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Repeated assessment of orthotopic glioma pO_2 by multi-site EPR oximetry: A technique with the potential to guide therapeutic optimization by repeated measurements of oxygen

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ABSTRACT

Tumor hypoxia plays a vital role in therapeutic resistance. Consequently, measurements of tumor pO_2 could be used to optimize the outcome of oxygen-dependent therapies, such as, chemoradiation. However, the potential optimizations are restricted by the lack of methods to repeatedly and quantitatively assess tumor pO_2 during therapies, particularly in gliomas. We describe the procedures for repeated measurements of orthotopic glioma pO_2 by multi-site electron paramagnetic resonance (EPR) oximetry. This oximetry approach provides simultaneous measurements of pO_2 at more than one site in the glioma and contralateral cerebral tissue.

The pO_2 of intracerebral 9L, C6, F98 and U251 tumors, as well as contralateral brain, were measured repeatedly for five consecutive days. The 9L glioma was well oxygenated with pO_2 of 27–36 mm Hg, while C6, F98 and U251 glioma were hypoxic with pO_2 of 7–12 mm Hg. The potential of multi-site EPR oximetry to assess temporal changes in tissue pO_2 was investigated in rats breathing 100% O_2. A significant increase in F98 tumor and contralateral brain pO_2 was observed on day 1 and day 2, however, glioma oxygenation declined on subsequent days.

In conclusion, EPR oximetry provides the capability to repeatedly assess temporal changes in orthotopic glioma pO_2. This information could be used to test and optimize the methods being developed to modulate tumor hypoxia. Furthermore, EPR oximetry could be potentially used to enhance the outcome of chemoradiation by scheduling treatments at times of increase in glioma pO_2.

1. Introduction

The imbalance between oxygen supply and demand along with tumor growth and atypical angiogenesis, often leads to the development of hypoxia (pO_2; partial pressure of oxygen < 10–15 mm Hg) in solid tumors. Subsequently, tumor hypoxia compromises treatment outcomes by facilitating DNA repair after radiation and chemotherapies. Hypoxia also leads to an alteration of gene expression, tumor progression, and metastases (Jensen, 2009; Oliver et al., 2009). In gliomas, the hypoxia responsive elements, such as Hypoxia Inducible Factor (HIF), are up-regulated and positively correlate with aggression and invasion (Jensen, 2009; Oliver et al., 2009). Consequently, tumor hypoxia has become a critical factor that must be addressed to optimize therapeutic outcome. Additionally, the dynamics of tumor pO_2 during the course of treatment is characteristic of the tumor microenvironment and proliferation. Therefore, tumor pO_2, if repeatedly measured, could be potentially used to follow efficacy, and identify responders early on during the treatments. This also will enable clinicians to prescribe alternate therapeutic interventions for non-responders.

Given the significance of hypoxia in tumor growth and response to therapies, considerable research has been carried out to develop methods for measurements of tumor oxygen, but the ability to make repeated measurements of tumor pO_2 has been challenging. Both invasive and non-invasive methods have been used to measure pO_2 in tumors, some via direct measurements of tumor pO_2 and several using parameters that relate to tumor oxygen. Each of these methods has certain benefits and limitations that will make them useful in certain settings but not appropriate for others. The polarographic (Eppendorf) and luminescence-based (OxyLite) methods do measure pO_2 directly in the tumor, but require a physical insertion of the probe in the tumor for pO_2 measurements. These can be used to obtain spatial information by making several tracks through the tumor. This invasive procedure makes these

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techniques unsuitable for repeated measurements of tumor pO_2 due to the trauma associated with their use. Also, measurements with Oxylite usually require a 15–30 min wait before the pO_2 readings are stabilized. Furthermore, the probes are somewhat fragile and very sensitive to minor movements (Brurberg et al., 2003). Methods based on nuclear magnetic resonance (NMR) have the advantage of widely available instrumentation. Blood oxygen level dependent (BOLD) imaging (Baudelet and Gallez, 2002) is widely available, including clinically, but provides only the relative amount of deoxyhemoglobin in the blood in the volume measured, which cannot be directly related to tissue oxygen in the tumor. Using more specialized equipments, ^{19}F NMR spectroscopy (Hunjan et al., 2001), and Overhauser methods (Krishna et al., 2002) have the potential to provide direct measurements of tumor oxygen but require the injection of the probe, and these injections need to be done each time for repeated measurements.

