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Skeletal Muscle and Glioma Oxygenation by Carbogen Inhalation in Rats: A Longitudinal Study by EPR Oximetry Using Single-Probe Implantable Oxygen Sensors

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Abstract

The feasibility of EPR oximetry using a single-probe implantable oxygen sensor (ImOS) was tested for repeated measurement of pO_2 in skeletal muscle and ectopic 9L tumors in rats. The ImOS (50 mm length) were constructed using nickel-chromium alloy wires, with lithium phthalocyanine (LiPc, oximetry probe) crystals loaded in the sensor loop and coated with AF 2400® Teflon. These ImOS were implanted into the skeletal muscle in the thigh and subcutaneous 9L tumors. Dynamic changes in tissue pO_2 were assessed by EPR oximetry at baseline, during tumor growth, and repeated hyperoxygenation with carbogen breathing. The mean skeletal muscle pO_2 of normal rats was stable and significantly increased during carbogen inhalation in experiments repeated for 12 weeks. The 9L tumors were hypoxic with a tissue pO_2 of 12.8 ± 6.4 mmHg on day 1; however, the response to carbogen inhalation varied among the animals. A significant increase in the glioma pO_2 was observed during carbogen inhalation on day 9 and day 14 only. In summary, EPR oximetry with ImOS allowed direct and longitudinal oxygen measurements in deep muscle tissue and tumors. The heterogeneity of 9L tumors in response to carbogen highlights the need to repeatedly monitor pO_2 to confirm tumor oxygenation so that such changes can be taken into account in planning therapies and interpreting results.

Keywords

Carbogen; Electron paramagnetic resonance (EPR) oximetry; Glioma; Implantable oxygen sensor (ImOS); Partial pressure of oxygen (pO_2)

1 Introduction

Tissue pO_2 is a key parameter in physiological and pathophysiological processes of biological systems and plays a key role in cancer therapy. For example, the efficacy of radiotherapy and chemotherapy dramatically depends on the level of oxygen in the tumors. Furthermore, tissue pO_2 varies in a temporal manner during disease progression and therapy. Accordingly, the assessment of tissue oxygenation is of great physiological and clinical interest.

In vivo electron paramagnetic resonance (EPR) oximetry is a powerful technique that provides repeated monitoring of tissue pO_2 as often as needed. In spite of successful applications in various pathologies, the sensitivity of EPR oximetry using particulate LiPc implants with direct detection has been limited to a depth of no more than 10 mm from the surface. To circumvent this problem, we have developed implantable oxygen sensors (ImOS) constructed with copper wires. However, these ImOS failed during rigorous testing in the skeletal muscle of rats and pigs due to mechanical damage. To overcome this problem, we have developed ImOS using nickel-chromium (Ni-Cr) alloy wires for pO_2 measurement in tissues such as skeletal muscle and 9L glioma.

2 Methods

2.1 Single-Probe Implantable Oxygen Sensors

Oxygen sensitive lithium phthalocyanine (LiPc) crystals were synthesized in our laboratory. The properties of LiPc have been reported earlier [1]. The ImOS of 50 mm in length was fabricated with Ni-Cr alloy and has two sets of loops, a large loop on one end and one small loop on the other end. These two loops are connected by a length of twisted wire. The small loop was loaded with LiPc aggregates (50–80 μg) and then the entire resonator was coated with a gas permeable biocompatible Teflon AF 2400[®] (Aldrich, Steinheim, Germany) (Fig. 13.1a). The small loop was implanted in the tissue of interest, and the large loop was placed below the skin for coupling with the external loop resonator of the EPR spectrometer [2].

2.2 Animal Preparation

All the animal procedures were approved by the Institutional Animal Care and Use Committee of Dartmouth Medical School. Fourteen male Fisher 344 rats, 200–250 g (Charles River Laboratories, Wilmington, MA) were used and divided into two groups: (i) Skeletal muscle group, N = 8; (ii) 9L tumor group, N = 6.

2.2.1 Tumor Model and Implantation of ImOS into the Skeletal Muscle and 9L Tumors—The 9L tumors were established by direct injection of 9L cells ($1\text{--}2 \times 10^6$ cells in 100 μl) into the subcutaneous tissue in the right thigh of the rats. One day or 4 days prior to the pO_2 measurement, the rats were anesthetized (2–2.5 % isoflurane in 30 % O_2) and the sensor loop was gently inserted into the skeletal muscle (5–6 mm depth, group i) or in the 9L tumor (2–3 mm depth, group ii) through a small skin incision, respectively. The remainder of the ImOS was inserted under the skin of the rats for the repeated measurement of pO_2 by EPR oximetry.

2.2.2 Hyperoxia Challenge—The rats were anesthetized (1.5 % isoflurane in 30 % oxygen) and baseline pO_2 was measured for 30 min and then the animals were allowed to breathe carbogen for 25 min. The inhaled gas was again switched back to 30 % O_2 for 25 min. This hyperoxia challenge was repeated either daily or weekly as shown in the results.

2.3 EPR Oximetry

EPR oximetry was performed with an L-band (1.2 GHz) EPR spectrometer using the method described earlier. Tissue pO_2 was determined by measuring the peak-to-peak line widths of the EPR spectra, which were converted to pO_2 by using appropriate calibration of the ImOS used in the study (Fig. 13.1a). The spectrometer parameters were: incident microwave power of 1–2 mW; modulation frequency 24 kHz; magnetic field 430 G; scan time 10 s and modulation amplitude not exceeding one third of the peak-to-peak line width.

