EPR Measurements of Tissue Oxygenation Status

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METHODS:

In order to make measurements *in vivo* with EPR low frequencies (usually 1200 MHz or lower) are required, in order to avoid excessive non-resonance absorption of the microwaves. Consequently, the magnet fields are 400 gauss or lower. EPR oximetry is based on the effects of molecular oxygen (which is paramagnetic) on the spectra of other paramagnetic materials. Both soluble and particulate paramagnetic materials have been used for this purpose. The soluble materials are especially useful for imaging. The particulates are especially useful for making repeated measurements from the same site.

Our approach is to use spectroscopy, because of the higher signal/noise and the sensitivity of EPR spectra to many parameters that are of potential clinical value but cannot be measured with other techniques. For direct oximetry, we have especially utilized the particulates, in combination with spectroscopy. By the use of field gradients and appropriate data processing, multiple sites can be measured simultaneously. The clinical *in vivo* EPR spectrometer uses a 400 gauss permanent magnet with 80 cm diameter pole pieces and a 50 cm gap, which is sufficient to accommodate a large adult in either a horizontal or a vertical orientation. For measurements of oxygen in tissues within 10 mm of the surface, we use implanted paramagnetic materials that are injected into the sites of interest and a surface resonator that is placed over these sites. Several very oxygen-sensitive materials have been developed for use in animals.

For oximetry measurements in human subjects, we have used India ink as the oxygen sensitive material (because it already is established for use in humans) or could enclose the paramagnetic materials in gas permeable biocompatible materials. Several sites can be measured simultaneously, using a magnet field gradient. This approach can be used to measure oxygen in critical regions of the legs of patients with peripheral vascular disease and in superficial malignant tumors. Oxygenation in wounds is measured using oxygen-sensing material that is placed within the wound in small oxygen permeable, biocompatible tubes. For deep-seated tumors, we will use an implantable resonator with lithium phthalocyanine in the small (1-3 mm) loop. The loop, covered with an oxygen permeable biocompatible coating, is placed in the tumor at the time of biopsy. The small loop is connected by a thin cable to a larger loop (about 10 mm diameter) that is placed subcutaneously, which is then noninvasively magnetically coupled to a surface resonator.

The measurements of redox status based on *in vivo* EPR provide information that is difficult to obtain *in vivo* with other methods. These include measurements of available thiols, following the rate of reduction of redox sensitive materials (e.g. nitroxides), and measurements of free radicals by spin trapping.

RESULTS AND DISCUSSION:

Direct repeated oximetry in animals has been very productive for studies of alterations in vasculature system (especially angiogenesis and ischemia-reperfusion damage in the brain and in the peripheral vascular system), oxygenation in tumors, oxygenation in the heart, and the effects on oxygenation of altered physiology (e.g. shock, hyperbaric oxygen) and therapeutic and pharmacological interventions (especially in the brain and tumors). Most of the experiments in animals have been done in small rodents, but very useful measurements also have been made in larger animals, including pigs. The initial measurements of tissue oxygen in human subjects have been made in the foot at several different sites chosen for their clinical relevance, using injected 10 – 25 μl of oxygen-sensitive India ink injected in the feet of volunteers. The technique has reliably measured tissue oxygen levels from the same sites for more than 18 months, and the measurements are continuing. Protocols also have been developed for using repeated measurements of pO_2 in tumors to guide radiation therapy, by using the information to optimize the timing and dose of radiation on an individual basis and for repeated measurements of oxygen in wounds to understand the detailed relationships between oxygen levels and healing, to follow the effects of therapeutic intervention, and ultimately to guide therapy.

The measurements of redox status currently are limited to animals, because the reagents needed for these studies have not been cleared for use in human subjects, although this situation may change in the near future. The capabilities of *in vivo* spin trapping, including nitric oxide, have been demonstrated in small rodents and applied for the study of oxidative damage and endotoxic shock. The feasibility of using the redox sensitive reduction of nitroxides and measuring thiols *in vivo* has been demonstrated in several studies in several laboratories.

CONCLUSION:

In vivo EPR techniques have been widely and effectively used in animal models and, more recently, in human subjects. These techniques provide information that is difficult or impossible to obtain with other techniques, and therefore it is likely that these applications will continue to expand in extent and usefulness.

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