Deep-Tissue Oxygen Monitoring in the Brain of Rabbits for Stroke Research

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The primary event in the ischemic stroke is a rapid decline in the oxygen levels after the loss of blood flow in specific areas of the brain. Subsequent pathological processes result in a central core area of severely ischemic tissue surrounded by a region of moderate ischemic tissue (penumbra) with a preserved cellular metabolism. The outcome of an ischemic stroke depends on the size of the infarct core and the potential to salvage the cells in the penumbra, which is hyperperfused, and therefore, at risk of infarction but still viable. Such viable penumbral tissue can be rescued by quick interventions that can increase oxygen levels or slow metabolism in the ischemic area to minimize oxidative injury on reperfusion.

Several strategies have been investigated to rescue ischemic tissue using experimental models, especially rodents, but largely failed in subsequent clinical trials. The rabbit model of ischemic stroke using embolic clot is a promising model for developing effective strategies. This model first led to the prediction of the clinical response of recombinant tissue-type plasminogen activator to restore blood flow in patients. The drug is currently recommended for administration within 3 hours for best outcomes and has also shown modest benefit when administered within 4.5 to 6 hours of clinical onset. The rabbit model of embolic clot is now considered as a pertinent model for translational research by the Stroke Therapy Academic Industry Roundtable recommendations.

To rationally develop effective therapies, it is important to understand the effect of ischemic stroke on oxygen levels (partial pressure of oxygen [pO2]) in the regions directly affected by the pathology, as well as contralateral regions of the rabbit brain. The potential changes in tissue pO2 of contralateral regions may provide crucial information on adaptive response, if any, of the brain to counteract ischemic stroke. Such research will greatly benefit from the availability of oximetry techniques that can directly and repeatedly measure tissue pO2 in several regions of the brain.

Several methods are currently available for the assessment of brain pO2, including oxygen electrodes. However, their limited capability to directly and repeatedly measure brain pO2 in a minimally invasive manner has restricted their effective application in ischemic stroke where monitoring oxygen levels are crucial for the development, and optimization of novel strategies for clinical translation. We report electron paramagnetic resonance (EPR) oximetry using implantable resonators to monitor brain pO2 in rabbit with the goal to develop and test novel strategies that can significantly reduce brain loss in ischemic stroke. EPR oximetry using particulate probes, such as lithium phthalocyanine crystals or its derivatives, have been used to study tissue pO2 in a wide range of experimental systems, including muscle, heart, brain, kidney, and liver in rodents and is now being developed for clinical applications. Despite the benefits of EPR oximetry, the currently available hardware technology limits pO2 measurement to a depth of 1 cm at L-band frequencies (1.2 GHz). This is largely because of nonresonant losses of the microwave energy in the tissue of interest. The penetration of microwave energy can be increased up to ≈7 cm by using lower frequencies but this decreases the signal/noise (S/N) ratio of the EPR signal, thus compromising the accuracy of measurements. To resolve this problem, we have pioneered an innovative design of implantable resonators for pO2 measurement at depths >1 cm (Figure 1).

We have implemented this approach to monitor tissue pO2 at 2 sites in each hemisphere of the rabbit brain simultaneously. Our overall goal is to optimize the outcome of ischemic stroke for clinical translation. To the best of our knowledge, this is the first report of monitoring brain pO2 in multiple sites and at depths >1 cm from the skull in rabbit by EPR oximetry.

Description of the Innovation

Implantable Resonator

The implantable resonator is assembled with thin nonmagnetic copper wire (0.3-mm wire gauge) and consists of a coupling...
loop (12–16 mm diameter) at one end and a transmission line with sensor loops (0.4–0.5 mm inner diameter) at the other end (Figure 1A). The sensor loops (or tips) are loaded with 30 to 50 μg of lithium phthalocyanine (LiPc, oximetry probe) crystals. The length of the transmission lines defines the depth and can be varied as needed for the experiment. The number of sensor loops and the distance between them can also be varied to measure pO2 at ≥1 sites in the brain of rabbits. The entire resonator is coated with a gas permeable and biocompatible Teflon AF2400. The mean area of the oximetry probe at the surface of each sensor loop is estimated to be 1.3 to 1.6 mm²; EPR oximetry, therefore, samples a region that includes many capillary segments and provides average pO2 at the site of sensor loop. Histological examination of the cerebral tissue in the rabbit euthanized 4 weeks after the placement of implantable resonator did not show any obvious accumulation of inflammatory cells or blood cells surrounding the sensor loops. Similar results were also evident as early as 7 days after the placement of implantable resonator with 6-mm length of transmission lines in the brain of rats.