We have focused on the development of EPR oximetry using particulates for the assessment of absolute pO_2 in the tumors. The basis of EPR oximetry is the paramagnetic nature of molecular oxygen, which therefore affects the EPR spectra of other paramagnetic species in its vicinity by altering their relaxation rates. The magnitude of the effects is directly related to the amount of oxygen that is present in the environment of the paramagnetic materials such as LiPc (lithium phthalocyanine) crystals. EPR oximetry using particulates requires one time implantation of the oximetry probe using a minimally invasive method, but all further procedures for pO_2 measurement are entirely non-invasive and can be repeated as often as desired. A temporal resolution of the order of several seconds, and oxygen sensitivity of 1 mm Hg could be achieved using lithium phathalocyanine (LiPc) in vivo. With RF fields at 1200 MHz and surface-loop resonators, pO_2 measurements can be made within tissues as deep as 10 mm from the surface; however implantable resonators are being developed to enable pO_2 measurements at much greater depths with excellent signal to noise ratio (Hou et al., 2011; Li et al., 2010). In order to asses oxygen heterogeneity, a spatial resolution of up to 1 mm are achievable using multiple implants of oxygen sensitive particulates, and by applying a magnetic field gradients to differentiate the independent signals from each implant (Khan et al., 2009, 2010). We describe the procedure for the oximetry of orthotopic gliomas using LiPc implants by EPR. The feasibility of this method is demonstrated by repeated assessments of experimental 9L, C6, F98 and human xenograft U251 glioma and contralateral brain pO_2 simultaneously by multi-site EPR oximetry. We also report the effect of 100% O_2 breathing on the F98 glioma and contralateral brain pO_2 in experiments repeated for five consecutive days. This oximetry approach could also be used to study the effect of other neuropathology, such as, the consequence of ischemia-reperfusion injury on the intracerebral tissue pO_2 and develop methods to minimize the tissue damage by investigating treatment protocols that can improve the oxygen levels in the affected areas of the brain (Hou et al., 2005, 2007; Williams et al., 2007). EPR imaging using water soluble probes can be used to obtain 3D oxygen maps of tumors with temporal resolution of approximately 10 min, oxygen sensitivity of 1 mm Hg, and spatial resolution of 1.5 mm, with repeated injection of probes required for each measurement (Epel et al., 2011).

2. Materials and methods

2.1. Animals and tumor models

All animal procedures were conducted in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Dartmouth Medical School. The experimental 9L gliomas have sarcomatous appearances histologically, while the C6 are classified as astrocytomas and have gene expressions similar to that of human gliomas (Barth and Kaur, 2009). However, both 9L and C6 are immunogenic, and therefore, caution should be exercised in analyzing therapeutic effects. F98 is an anaplastic glioma with minor sarcomatous characteristics (Barth and Kaur, 2009). The F98 glioma is weakly immunogenic with growth and invasive characteristics consistent with human glioblastomas. The U251 xenograft glioma is an astrocytic phenotype with histological features similar to those of the human glioblastoma, including angiogenesis, and tumor cell infiltration (Candolfi et al., 2007). Fischer and Wistar rats (200–250 g), which are syngeneic hosts for 9L/F98 and C6 gliomas respectively, were purchased from Charles River Laboratory (MA) and housed in the animal resource facility at Dartmouth Medical School. The athymic nu/nu (homozygous, 15–20 g) mice were purchased from Charles River Laboratory (MA) and housed in the quarantine quarters of the animal resource facility at Dartmouth Medical School.

2.2. Glioma cells culture and intracerebral tumor inoculation

The 9L, C6, and F98 cells were purchased from ATCC (Manassas, VA) and propagated in Dulbecco's Modified Eagle's Medium with 4.5 g/L glucose, 1 mM sodium pyruvate, 10% FBS and 1% penicillin–streptomycin. For tumor inoculation, the cells were detached from the culture flask by trypsinization (0.25% trypsin, Mediatech Inc., Manassas, VA), and washed three times with the medium without serum or additives. The cell numbers were determined by Countess automated cell counter (Invitrogen, CA) and a suspension of 50,000 cells/10 μL was prepared for injection in rat brain.

The rats were anesthetized using 2.5% isoflurane with 30% oxygen through a nose cone, and the head was immobilized on a stereotaxic apparatus (ASI Instruments, MI) (Fig. 1). The head was shaved and aseptically prepared with Betadine and 70% ethanol. Each rat was inoculated with one tumor in the left hemisphere by slow injection of the cells over 2 min with a 25-gauge needle through a burr hole at 3 mm behind bregma (anteroposterior, −3.0 mm), 1.5 mm from midline (mediolateral, 1.5 mm) and at 3.5 mm depth from the skull (dorsoventral, 3.5 mm). After injection of the tumor cells, the burr hole was cleaned and sealed with bone wax, and the skin was sutured.

The U251 cells were obtained from NCI and cultured in RPMI 1640 medium using the procedure described above. The U251 tumors were established by slow injection of 1×10^6 cells/10 μL over 5 min with a 25-gauge needle through a burr hole at the following coordinates: anteroposterior, −1.7 mm; mediolateral, 1.5 mm; and dorsoventral, 2.0 mm in the left hemisphere. After injection of the tumor cells, the burr hole was cleaned and sealed with bone wax, and the skin was sutured.

2.3. Implantation of oxygen sensitive particulate probe

The paramagnetic LiPc crystals are synthesized in our laboratory by an electrochemical method and their physicochemical properties have been described previously (Liu et al., 1993). After implantation, the LiPc deposits remain in the interstitial compartment of the tumor with minimal evidence of edema or infiltration of inflammatory cells. A minor accumulation of red blood cells and some necrotic cells around the LiPc deposits is typically observed, which perhaps reflect the normal histological pattern of the tumor. In order to enhance the biocompatibility, in particularly for clinical applications, various encapsulations of the oximetry probes in biocompatible and inert polymers have been developed, which could be potentially retrieved after the treatments (Dinguizli et al., 2006; Meenakshisundaram et al., 2009a,b).