Chorioretinal Vascular Oxygen Tension in Spontaneously Breathing Anesthetized Rats

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Key Words
Choroid · Oxygen · Retina

Abstract
Purpose: To establish baseline and variability of oxygen tension (PO$_2$) measurements in the choroid, retinal arteries, capillaries, and veins of spontaneously breathing anesthetized rats and determine the effect of a moderate surgical procedure on the chorioretinal PO$_2$. Methods: Our previously established optical section phosphorescence imaging technique was utilized to measure PO$_2$ in the chorioretinal vasculatures. Imaging was performed in 29 spontaneously breathing rats under ketamine/xylazine anesthesia. In 7 rats, blood was drawn using a surgically implanted femoral arterial catheter and analyzed to determine the systemic arterial PO$_2$. The PO$_2$ measurements in 22 rats without surgery (group 1) and 7 surgically instrumented rats (group 2) were statistically compared. The intrasubject variability was calculated by the average standard deviation (SD) of repeated measurements. Results: The average systemic arterial PO$_2$ was 52 ± 7 mm Hg (mean ± SD) in group 2. In group 1, the average PO$_2$ measurements in the choroid, retinal arteries, capillaries, and veins were 50 ± 11, 40 ± 5, 39 ± 6, and 30 ± 5 mm Hg, respectively. No statistically significant PO$_2$ differences in any of the chorioretinal vasculatures were found between the two groups (p > 0.4). The intrasubject variability was 3 mm Hg in the choroid, retinal arteries, capillaries, and veins. Conclusions: Chorioretinal PO$_2$ measurements in spontaneously breathing anesthetized rats have a relatively low variability, indicating that PO$_2$ changes due to various physiological alterations can be reliably assessed.

Introduction
Oxygen plays a pivotal role in the maintenance of normal retinal function. Deranged retinal oxygenation is implicated in the development of many retinal diseases including diabetic retinopathy, glaucoma and age-related macular degeneration [1–4]. Rat models of diabetes, glaucoma, and retinal degeneration [5–11] have become available and can be utilized to investigate the role of oxygen in the development of disease-related retinal pathologies.

Several techniques have been utilized to study retinal oxygenation in animals. Retinal tissue hypoxia has been shown to be present in experimental diabetes by inserting oxygen-sensitive microelectrodes through the eye into the retina [12–14]. This technique has also been utilized to measure retinal tissue oxygen consumption in normal animals [15–17], diabetic cats [12], and in a rat model of retinal degeneration [18]. However, placement of microelectrodes has the potential to disturb the retinal microenvironment. Also, oxygenation in only a few retinal locations can be studied, and repeated PO$_2$ measurements at the same location may affect the retinal microenviroment.
iment. Subnormal retinal oxygenation response to hyperoxic challenge has been demonstrated in diabetes by magnetic imaging [19, 20]. However, this technique does not provide a direct measure of tissue PO$_2$ and is limited by its lower resolution compared with optical techniques. Intravascular oxygen tension (PO$_2$) has been measured in normal mice and rats by a phosphorescence imaging technique [21–24]. However, due to the limited depth discrimination, the retinal vascular PO$_2$ measurements were likely to have been influenced by the underlying choroid.

We have previously developed a method to measure PO$_2$ separately in the chorioretinal vasculatures noninvasively with respect to the eye [25–27]. The purpose of the current study was to establish the variability and baseline measurements of intravascular chorioretinal PO$_2$ in spontaneously breathing, anesthetized rats. Establishment of these parameters is needed to assess the PO$_2$ in spontaneously breathing, anesthetized rats. Establishment of these parameters is needed to assess the capability of the system for monitoring PO$_2$ changes over time and detecting PO$_2$ abnormalities due to disease. Additionally, the effect of a moderate surgical procedure on the chorioretinal PO$_2$ was determined.

**Materials and Methods**

**Animals**

Twenty-nine male Long Evans pigmented rats (450–650 g) were used for the study. The animals were treated in compliance with the Statement for the Use of Animals in Ophthalmic and Vision Research of the Association for Research in Vision and Ophthalmology. The rats were anesthetized using ketamine (85 mg/kg i.p.) and xylazine (3.5 mg/kg i.p.). Anesthesia was maintained by an intraperitoneal infusion of ketamine and xylazine at the rate of 0.5 and 0.02 mg/kg/min, respectively. No surgical procedures were performed in 22 rats (group 1). Blood gas analysis was performed in 7 surgically instrumented rats (group 2). The left femoral artery was cannulated and the heparinized catheter was attached to a stopcock. During imaging, blood was drawn from the catheter into a heparinized 0.5-ml syringe that was immediately attached to a stopcock. During imaging, blood was drawn from the catheter into a heparinized 0.5-ml syringe that was immediately subjected to blood gas analysis.