**Procedure for the Placement of Implantable Resonator in the Brain of Rabbits**

The surgical procedure for the placement of implantable resonator in the brain of rabbit was in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of Geisel School of Medicine at Dartmouth. The head of the anesthetized rabbit was antiseptically treated with Betadine, and 70% alcohol scrubs. A small incision (2–3 cm) was made on the skin and burr holes were gently created by using 18-gauge needle on the skull at predefined coordinates (anterior–posterior from bregma, −2.0 mm; medial–lateral from midline, 4 and 8 mm on each hemisphere; dorsal–ventral from surface of skull, 15 and 10 mm in each hemisphere). The sensor loops, SL1 and SL4, were located in the parietal cortex, whereas SL2 and SL3 were located in the subcortex (basal forebrain/internal capsule) region of the brain. The position of the sensor loops can be altered depending on the coordinates of the compromised tissue after ischemic stroke. The sensor loops were placed at the desired depth and the coupling loop was placed on the skull below the skin, to allow inductive coupling with the external surface loop resonator of the EPR spectrometer (Figure 1B). The incision on the skin was closed with nonabsorbable 3-0 nylon suture and the rabbit was monitored for recovery as per the Institutional Animal Care and Use Committee protocol. The repeated measurements of brain pO2 by EPR oximetry was started 72 hours (day 3) after the placement of implantable resonator and the measurements were repeated for 4 weeks.

**In Vivo EPR Oximetry**

EPR oximetry requires one-time implantation of the oxygen probes (LiPc or implantable resonator), but rest of the procedure for pO2 measurement is entirely noninvasive and can be
repeated as desired.\cite{8,17–19} The basis of oximetry is the paramagnetic nature of oxygen, which broadens the EPR signal of the probe in proportion to the amount of oxygen. EPR oximetry has unique capabilities and advantages compared with other techniques, such as (1) direct measurement of absolute pO2 in the tissue of interest, (2) pO2 is quantified through a physical interaction of oxygen with the probe (does not require oxygen consumption), (3) pO2 measurements can be made continuously and repeatedly as desired, without a confounding influence of previous measurements, (4) The oxygen sensors are metabolically inert and coated with Teflon, therefore, do not perturb the tissue microenvironment, including oxygen content, and (5) there is no other technique available at present to make repeated measurement of tissue pO2 without the need to reintroduce the probe for each measurement.

A 1.2-GHz EPR spectrometer equipped with a surface loop resonator and a set of gradient coil for multisite oximetry was used for monitoring brain pO2 in the rabbit. The anesthetized rabbit (2.5% isoflurane in 30% O2) was positioned in the EPR magnet and the external surface loop resonator was gently placed over the head region. A magnetic field gradient of 1.7 G/cm per ampere was used to separate the EPR spectra from each sensor loop for simultaneous multisite oximetry.\cite{20} The peak-to-peak line widths of the EPR spectra were used to determine pO2 by using the calibration of implantable resonator (Figure 1C and 1D). The rabbit was maintained at 38±1.0°C (monitored via a rectal probe) by keeping the animal under warm air during the EPR measurements. The EPR settings were as follows: incident microwave power, 0.4 to 1.2 mW; modulation frequency, 24 kHz; magnetic field center, 410 G; scan time, 10 s, scan range, 8 to 12 G, and modulation amplitude not exceeding one third of the line width. The implantable resonator appears as a signal void in T1-weighted MRI scans, which can be used to confirm their position in the brain of rabbits.

