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Comparison of two probe designs for determining intraocular oxygen distribution

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Abstract

Introduction—Alterations in intraocular oxygen levels are important contributors to, or indications of ocular disease. Polarographic electrodes and fiberoptic sensors (optodes) have been used to measure oxygen and to map the distribution of oxygen in animal models and in human eyes. A recent study reported the use of a commercial electrode to compare oxygen distribution in the vitreous of patients undergoing vitrectomy related to central retinal vein occlusion, macular hole or preretinal membrane. The results of this study were at variance with previous measures of oxygen distribution in the human vitreous using polarographic or optical sensors. To resolve this discrepancy, the present study compared measurements made in vitro or in animal eyes, using the electrode employed in the previous study or a fiberoptic sensor of different design.

Study design—Comparative in vitro and in vivo measurements.

Results—In vitro, the two devices reported similar levels of oxygen, although the electrode consistently detected levels above the calculated values. In rabbit eyes, the electrode had a slow response time and was unable to detect oxygen gradients that were readily measured by the smaller optode. When the electrode was inserted into an eye of similar size to the human eye, the reference thermistor measured the temperature outside the eye, not in the vitreous.

Conclusions—The design of the electrode used in the previous study makes it unsuitable for measurements of oxygen distribution in the eye.

1. Introduction

Accurate measurement of oxygen partial pressure (pO_2) in biologic tissues has been of interest to scientists and clinicians for many years.¹ The past few years have seen heightened interest in measuring O_2 levels in the retina, vitreous body and lens, because decreased or increased levels of intraocular oxygen have been linked to age-related macular degeneration, diabetic retinopathy, cataract and glaucoma.^{2–9}

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There are two types of commercially-available systems for oxygen measurement: polarographic oxygen electrodes and fiberoptic probes (optodes). These devices have been used in many basic and clinical studies, including measurement of tumor and brain tissue oxygenation. Each type of probe has been used to map the oxygen distribution in animal and human eyes.^{2 9–15}

Recently, Williamson et al. used a commercially-available electrode to measure the oxygen distribution in the eyes of patients during vitrectomy for central retinal vein occlusion.¹⁶ The design of this electrode differed from the polarographic probes used in previous measures of oxygen distribution in the eye. The results obtained also differed from previous studies of oxygen distribution in the human vitreous, whether these studies employed polarographic or fiberoptic probes.^{4 12 13 15 17–19} To better understand the possible sources of this difference, we compared measurement of pO₂ made with a fiber-optic sensor (Oxylab pO₂ optode; Oxford Optonix, Oxford, UK) with those obtained using the Licox polarographic electrode (Licox system; Integra LifeScience, Plainsboro, NJ) employed in the Williamson study. The performance of the two probe designs was compared in vitro and in rabbit eyes. These studies revealed possible explanations for the different values obtained with the two sensors.

2. Materials and methods

In vitro comparison

A two-chamber apparatus was used to determine the accuracy of the probes at different oxygen concentrations. Gas entering the first chamber was bubbled through distilled water. The humidified gas then passed through a sparger into a second fluid-filled chamber, where the oxygen partial pressure was recorded by the Oxylab optode and the Licox electrode. Gases of known oxygen concentration were used as standards (100% nitrogen: 0% O₂; oxygen: 1% and 5%; Airgas Mid America, St. Louis, MO). Measurements were repeated six times for each probe type.

The partial pressure was calculated depending on O₂ concentration in each gas by the following equation:

$$\text{Calculated pO}_2 \text{ [mmHg]} = ((\text{Barometric pressure [mmHg]} - \text{partial pressure of H}_2\text{O [mmHg]}) \times \text{O}_2 \text{ concentration [mmHg]}) / 100$$

Data are presented as mean $\pm$ standard deviation. Statistical analysis (Mann-Whitney-U-Test) was performed by SigmaStat for Windows Version 3.10. A *P*-value of < 0.05 was considered to be statistically significant.

In vivo comparison

Animals—Adult albino rabbits (2.5–4.5 kg; age, 2–6 months) were obtained from Myrtle's Rabbitry (Thompson Station, TN). All animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and with the approval of the Animal Studies Committee of the Washington University School of Medicine.