The pupils were dilated with 2.5% phenylephrine and 1% tropicamide. Rectal temperature was maintained between 37 and 38°C via a copper tubing water heater. An oxygen-sensitive molecular probe, either Pd-porphyrin (Frontier Scientific, Logan, Utah, USA) or Oxyphor R2 (Oxygen Enterprises, Philadelphia, Pa., USA), was prepared and injected intravenously [27]. Initially, Pd-porphyrin was used in 22 animals and later Oxyphor R2, which is a formulation of the Pd-porphyrin that is simpler to prepare, was used in 7 animals. Pd-porphyrin oxygen probe was selected due to its sensitivity to oxygen over the range of PO$_2$ typically found in vivo, its peak excitation wavelength that is within the transmission spectrum of the ocular media, and its phosphorescence emission that is minimally absorbed by retinal tissue. Prior to imaging, 1% hydroxypropyl methylcellulose was applied to the cornea and a glass coverslip was placed on the cornea in order to eliminate its refractive power and to prevent corneal dehydration. The rat was placed in front of the imaging instrument. The laser power was adjusted to 100 µW, which is safe for viewing according to the American National Standard Institute for Safety Standards [28].

**Imaging**

The instrument used for measurements of PO$_2$ separately in the retinal and choroidal vasculatures has been described previously [27]. A laser beam (λ = 532 nm) was projected at an oblique angle on the retina following intravenous injection of the probe, and phosphorescence emission was imaged. On the phosphorescence optical section image, choroidal and retinal vasculatures appeared laterally displaced according to their depth location, because the incident laser beam was not coaxial with the viewing axis. Imaging was performed at locations within one-disk diameter from the edge of the optic nerve head.

A frequency-domain approach was used for the measurement of phosphorescence lifetime of the oxygen-sensitive molecular probe. The incident laser light that was used for excitation of the molecular probe and the sensitivity of the camera that was used for detection of the phosphorescence emission were independently modulated. In each eye, phase-delayed images were acquired by varying the phase relationship between the two modulators. The images were analyzed to derive phosphorescence lifetime in the retinal vein, artery, capillaries, and in the choroid [27]. The phosphorescence lifetime measurements were converted to provide measurements of PO$_2$, according to the Stern-Volmer expression:

$$\tau = \frac{1}{\tau_0} = 1 + Q \left( \frac{[O_2]}{[O_2]} \right)$$

where $\tau_0$ (s) is the phosphorescence lifetime, $\tau$ (µs) is the phosphorescence lifetime, $K_Q$ (1/mm Hg µs) is the quenching constant for the triplet-state phosphorescence, and $[O_2]$ is the concentration of oxygen in the tissue. The molecular probe and the sensitivity of the camera that were used for detection of the phosphorescence emission were independently modulated.

**Results**

The systematic arterial and chorioretinal PO$_2$ measurements in group 2 rats are shown in table 1. The average systemic arterial PO$_2$ was 52 ± 7 mm Hg (mean ± SD; n = 7). The average PO$_2$ in the choroid, retinal arteries, capillaries, and veins were 52 ± 7, 43 ± 6, 38 ± 6, and 30 ± 5 mm Hg, respectively. PO$_2$ measurements in each of the four vasculatures were divided by the systemic arterial PO$_2$ to derive relative values. Relative to the systematic arterial PO$_2$, the PO$_2$ measurements in the choroid, retinal arteries, capillaries, and veins were 1.0 ± 0.1, 0.8 ± 0.1, 0.7 ± 0.1, and 0.6 ± 0.1, respectively. No statistically significant PO$_2$ difference in the chorioretinal vasculatures was found between the two groups (p > 0.3). The mean chorioretinal PO$_2$ measurements in group 1, group 2 and combined groups 1 and 2 are shown in ta-
ble 2. The average combined PO$_2$ measurements in the choroid, retinal arteries, capillaries, and veins were $51 \pm 10$, $41 \pm 6$, $39 \pm 6$, and $30 \pm 5$ mm Hg, respectively (fig. 1). The intrasubject variability was $3$ mm Hg in the retinal veins, arteries, capillaries, and in the choroid. The retinal arteriovenous PO$_2$ difference was $10 \pm 3$ mm Hg in group 1, $13 \pm 6$ mm Hg in group 2, and $11 \pm 4$ mm Hg in combined groups 1 and 2. The retinal arteriovenous PO$_2$ difference was determined in each rat and shown in figure 2. The distribution appeared relatively constant, suggesting a consistent oxygen tension drop across the vascular bed. The measured chorioretinal PO$_2$ using Pd-porphyrin and Oxyphor R2 oxygen probes were statistically compared, indicating no significant difference ($p > 0.1$).

