suitable for *in vivo* studies; (3) identification and development of paramagnetic compounds with properties suited for particular applications (especially for the measurement of pO_2); and (4) improved methods for data acquisition and analysis. Thanks to the combination of efforts of pioneer groups in the field, *in vivo* EPR is now widely recognized as a powerful tool in specific areas. Therefore it is appropriate to consider: when, how and why to use *in vivo* EPR?

WHEN AND WHY TO USE *IN VIVO* EPR?

The value of *in vivo* EPR should derive from capabilities that cannot be achieved as well by using other approaches.[1] Often these are related to the unique ability of EPR to detect and characterize unpaired electron species and that the resulting spectra are affected by the environment in the vicinity of the unpaired electrons. The potential values of *in vivo* EPR include: direct measurements of free radicals (for example, nitric oxide[2]), confirmation of the involvement of an unpaired electron species in the occurrence of a physiological or a pathophysiological process,[3,4] measurements that are easier and/or more robust are obtained using another technique (for example, repeated measurements of pO_2 in tissues[5]) and measurements of a microenvironment in which a spin label has been introduced (for example to measure the redox state,[6,7] microviscosity[8,9] or pH[10]). These capabilities open the window to study how physiological factors,[7,11,12] treatments[13,14] and drugs[15–18] are able to modulate these parameters in tissues.

The manuscripts published in this issue provide information on the 'state-of-the-art' in specific areas such as oncology (reviewed by Gallez *et al.*), cardiology (reviewed by Kuppusamy and Zweier), neuroscience (research article by Liu *et al.*), metabolism (reviewed by Fujii and Berliner), and in particular diseases such as sepsis (reviewed by James *et al.*). *In vivo* EPR is now routinely used in laboratories and recognized for the unique information it can provide. As the development of *in vivo* EPR has been very successful in animals, these results have made possible very attractive potential clinical applications. An overview by Swartz *et al.* describes the ongoing developments at Dartmouth Medical School, the instrumental challenges, and the first clinical results.

HOW TO USE *IN VIVO* EPR?

Looking to the unique information that *in vivo* EPR is able to provide in specific areas, the readers may be very interested to acquire and use this methodology in their own laboratory. Fortunately, the technology is no longer limited to laboratories that are developing EPR instrumentations. It is very likely that the technology will expand, as several EPR companies are now manufacturing commercial low frequency spectroscopy EPR and imaging systems for small animal research. Interestingly, several new biomedical-oriented laboratories without strong engineering support, emerged during the last few years, directed by 'academic sons' of pioneers who developed *in vivo* EPR. Still, the development of instrumentation will be a key factor to continue to gain in sensitivity and to make it easier to extend applications to larger biological samples or even to humans. In this issue, Subramanian *et al.* describe the principles and compare the relative merits of continuous wave EPR, time-domain EPR imaging,[19] and Overhauser enhanced magnetic resonance imaging.[20] As this group has expertise in all three of these types of instrumentation, they are in an excellent position to provide comprehensive information on the advantages and disadvantages of each approach. The applications of *in vivo* EPR will continue to benefit from new developments in control systems for recording the EPR data (research article by Hirata *et al.*) and in new types of resonators.

Finally, the value of an *in vivo* EPR experiment will strongly depend on the right choice of approach and the paramagnetic sensor that is introduced in the system. When is it preferable to use spectroscopy vs imaging? When is it preferable to use soluble paramagnetic compounds or particulates for measuring oxygen? These questions are reviewed in the manuscript of Gallez *et al*. There has been considerable effort made by several groups to develop and increase the capabilities of paramagnetic probes such as spin traps or paramagnetic materials for oximetry, including the suitability of the substances for use in animals and human subjects. An example of these types of studies is described by Charlier *et al.*, in which they describe approaches to increase the biocompatibility of an oxygen sensor that may be used in human subjects.

CONCLUSION

In summary, EPR has a unique capability to detect species with unpaired electrons. This is the method of choice to measure key bioradicals such as oxygen, nitric oxide or radical metabolites in living systems. Major advances in the last few years have overcome many of the technical difficulties for doing EPR in living subjects. Thanks to instrumental developments and research on new paramagnetic sensors, unique and very elegant

studies have demonstrated the unique capability of *in vivo* EPR in most biomedical fields. Further developments are very likely, but it already is clear that *in vivo* EPR already is a very productive and valuable set of techniques, which should be known and often utilized by investigators using all types of magnetic resonance techniques.