**Results**

Brain pO2 was measured for 20 to 25 minutes on day 3 and the measurements were repeated on days 5, 7, 14, 21, and 28 (Figure 2). The mean (SD) baseline pO2 at each site (SL1–SL4) in the brain on day 3 was 39.2 (2.2), 41.6 (1.4), 41.3 (1.7), and 43.6 (2.0) mmHg, respectively, and only a modest variation was observed in the measurements repeated subsequently for ≤4 weeks. To mimic the ischemic situation with low levels of oxygen and test the response of implantable resonator, the breathing gas was switched to 15% O2 for 15 minutes and then returned to 30% O2 (Figure 3A). To test the potential effect of hyperoxia on brain pO2, the breathing gas was switched to carbogen (95% O2+5% CO2) for 20 to 25 minutes and then returned to 30% O2 (Figure 3B). These experiments were performed on days 7, 14, 21, and 28. The brain pO2 measured at 4 sites decreased by ≈30% from baseline in rabbit breathing 15% O2. However, brain pO2 measured at 4 sites increased significantly by ≥75% during carbogen breathing. An exponential quadratic function of time was used to determine minimum pO2 (PO2min) attained during 15% O2 on each day and the time to reach the PO2min (Tmin) (Figure 4). Similar analysis was used to determine maximal pO2 (PO2max) attained on each day and the time to reach maximum pO2 (Tmax) during carbogen inhalation. These analyses suggest that it took 10 to 14 minutes to reach a minimal or maximal pO2 when the breathing gas was switched from 30% O2 to 15% O2 or carbogen, respectively. A similar time scale was noted for the brain pO2 to return to the baseline level when the breathing gas was switched from 15% O2 or carbogen to 30% O2. We anticipate that such temporal pO2 information will be extremely useful in designing hyperoxic therapies to modulate oxygen levels in ischemic stroke.

![Figure 2. Tissue pO2 in the rabbit brain at 2 sites in each hemisphere measured simultaneously by multisite electron paramagnetic resonance oximetry. The sensor loops (SL) 1 and SL4 were at a depth of 10 mm; SL2 and SL3 were at a depth of 15 mm in the left and right hemisphere, respectively. The brain pO2 measurements were repeated on days 3, 5, 7, 14, 21, and 28.](image)

![Figure 3. Typical changes in tissue pO2 of the rabbit brain at 2 sites in each hemisphere during (A) 30% O2 (baseline), 15% O2, and return to 30% O2 breathing, (B) 30% O2, carbogen and return to 30% O2 breathing. The experiment was repeated for 4 consecutive weeks.](image)
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the length of transmission lines, number, and distance between
the design of the implantable resonator, including
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that may occur in ischemic stroke. The extent of increase in
tissue pO2 during carbogen breathing is encouraging and can
be potentially used as a therapeutic strategy to improve oxygen
levels and thus save vital tissue loss in ischemic stroke.
For the multisite oximetry approach, the position of sensor
loops should be carefully selected so that they are located in the
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experiment. A stable brain pO2 was observed from day 3, which
suggest that the experiments to investigate ischemic stroke can
be initiated as early as 3 days after the placement of implantable resonator in the brain of rabbits. The measurement of tissue pO2
in the nonischemic contralateral brain can be used as internal
control and investigate adaptive response of the brain to isch-
emic stroke. The brain pO2 data presented here was obtained in
a rabbit to illustrate the capability of temporal monitoring by EPR oximetry. We are currently implementing this technique
to investigate temporal changes in the brain pO2 in additional rabbits during hyperoxia and ischemic stroke.

Discussion
To fully comprehend the pathology of stroke and rationally
develop strategies to rescue ischemic tissue, there is an unmet
need to understand the complex temporal changes in tissue pO2 that occur during the course of ischemic stroke, a capability
that previously has not been available. The results highlight
the ability of EPR oximetry using implantable resonator for
pO2 measurements at 4 sites simultaneously in the brain of a rabbit. The pO2 measurements can be repeated as desired. A
rapid decline in tissue pO2 during 15% O2 breathing potentially
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Conclusions
We have demonstrated a direct and longitudinal measurement of absolute tissue pO2 at several sites simultaneously in the
brain of rabbit by EPR oximetry using implantable resonators, a capability, which was not available hitherto. Dynamic
information of cerebral pO2 can be used to test and to optimize strategies for improving treatment outcome of ischemic
stroke. EPR oximetry with implantable resonators should
also be useful to investigate the effect of other pathologies,
such as traumatic brain injury and cold injury, on the oxygen
levels in the brain of clinically pertinent animal models.

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