Measurement of Tumor Oxygenation: A Comparison between Polarographic Needle Electrodes and a Time-Resolved Luminescence-Based Optical Sensor


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A novel oxygen sensor which does not rely on electrochemical reduction has been used to measure the oxygenation of the murine sarcoma F in a comparative study with an existing polarographic electrode that is available commercially. The prototype luminescence sensor yielded an oxygen distribution comparable with readings made using a pO2 histograph. The percentage of regions detected that had a pO2 less than 5 mm Hg was 79 and 75 using the Eppendorf pO2 histograph and the luminescence fiber optic sensor, respectively. These values were compatible with a measured radiobiologically hypoxic fraction of 67% in this tumor. The polarographic method detected more regions with a pO2 of 2.5 mm Hg or less (69%) compared with the optical sensor (50%) (P < 0.05). This could reflect differences in the oxygen use of the sensing devices. This initial assessment indicates the potential of a fiber-optic-based oxygen-monitoring system. Such a system should have several advantages including monitoring temporal oxygen changes in a given microregion and use with NMR procedures. © 1997 by Radiation Research Society

INTRODUCTION

Microregional oxygen deficiency in human tumors is regarded as a major contributing factor in local radiation response (1). In particular, the proportion of cells existing at oxygen tensions between 0-10 mm Hg will be of importance, since radiosensitivity varies from its minimum to near maximum value over this range. Indeed, nearly three times more radiation dose is needed to kill hypoxic cells relative to normally aerobic cells (2). The measurement of tissue oxygenation is therefore of great clinical interest both as a predictive assay and as a means of understanding tumor pO2, which may aid future treatment development for both radiotherapy and chemotherapy.

At present, the most commonly used commercial system for the measurement of tissue oxygenation in vivo is an electrode designed for rapid assessment of oxygen distribution throughout a tissue: the Eppendorf pO2 histograph (3). This instrument relies on polarography. Clear advantages exist using this mode of measurement, for example a linear relationship between current and pO2, and by optimizing the polarization voltage along with the addition of a Teflon membrane at the probe tip, contamination in vivo from molecules other than oxygen is reduced significantly. However, technically there are several potential limitations which can be minimized but not eliminated. These include the consumption of tissue oxygen by electrochemical reduction, which can introduce underestimation to the oxygen value. It should be noted that the Eppendorf histogram is designed to minimize this by incorporating a recessed tip electrode, a feature examined by Whalen et al. (4), and by moving the electrode progressively through the tissue. Nevertheless, oxygen depletion will still occur, albeit lower than that resulting from blunt-ended nonmovable electrodes of, for example, the original Clark design (5). A second technical problem involves the difficulties of accurately detecting the very small currents associated with low oxygen concentrations over and above the general background noise. This problem becomes more important as electrode geometry is miniaturized.

Theoretically, these limitations can be overcome using fiber-optic sensors which use dyes whose luminescence is quenched by oxygen. Essentially, these sensors rely on the measurement of the oxygen-quenched lifetime of a luminescent molecule immobilized at the tip of an optical fiber. The optical fiber is used to guide the excitation and emission light transients to the measurement system. However, as with the development of polarographic electrodes for routine use in living tissue, several technical challenges had to be overcome. These included the identification of a suitable luminophor, attachment of this to a fiber optic and the development of real-time signal analysis methods. Recent work at the Gray Laboratory has resulted in the development of a prototype device which uses the fluorophor, tris(4,7-diphenyl-1,10-phenanthroline) ruthenium chloride. Technical details and some preliminary measurements with the device have recently been reported (6, 7).
The fiber-optic sensor operates most effectively in the 0–15 mm Hg pO2 range with fast response and settling times (<2 s). The object of the current study was to evaluate the applicability of the device for measuring tumor oxygen tension and to compare the measurements to those obtained using the Eppendorf pO2 histograph as well as to radiobiological measurements of hypoxia.

**MATERIALS AND METHODS**

**Tumor Model**

The tumor used in this investigation was the sarcoma F (SaF) murine tumor transplanted subcutaneously into the rear dorsum of female CBA/Gy f TO mice. These tumors were serially maintained by injecting 0.05 ml of a crude cell suspension and selected for treatment when they had reached 5–6 mm in diameter.

**Measurement of Radiobiological Hypoxia**

**Tumor irradiation.** All irradiations were performed using a 250 kV Pan-tak X-ray machine set at 240 kV and 15 mA. The mean dose rate for these series of experiments was 3.6 Gy/min. Tumors were irradiated in a similar fashion to the method originally developed by Sheldon and Hill (9). The mice were unanesthetized and exposed to either breathing air or hypoxia during irradiation. Hypoxia was induced by sacrificing the tumor-bearing mouse by cervical dislocation 10 min before the start of irradiation.