**Discussion**

In the current study, systemic arterial PO$_2$ and intravascular chorioretinal PO$_2$ was measured in spontaneously breathing anesthetized rats. The systemic arterial PO$_2$ of the rats in the current study was lower than that reported in previous studies [29, 30], which may be at-
tributed to the respiratory-depressant effect of ketamine and xylazine anesthesia. Anesthesia with ketamine and xylazine administered over a 4-hour period has been shown to have a progressive deleterious effect on systemic oxygenation [31]. In a previous study, retinal venous PO₂ in mice with only one dose of anesthesia was measured by phosphorescence imaging to be between 30 and 45 mm Hg [23]. The measured retinal venous PO₂ in our study was 30 mm Hg and relatively constant, which may be attributed to the higher dosage and continuous administration of anesthesia. The degree of systemic hypoxia due to anesthesia was relatively constant among the rats in the current study, indicating similar cardiorespiratory conditions. Therefore, reliable baseline PO₂ measurements in spontaneously breathing anesthetized rats were established.

Previous studies have demonstrated significant changes in systemic hemodynamics [32] and systemic oxygenation [33] during and immediately following major surgery, which may potentially alter ocular oxygenation. Since blood is drawn from the femoral artery to monitor systemic arterial PO₂ and establish the physiologic conditions of animals during experiments, it is beneficial to determine the effect of this surgical procedure on retinal oxygenation. In the current study, no significant difference was observed in the PO₂ in the chorioretinal vasculatures between rats that had been subjected to surgery and those that had not. The surgical procedure of femoral artery catheterization and the associated increased time under anesthesia did not significantly affect the choroidal PO₂.

Retinal arterial PO₂ measurements were similar to previously reported measurements using a fluorescence quenching technique that measured juxta-arteriolar PO₂ in spontaneously breathing anesthetized rats [34]. However, the retinal arterial and venous PO₂ measurements in the current study were different compared to previously published values obtained in rats with similar systemic arterial PO₂, but using a phosphorescence imaging system that lacked depth discrimination [21]. The difference in the PO₂ measurements is likely related to the capability for differentiating the contribution of the signals from the choroidal and retinal vasculatures. Without depth discrimination, the retinal vascular PO₂ measurements can be significantly influenced by the underlying choroid and choriocapillaris that have higher PO₂ values.

As anticipated, the choroidal PO₂ was comparable to the systemic arterial PO₂, since the choroid is a high-flow vascular system. However, the choroidal PO₂ values measured in the current study were 20% higher than measurements obtained in a previous study using oxygen-sensitive microelectrodes in hypoxic cats with comparable systemic arterial PO₂ [35]. The difference between the measurements may be due to the fact that microelectrodes can penetrate both choroidal tissue and vasculature, while the molecular probe used for obtaining the majority of the data in the current study was bound to albumin and remained in the choroidal vasculature. Additionally, the use of different animal species may have also contributed to the lack of correspondence between the findings of the two studies. Though, to our knowledge, retinal capillary PO₂ has not been previously measured, the PO₂ is expected to be intermediate between the arterial and venous PO₂. However, in the current study, the PO₂ measured in the retinal capillaries was not statistically different from measurements in the retinal artery PO₂ (p > 0.1), which seems to suggest that PO₂ was measured predominantly in the capillaries near the arterial end. Also, the proximity of the measurements to the optic nerve head may have contributed to the above finding. Since there is progressive oxygen loss from the arteries, the blood in the peripheral retina has a lower PO₂. The measurements in the current study were performed in areas near the optic nerve head, where venous blood contained contributions from peripheral retina and had reduced PO₂, hence resulting in similar retinal capillary and artery PO₂ measurements. The intrasubject variability in PO₂ measurements was 3 mm Hg, indicating that changes of about 6 mm Hg (2*SD) in the chorioretinal PO₂ can be detected over time with 95% confidence. The intersubject variability measurements imply that pathologic PO₂ alterations around 8, 12, 12, and 14 mm Hg can be measured with 95% confidence in retinal veins, arteries, capillaries and the choroid, respectively. In future studies, mechanical ventilation of animals will likely further reduce the variability in the measurements. Measurement of chorioretinal PO₂ in experimental animal models of retinal diseases may help elucidate the role of hypoxia in the development of retinal vascular pathologies. Overall, chorioretinal PO₂ measurement has potential value for assessment of changes due to various physiological alterations.

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References


