**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 are also 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. Copyright © 2004 John Wiley & Sons, Ltd.

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...
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 \( \text{pO}_2 \)); 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\(^5\)), 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 \( \text{pO}_2 \) 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\(^5-18\) 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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We appreciate very much the invitation of John R. Griffiths, editor-in-chief of NMR in Biomedicine, to develop this special issue about in vivo EPR. This has been a real opportunity to describe the last advances in the field. This issue would not have been possible without the dedication of many authors. We wish to express our sincere thanks to all of them, and to the reviewers who make the contributing articles instructive for the large audience.

REFERENCES