**Clonogenic survival.** Full details of this procedure have been published (9). Briefly, after irradiation the tumors were immediately excised, weighed, chopped and enzymatically digested (each dose point consisted of data from the pool of two tumors). After cell recovery and counting, known numbers of cells were dispensed into petri dishes and incubated at 37°C in a humidified incubator gassed with 2% O2/5% CO2/93% N2 for 7–10 days. The colonies were fixed, stained with methylene blue and counted.

**Calculation of hypoxic fraction.** Colony-forming efficiency (CFE) was calculated by counting the number of colonies on the petri dish and dividing this by the number of cells originally seeded. Three dilutions were made of each sample and each dilution was duplicated; thus the average of six samples gave the average CFE for each group and a standard error on the mean was calculated. The surviving fraction (SF) was determined by calculating the number of clonogens per 100 mg of treated tumor and dividing this by the average number of clonogens per 100 mg of control tumor. The SFs of the aerobic and hypoxic treatments were averaged for the 35 animals involved. The oxygen distribution, expressed as a histogram, was calculated from the pooled data for all the animals.

**Statistical analyses.** After the preliminary data analysis outlined above, each tumor had four oxygen values associated with it: median, percentage less than 2.5 mm Hg, less than 5 mm Hg and less than 10 mm Hg. By pooling these values for each of the oxygen tension treatments, a stable reading for the site. Data collection was made at a frequency of 15 readings per minute.

**RESULTS**

Figure 1 shows the radiation response data for tumors irradiated in air-breathing mice and for tumors rendered hypoxic tension.
100% hypoxic. It can be seen that the SaF tumor has a radiobiologically hypoxic fraction, assessed from the displacement of the two survival responses, of 67%. In clinical radiation oncology such measures of radiobiological hypoxia are impossible, and assessment has to be made using oxygen sensors. In the present study, we have assessed the oxygen tension in SaF tumors using two different oxygen sensors, namely the commercially available Eppendorf \(p\text{O}_2\) histograph and an in-house fiber-optic luminescence sensor. The calibration curve for the latter is shown in Fig. 2. The curve illustrates a nonlinear response and is thus quite different from the well-established linear relationship between current and \(p\text{O}_2\) obtained with polarographic measurements. This nonlinearity highlights an interesting feature of the fiber-optic device. The error in the lifetime (\(\tau\)) reading will attribute varying degrees of error to the oxygen reading. At lower oxygen values of less than about 5%, the errors will be smaller than those attributed to readings of 21% oxygen: hence an increased accuracy at lower oxygen tensions. However, this does mean decreased accuracy at higher oxygen levels. (The linear response of a polarographic electrode ensures the same degree of uncertainty at any oxygen measurement.)

The histograms of \(p\text{O}_2\) values obtained from SaF tumors using the Eppendorf \(p\text{O}_2\) histograph and using the fiber-optic luminescence sensors are shown in Figs. 3 and 4, respectively. Both the histogram and luminescence

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**FIG. 1.** Survival curves for SaF tumor cells irradiated in vivo. (●) Survival in tumors made 100% hypoxic during the radiation procedures (\(n = 8\)). (○) Survival in tumors unperturbed during the radiation treatment (\(n = 12\)). Hypoxic fraction, calculated from the displacement of the two lines as described in the Materials and Methods, equalled 66.6% (± 2.79). \(n = \) number of experiments. The data points are the mean ± 1 SEM.

**FIG. 2.** Calibration curve for the fiber-optic oxygen sensor.

**FIG. 3.** \(p\text{O}_2\) histogram representing the oxygenation of the sarcoma F murine tumor as determined polarographically. \(N = 35; n = 1981\). (\(N = \) number of animals; \(n = \) number of measurements.)

**FIG. 4.** The oxygen distribution of the sarcoma F as determined with the fiber-optic oxygen sensor. \(N = 20; n = 240\). (\(N = \) number of animals; \(n = \) number of measurements.)
sensor identify a range of oxygen tensions from 0 to 60 mm Hg. However, the majority of values are in the range of 0 to 5 mm Hg, consistent with the existence of radiobiological hypoxia.

To assess whether the high proportion of values less than 2.5 mm Hg measured by both of these techniques could be attributed to sampling large regions of necrosis, a histological study was performed. From this the necrotic fraction was found to be very low at 8.3% (SEM ± 0.5) and thus would not be expected to have a major influence on the overall oxygen distribution.

A direct comparison of values obtained with the Eppendorf histograph and the fiber-optic sensor are shown in Table I. It can be seen that overall the values are broadly comparable. Indeed, the percentage of values less than 5 mm Hg (considered to be a measure of radiobiological hypoxia) is almost identical and furthermore is similar to the measured radiobiologically hypoxic fraction in this tumor. However, the median value obtained with the fiber-optic sensor is higher than that obtained with the Eppendorf histograph.