Anesthesia and oxygen delivery

Animals were first anesthetized with ketamine-xylazine, and then isoflurane was administered through a mask for 3 to 5 minutes before intubation. A 3-0 cuffed endotracheal tube was inserted and connected to an anesthesia machine (Narkomed 2A; North American Drager, Louisville, KY). Anesthesia was then maintained with 2% isoflurane. During the oxygen measurements, hemoglobin saturation (SaO₂), heart rate, respiratory rate, CO₂ inspiration and expiration, and body temperature were monitored in conditions that resembled normal breathing. Rabbits received 20% oxygen, and the rate of respiration was

adjusted to keep the SaO_2 at the normal level of ~97%. The partial pressure of oxygen in different regions of the vitreous chamber was then measured.

Oxygen measurements in rabbit eyes

An optical oxygen probe (Oxylab PO_2 optode; Oxford Optronix, Oxford, UK) and a polarographic microelectrode (Licox REF CC1.P1; Integra LifeScience, Plainsboro, NJ) were used to measure oxygen levels in several locations within the eye. Six series of measurements were obtained for each probe type in the eyes of three rabbits. To measure oxygen partial pressures in the rabbits under circumstances that were as close as possible to physiological, we avoided the use of drugs that might affect ocular blood flow. To avoid damaging the rabbit lens, which occupies a larger proportion of the globe than in the human eye, we used a posterior sclerotomy, 6.5 mm posterior to the corneal limbus. The partial pressure of oxygen was measured, in sequence, in the center of the vitreous chamber (mid-vitreous), in the vitreous body near the avascular retina away from major retinal vessels (near the avascular retina), and in the vitreous body just anterior to major retinal vessels (near the vascular streak). Because bright light can alter the values obtained with the optode, only normal laboratory illumination was used during measurements. We confirmed that this amount of light did not alter the readings by checking oxygen levels with the lights on or off. In the low light that reached the interior of the eye, the blue excitation light at the tip of the probe permitted accurate positioning for each of the oxygen measurements. The mean $\text{pO}_2 \pm \text{SD}$ are reported for each location.

Calibration

The response profile of each optode and microelectrode to oxygen and temperature is calibrated by the manufacturers. A thermistor is incorporated into each probe, permitting the output of the probe to be adjusted for temperature during measurements. Before use, each optode and microelectrode was checked against water samples bubbled with 100% N_2 and 5% O_2 , to confirm the accuracy of the probe in the range of oxygen levels that are typically encountered in the eye (0 to 38 mmHg). Probes were rechecked after use, to assure the accuracy of the measurements.

Comparison of position of the probes using pig eyes

We used pig eyes to illustrate the position of the two probes in an eye of similar size to a human eye.

3. Results

In vitro comparison

Fig. 1A shows the distribution of measured pO_2 concentrations in comparison with the calculated oxygen values using different test gases (pure N_2 , 1% O_2 , and 5% O_2). There were statistically-significant differences between the two probes in all test gases, with the Licox probe consistently reporting higher values ($P < 0.05$). However, there was good correlation between the data generated by the two probes over this test range (Fig. 1B; $R^2 = 0.997$, $P < 0.001$). Consideration of the Bland-Altman plot of the two measurement techniques (Fig. 1C) showed that the consistently higher values for the electrochemical detector explained this correlation. Fig. 1A and the Bland-Altman plot show that the two methods had comparable accuracy at 1% O_2 . However, in pure N_2 , the Oxylab probe reported values closer to the calculated pO_2 (0 mmHg). At 5% O_2 the Oxylab probe slightly underreported, while the Licox probe slightly overestimated pO_2 . Thus, in the physiological range of oxygen levels, either probe provided reproducible measures that were close to the calculated values.

Oxygen gradients measured in vivo

Fig. 2 shows the results of measuring intraocular pO_2 in the eyes of anesthetized rabbits. First, there was a striking difference between the response times of the two probes. The Oxylab probe reached a stable value within 30 seconds, the shortest time recorded, while the Licox probe required at least three minutes to reach a stable value, as reported previously.¹⁶

The spatial sensitivity of the two probes was evaluated by attempting to measure oxygen gradients in the eyes of anesthetized rabbits. The rabbit retina has a vascular streak that extends nasally and temporally from the optic nerve head. Above and below this streak, the retina is avascular. Oxygen partial pressure was measured near the central retina, just over the vascular streak, near the avascular dorsal retina, and in the mid-vitreous chamber. The Licox probe reported the same pO_2 at the three locations. In agreement with previous reports, the Oxylab probe recorded highest pO_2 near the vascular streak, intermediate pO_2 in the mid-vitreous and lowest pO_2 near the avascular retina (Fig. 2).¹⁴