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REFERENCES

1. Swartz HM, Berliner LJ. Introduction to *in vivo* EPR. In *In vivo EPR (ESR): Theory and Applications. Biological Magnetic Resonance*, Vol. 18., Berliner LJ (ed.). Kluwer Academic/Plenum: New York, 2003; 1–20.
2. James PE, Swartz HM. Simultaneous detection of pO2 and NO by electron paramagnetic resonance. *Meth. Enzymol.* 2002; **359**: 52–66.
3. Fujii H, Berliner LJ. *In vivo* EPR evidence for free radical adducts of nifedipine. *Magn. Reson. Med.* 1999; **42**: 691–694.
4. Mäder K, Bacic G, Swartz HM. *In vivo* detection of anthralin-derived free radicals in the skin of hairless mice by low-frequency electron paramagnetic resonance spectroscopy. *J. Invest. Dermatol.* 1995; **104**: 514–517.
5. Dunn JF, Swartz HM. *In vivo* electron paramagnetic resonance oximetry with particulate materials. *Methods* 2003; **30**: 159–166.
6. Gallez B, Bacic G, Goda F, Jiang J, O'Hara JA, Dunn JF, Swartz HM. 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. Kuppusamy P, Li H, Ilangovan G, Cardounel AJ, Zweier JL, Yamada K, Krishna MC, Mitchell JB. Noninvasive imaging of tumor redox status and its modification by tissue glutathione levels. *Cancer Res.* 2002; **62**: 307–312.
8. Halpern HJ, Chandramouli GV, Barth ED, Yu C, Peric M, Grdina DJ, Teicher BA. Diminished aqueous microviscosity of tumors in murine models measured with *in vivo* radiofrequency electron paramagnetic resonance. *Cancer Res.* 1999; **59**: 5836–5841.
9. Mäder K, Gallez B, Liu KJ, Swartz HM. Non invasive *in vivo* characterization of release processes in biodegradable polymers by low-frequency electron paramagnetic resonance spectroscopy. *Biomaterials* 1996; **17**: 457–461.
10. Gallez B, Mä der K, Swartz HM. Non invasive measurement of the pH inside the gut by using pH-sensitive nitroxides. An *in vivo* EPR study. *Magn. Reson. Med.* 1996; **36**: 694– 697.
11. Jordan BF, Gregoire V, Demeure RJ, Sonveaux P, Feron O, O'Hara JA, Vanhulle VP, Delzenne N, Gallez B. Insulin increases the sensitivity of tumors to irradiation: involvement of an increase in tumor oxygenation mediated by a nitric oxide-dependent decrease of the tumor cells oxygen consumption. *Cancer Res.* 2002; **62**: 3555–3561.
12. Jordan BF, Sonveaux P, Feron O, Gregoire V, Beghein N, Gallez B. Nitric oxide mediated increase in tumor blood flow and oxygenation of tumors implanted in muscles stimulated by electric pulses. *Int. J. Radiat. Oncol. Biol. Phys.* 2003; **55**: 1066–1073.
13. Goda F, O'Hara JA, Rhodes ES, Liu KJ, Dunn JF, Bacic G, Swartz HM. Changes of oxygen tension in experimental tumors after a single dose of X-ray irradiation. *Cancer Res.* 1995; **55**: 2249–2252.
14. Goda F, Bacic G, O'Hara JA, Gallez B, Swartz HM, Dunn JF. The relationship between partial pressure of oxygen and perfusion in two murine tumors after X-ray irradiation: a combined gadopentetate dimeglumine dynamic magnetic resonance imaging and *in vivo* electron paramagnetic resonance oximetry study. *Cancer Res.* 1996; **56**: 3344–3349.
15. Gallez B, Jordan BF, Baudelet C, Misson PD. Pharmacological modifications of the partial pressure of oxygen in murine tumors: evaluation using *in vivo* EPR oximetry. *Magn. Reson. Med.* 1999; **42**: 627–630.
16. Jordan BF, Misson P, Demeure R, Baudelet C, Beghein N, Gallez B. Changes in tumor oxygenation/perfusion induced by the no donor, isosorbide dinitrate, in comparison with carbogen: monitoring by EPR and MRI. *Int. J. Radiat. Oncol. Biol. Phys.* 2000; **48**: 565–570.
17. Jordan BF, Sonveaux P, Feron O, Grégoire V, Beghein N, Dessy C, Gallez B. Nitric oxide as radiosensitizer: evidence for an intrinsic role additive to its effect on oxygen delivery and consumption. *Int. J. Cancer* 2004; **109**: 768–773.
18. Lei H, Grinberg O, Nwaigwe CI, Hou HG, Williams H, Swartz HM, Dunn JF. The effects of ketamine-xylazine anesthesia on cerebral blood flow and oxygenation observed using nuclear magnetic resonance perfusion imaging and electron paramagnetic resonance oximetry. *Brain Res.* 2001; **913**: 174–179.
19. Subramanian S, Yamada KI, Irie A, Murugesan R, Cook JA, Devasahayam N, Van Dam GM, Mitchell JB, Krishna MC. Non invasive *in vivo* oximetric imaging by radiofrequency FT EPR. *Magn. Reson. Med.* 2002; **47**: 1001–1008.
20. Krishna MC, English S, Yamada K, Yoo J, Murugesan R, Devasahayam N, Cook JA, Golman K, Ardenkjaer-Laersen JH, Subramanian S, Mitchell JB. Overhauser enhanced magnetic resonance imaging for tumor oximetry: co-registration of tumor anatomy and tissue oxygen concentration. *Proc. Natl Acad. Sci. USA* 2002; **99**: 2216–2221.