### DISCUSSION

Hypoxia is known to be a key factor responsible for tumor resistance to radiation. *In vitro* studies have also indicated that it can induce resistance to chemotherapeutic drugs (14–16) and alter the cellular responses to cytokines (17–19). The advent of the Eppendorf pO2 histogram has enabled clinical measurements of tumor oxygenation to be made. These studies have shown that hypoxia (pO2 < 10 mm Hg) is a common feature of solid tumors in humans (20). Moreover, hypoxia has been shown to be a prognostic indicator for local control after radiotherapy in head and neck and cervical cancer (21, 22). Likewise, recent studies indicate that hypoxia can predict for distant metastasis and survival in patients with soft tissue sarcomas (23). These studies have shown that routine measurements of tumor oxygenation is the Eppendorf histograph, which uses polarographic techniques for oxygen detection. Recent technical developments have indicated the potential of fiber-optic-based oxygen sensors (6, 7).

The present study has provided the first direct *in vivo* comparison between a recently developed fiber-optic oxygen sensor and the established “gold standard” polarographic oxygen electrode, the Eppendorf histograph. The machines use two totally different processes to measure tissue oxygen tension. The calibration curve for the fiber optic, shown in Fig. 2, illustrates that, while measurements of oxygen tension over the physiological range are possible, increased accuracy at low oxygen tensions (<5%) is a fortuitous consequence of the luminophor’s calibration behavior.

The electrode and the fiber-optic sensor both have similar dimensions with external diameters of 200–300 μm. The insertion of these sensors would be expected to result in similar tissue perturbation. The volume of measurement has been estimated for the Eppendorf as a region of 50–100 cells around the probe tip (3). The sampling volume of the fiber-optic sensor is difficult to estimate accurately. Further work is ongoing to examine the effect of the outer polymer coating upon the accuracy of oxygen measurement; however, it is expected that its contribution will be small and as such probe measurement should be confined primarily to the sensor tip. Coupled with the expected lower oxygen consumption by this technique, the sampling volume should be less than that of the Eppendorf histograph.

Despite the differences in the method of oxygen detection and the different methods of probe insertion and movement, the instruments provided very similar oxygen tension profiles in the murine sarcoma F. Based on previous radiobiological data, radioresistance is associated with pO2 values less than 10 mm Hg and often less than 5 mm Hg. It can be seen that, if compared with the 67% radiobiologically hypoxic fraction actually measured, both sensors recorded percentages of values <5 mm Hg almost identical with this (79% for Eppendorf histogram and 75% for the optical sensor). However, there are some slight but significant differences in the measurements made by the two sensors when other parameters are compared. For example, the median pO2 obtained for the tumors using the fiber optic or the Eppendorf are signifi-
cantly different (2.8 compared to 1.4 mm Hg). This reflects the fact that significantly more readings in the 0–2.5 mm Hg range are detected using the Eppendorf polarographic electrode. While this may be due to sampling differences, oxygen use may be another contributing factor, particularly when comparing a process of measurement that can induce a small degree of depletion with one whose oxygen consumption is probably lower.

This initial study has demonstrated the feasibility of using fiber-optic sensors for detection of tissue oxygenation. Further work is now required in other systems to demonstrate their utility. It is clear that a fiber-optic-based system could offer several advantages over a polarographic method. These include the ability to adapt the system to a multichannel device enabling the detection of temporal changes in tumor oxygenation on a microregional scale, an application that is difficult because of the oxygen use associated with polarographic electrodes.

In summary, these results indicate that the same degree of accuracy regarding oxygen measurement can be obtained with luminescence-based optical oxygen sensors as with polarographic electrodes. The optical sensor used in the current study had an external diameter of 230 μm compared with the Eppendorf needle electrode (300 μm). However, it is possible to develop fiber-optic probes with smaller diameters, thus reducing the degree of tissue damage induced by probe insertion and movement. At present the fiber-optic sensor is not automated to advance through tissue in a manner comparable with the Eppendorf histograph. While this aspect is a major drawback since sampling differences may contribute to a less rigorous average of the overall tissue oxygenation, work is ongoing to allow the addition of a stepper device similar to the histograph. Furthermore, with the expansion into a multi-sensor arrangement, the instrument could provide an oxygen-sensing device with improved clinical potential. However, as with the Eppendorf, the measurement process remains invasive, even if smaller-diameter probes are used. Clearly, the ideal routine clinical procedure should be noninvasive. Much effort is ongoing into the development of such procedures, particularly using magnetic resonance (MR) imaging (24, 25). If such efforts succeed, the development and calibration of these imaging procedures will, in turn, require MR-compatible sensors. Fiber-optic devices, such as the one described here, being nonmagnetic and nonconducting, are ideally compatible with MR technology.

ACKNOWLEDGMENTS

This work was supported by the Cancer Research Campaign (CRC). DRC is funded by a Mount Vernon and Watford Hospitals Trust Studentship award. EML is funded by a Scott of Yews Studentship. The authors would like to thank Mr. P. Russell and his staff for care of the animals in this study.

Received: September 12, 1996; accepted: November 11, 1996

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