Comparison of the structural features of the two probes

The Licox probe was developed to monitor O_2 in brain tissue. The length of the probe is 462 mm, which permits it to be fastened to the skull while monitoring tissue oxygen levels in the brain. Fig. 3 shows the locations of the oxygen and the temperature sensors in the two probes and the effects of their different designs on the position of the sensors in the eye. In the Oxylab probe, the oxygen and temperature sensors are located at the tip of the probe (Fig. 3A). The temperature and oxygen sensors of the Licox probe are separated by about 15 mm (Figs. 3A, B). About 6 mm separates the tip of the Licox probe from the oxygen sensor, preventing it from recording oxygen close to the probe tip and limiting its use in small areas like the vitreous chamber (Figs. 3B, C). When the Licox probe was inserted into the vitreous cavity of the pig eye, the temperature sensor was outside of the eye.

4. Discussion

In this study, pO_2 measurements using a commercially-available polarographic microelectrode and a fiberoptic optode were compared in vitro and in vivo. Measurement of pO_2 in vitro showed good correlation between the data generated by the two systems, although the electrochemical probe consistently reported values that were higher than those calculated. This result is consistent with previous studies of the accuracy of the Licox probe.^{20 21}

Polarographic electrodes, which measure the current generated when a cathode and anode immersed in an electrolyte solution come into contact with oxygen, have been widely used for monitoring tissue oxygen in clinical and laboratory settings. To measure oxygen, polarographic sensors consume oxygen by electrochemical reduction. Therefore, depending on the physiological conditions, the electrode may underestimate the level of tissue oxygen, especially under conditions of tissue hypoxia or when measuring in a gel, in which oxygen availability is limited by diffusion.

Peterson et al. developed a fiber-optic sensor for the determination of pO_2 .^{22 23} This device is based on the quenching of dye fluorescence by oxygen. The dye is encased at the end of an optical fiber which is connected to instrumentation that measures the fluorescence and interprets it as pO_2 . Short pulses from a light-emitting diode are transmitted along the fiberoptic sensor to excite a platinum-based fluorophore situated at the sensor tip. The resulting emission of fluorescent light, quenched by the presence of oxygen molecules, travels back up the fiber and is detected by the instrument.^{15 22–25} Unlike the polarographic electrode, the optode does not consume O_2 . It also measures equilibrium partial pressure, so it is not dependent on the rate of diffusion to the sensor or through a membrane. As a result,

it is less sensitive to motion or stirring. It has good stability and reproducibility in vitro, is small in size, made of materials that are not toxic to or reactive with body fluids, has little temperature dependence and, in the form used in the current study, can be sterilized for re-use.^{4 15 22 23}

Operating characteristics of the oxygen sensors used in the present study

Optode (OxyLab pO₂ system): Short pulses of LED light are transmitted along the fiberoptic sensor to excite a platinum-based fluorophore at the sensor tip. The fluorophore is enclosed within a silicon matrix. Emitted light travels back up the fiber and is detected by the instrument. The lifetime of fluorescence is inversely proportional to the concentration of dissolved O₂. Temperature is measured by a thermocouple, near the tip of the optode. The temperature calibration curve for each probe is scanned into the monitor using a barcode. The pO₂ signal from the OxyLab probe was averaged every second and recorded with the use of a data-acquisition system (PowerLab; AD Instruments Pty Ltd, Castle Hill, Australia).

O₂ microelectrode (Licox system): The Licox probe (Integra LifeScience) is based on the Clark polarographic electrode, which measures the current generated when a cathode and anode, immersed in an electrolyte solution, come into contact with oxygen.^{26 27} The polarographic cathode and anode are separated from the tissue by a polyethylene membrane (80 µm thickness) that prevents a liquid junction with tissue. Measured current is linearly correlated with pO₂. Since this relationship varies with temperature, each probe has its own current-temperature function. This function is calculated at the time of manufacture and recorded onto a "smart card," which is inserted into the monitor at the time of use. Temperature is measured by a thermocouple in the probe.