2.4 Histological Analysis

At the end of the experiments, the rats were euthanized and muscle tissue surrounding the ImOS was excised and fixed with 10 % formalin, embedded in paraffin, and stained with hematoxylin–eosin for histological studies.

2.5 Statistical Analysis

Data were analyzed by Student's t-test. A paired t-test was used to compare pO₂ changes within the same group. The tests were two-sided, and a change with a p-value <0.05 was considered statistically significant. All data are expressed as mean±SE. N is the number of animals in each group.

3 Results

The pO₂ measurements were started 4 days after the surgical implantation of the ImOS in the skeletal muscle and continued for up to 12 weeks (Fig. 13.1b). No significant difference in the skeletal muscle pO₂ was evident while breathing 30 % O₂ from day 4 to day 84. The mean skeletal muscle pO₂ increased significantly during carbogen inhalation (Fig. 13.1b). Histological examination showed no obvious blood cells along the track of the ImOS; however, the presence of a thin capsule of inflammatory cells and fibroblasts was observed (Fig. 13.1c). These results demonstrate minimal histological changes around the ImOS and are similar to our earlier observation in the brain of the rats [2] and in the muscle of the rabbits [3].

A typical variation in the response of three ectopic 9L tumors to carbogen inhalation is shown in Fig. 13.2a–c. A small (Fig. 13.2a) to modest (Fig. 13.2b) and significant (Fig. 13.2c) response of the 9L tumor pO₂ to carbogen inhalation was evident in these individual tumors. The pO₂ data including these tumors were pooled to obtain mean baseline pO₂ and response to carbogen inhalation (Fig. 13.2d). The mean baseline pO₂ of the 9L tumors was 12.8 ±6.4 mmHg on day 1 (Fig. 13.2d). A significant increase in the mean tumor pO₂ was observed during carbogen inhalation on day 9 and day 14 (Fig. 13.2d).

4 Discussion

The goal of current study was to test ImOS fabricated with Nickel–Chromium (Ni-Cr) alloy wires for a longitudinal measurement of skeletal muscle and 9L tumor pO₂ and the response to carbogen inhalation. Our results indicate that the ImOS fabricated with Ni-Cr alloy wires remained intact and thus enabled repeated measurement of tissue pO₂ in the skeletal muscle and ectopic 9L tumors for several weeks using EPR oximetry.

The mean baseline pO₂ values of the skeletal muscle were stable and a consistent response to carbogen inhalation was observed. The ectopic 9L tumors were hypoxic, which are consistent with the earlier reports [4]. However, the response of individual 9L tumors to carbogen varied among the animals. Lanzen et al. [5] used microelectrodes to detect the changes in tumor pO₂ in R3230Ac tumors transplanted in the quadriceps muscle or subcutis. The tumors in the muscle showed an increase in pO₂ from 14.6±3.2 to 34.5±8.2 mmHg with carbogen breathing; however, no significant change in the pO₂ of the subcutaneous tumor was observed. Bussink et al. used OxyLite to measure the changes in pO₂ on carbogen breathing in three human xenograft tumor lines and found that EI02 and EI06 tumors showed a significant increase in tumor pO₂. In an additional cell line (SCCNij3 tumor xenograft), 9 out of 17 tumors had an increase in pO₂ during carbogen breathing [6]. We speculate that the differences in response of individual 9L tumor pO₂ to carbogen might be

due to a different location of the sensor loop of the ImOS in each tumor. Additionally, differences in the status of tumor vasculature, blood flow and interstitial pressure among tumors can also lead to a difference in responses to carbogen inhalation over days. These variables should be further investigated.

The temporal changes in mean tumor pO_2 on carbogen inhalation varied over days with a statistically significant increase on days 9 and 14. These results signify the need for a repeated measurement of tumor pO_2 during interventions designed to improve the levels of oxygen for therapeutic intention.

In conclusion, temporal and longitudinal changes in skeletal muscle and glioma pO_2 can be measured using EPR oximetry with ImOS. The ability to repeatedly assess skeletal muscle and tumor pO_2 is likely to play a vital role in understanding the dynamics of tissue pO_2 during carbogen inhalation and other therapies designed to modulate tumor hypoxia. EPR oximetry with ImOS can provide this information to potentially optimize therapies by scheduling treatments at times of increase in tissue oxygenation.

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Fig. 13.1.

(a) Calibration plot of an ImOS. The response of ImOS to different concentrations of perfused oxygen and regression coefficients (R^2). *Inset*: a sample of 50 mm length ImOS used in this study, (b) Mean skeletal muscle pO₂ prior to and during carbogen inhalation. Mean±SE, N = 8. *p<0.05, **p<0.01, compared to baseline on the same day (paired t-test). (c) H&E stained sections of the skeletal muscle tissue obtained from a rat after 84 days of ImOS implantation. The *long arrows* indicate the track of the ImOS in the muscle and *short arrows* indicate the presence of a thin capsule formed by few layers of inflammatory cells around the track of the ImOS. The thickness of each section is 5 μm. Magnification: 40×

Fig. 13.2.

Dynamic changes in ectopic 9L glioma pO₂ prior to and during carbogen inhalation in three typical rats (**a**: rat#4; **b**: rat#1; **c**: rat#6) and mean pO₂ observed from a group of six rats (**d**, *open diamond*: baseline tumor pO₂; *filled diamond*: tumor pO₂ during carbogen inhalation. mean±SE, N=6). *Black arrows* indicate the time to start carbogen inhalation. *p<0.05, compared with baseline on same day (paired t-test)