Measurement of oxygen in the human eye in vivo

Previous investigators measured oxygen gradients in the vitreous chamber of animals and humans.^{12–14} Oxygen measurements made close to the retina were typically higher than near the lens. Microelectrode measurements showed that this was due to oxygen diffusing from the larger retinal vessels.²⁸ These studies indicate that the retinal vasculature is the primary source of oxygen for the vitreous in both humans and animals. However, the results reported by Williamson were the opposite; oxygen levels were consistently greater in the mid-vitreous than near the retina (33.7 vs. 15.0 mmHg).¹⁶ Williamson, et al. cite an earlier study by Sakaue as consistent with their findings. However, Sakaue reported higher pO₂ in the pre-retinal vitreous (19.9 mmHg) than in the central or anterior vitreous (15.9 and 16.7 mmHg, respectively).^{12 13} There were also significant differences in the absolute values of pO₂ obtained using the Licox electrode and previous studies using polarographic or fiberoptic sensors. As reported above, Sakaue reported a mean pO₂ of 15.9 mmHg in the central vitreous using an electrode^{12 13}, while Holekamp, et al. found mean oxygen partial pressure in the mid-vitreous to be 7.1 mmHg using a fiberoptic sensor.⁴ This value decreased to 5.7 mmHg in diabetic patients.¹⁷ By contrast, Williamson et al. reported that the pO₂ in the central vitreous was 33.7 mmHg in normal eyes and 19.8 mmHg in patients with central retinal vein occlusion.¹⁶ Given the similar performance of the OxyLab and Licox probes in vitro, these differences are difficult to explain, based solely on the ability of the two probes to measure oxygen.

Using the Licox system in the rabbit eye, we measured no difference in oxygen tension between in central and preretinal vitreous when sufficient time was allowed for the probe to reach a stable value. This appeared to be due to the lower spatial sensitivity of the Licox probe and the possibility that temperature compensation was inaccurate, since the thermistor was located outside the eye. In studies in human eyes, the slow response time of the Licox electrode may also be a burden to the surgeon and the patient.¹⁶

The Licox probe is an advanced example of commercially-available polarographic electrode for clinical use. Our analysis confirmed that it accurately reported pO₂ in vitro. However the probe was developed for continuously monitoring pO₂ in brain tissue, where its longer response time and the distance between the probe tip, electrode and thermistor are not likely to be an issue. However, in small areas like the vitreous cavity of the eye, the probe design is limiting. In contrast, the Oxylab probe showed good spatial resolution, revealing a gradient of O₂ tension in the rabbit vitreous cavity, as reported previously for fiber-optic and polarographic electrodes.^{4 12–14 17 28} We suggest that the differences in probe design account for the atypical results obtained when the Licox probe was used to measure pO₂ in human eyes.¹⁶

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Figure 1.

Distribution of measured pO₂ concentrations [mmHg] in comparison with the calculated oxygen values (A), correlation plot of the OxyLab fiber-optic optode and the Licox polarographic probe measurement techniques (B), and comparison of the two oxygen measurement methods using a Bland-Altman plot (C). There was a statistically significant difference between the two probes in all test gases (pure N₂, 1% O₂, and 5% O₂) ($P < 0.05$) (A). However, there was good correlation between the data generated by the OxyLab and the Licox systems ($R^2 = 0.997, P < 0.001$) (B). The Bland-Altman plot indicates that there was a measurement difference (mean 2.5 mmHg) between two systems, with the Licox probe consistently higher. Both methods were comparable at 1% O₂, but in pure N₂ the OxyLab probe reported values closer to the calculated pO₂ (0 mmHg) (*, $P < 0.05$)

Figure 2.

Intraocular oxygen tension in the rabbit eyes using the Licox and the Oxylab systems. The Licox system had a relative long response time and poor spatial resolution in the rabbit eyes. No differences in pO₂ were measured at different intraocular locations (mid-vitreous, near the avascular retina, and near the vascular streak) using the Licox system (A). In contrast, the Oxylab probe detected oxygen gradient in different locations in the vitreous cavity of rabbit eyes (B).

Figure 3.

Comparison of the structural features of the Licox and the Oxylab probes using a pig eye as a reference. Red arrows point to the thermistors (temperature sensors) and white arrows indicate the location of the oxygen-sensitive regions. The thermistor of the Licox probe is also circled with a red dotted line. (A) A magnified view of the two probes. The oxygen-sensitive region of the Licox probe is about 7 mm wide and is located about 6 mm from the probe tip, while the thermistor is an additional 15 mm away from the oxygen sensor. Both sensors are located near the tip of the Oxylab probe. (B) When the probe tips are located in the mid-vitreous, the oxygen-sensitive region of the Licox probe is close to the surface of the eye and may lie partially outside of it. The thermistor is outside the eye. (C) When the probe tips are close to the retina, the oxygen-sensitive region of the Licox probe is near the middle of the vitreous; the thermistor is still outside the eye.