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 $\pm$ 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 measure-

ments in spontaneously breathing anesthetized rats have a relatively low variability, indicating that PO_2 changes due to various physiological alterations can be reliably assessed.

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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 microenviron-

ment. 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 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 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 μ W, 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 ($\lambda = 532$ 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: $\tau_0/\tau = 1 + (\kappa_Q)(\tau_0)(PO_2)$, where PO_2 (mm Hg) is the oxygen tension, τ (μ s) is the phosphorescence lifetime, κ_Q (1/mm Hg μ s) is the quenching constant for the triplet-state phosphorescence probe, and τ_0 is the lifetime in zero oxygen environment. The intrasubject variability was calculated by the average standard deviation (SD) of repeated measurements. Unpaired Student's *t* test was performed to compare the PO_2 measurements between groups. Significance was accepted at $p < 0.05$.

Results

The systemic 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 systemic 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-

Fig. 1. Chorioretinal PO₂ measurements obtained in 29 spontaneously breathing rats under anesthesia. The error bars represent the standard error of the mean.

Fig. 2. The distribution of retinal arteriovenous PO₂ difference in spontaneously breathing anesthetized rats (n = 29).

Table 1. PO₂ (means ± SD) measurements in the systemic artery and chorioretinal vasculatures in spontaneously breathing anesthetized surgically instrumented rats (group 2)

Group 2	PO ₂ , mm Hg				
	systemic artery	choroid	artery	capillaries	vein
Rat 1	61	61 ± 2	51 ± 4	48 ± 5	38 ± 3
Rat 2	46	48 ± 3	41 ± 3	38 ± 5	30 ± 1
Rat 3	58	60 ± 3	52 ± 5	41 ± 4	27 ± 3
Rat 4	52	49 ± 4	38 ± 3	38 ± 2	31 ± 2
Rat 5	44	44 ± 2	42 ± 2	38 ± 2	25 ± 5
Rat 6	46	47 ± 3	35 ± 3	28 ± 2	24 ± 3
Rat 7	60	56 ± 7	39 ± 5	33 ± 5	32 ± 3

Table 2. Average PO₂ (mean ± SD) measurements in the chorioretinal vasculatures in spontaneously breathing anesthetized instrumented rats

Group	PO ₂ , mm Hg			
	choroid	artery	capillaries	vein
1	50 ± 11	40 ± 5	39 ± 6	30 ± 5
2	52 ± 7	43 ± 6	38 ± 6	30 ± 5
Combined 1 & 2	51 ± 10	41 ± 6	39 ± 6	30 ± 5

Group 1 = No surgical procedure (n = 22); group 2 = surgically instrumented (n = 7).

ble 2. The average combined PO₂ measurements in the choroid, retinal arteries, capillaries, and veins were 51 ± 10, 41 ± 6, 39 ± 6, and 30 ± 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 PO₂ difference was 10 ± 3 mm Hg in group 1, 13 ± 6 mm Hg in group 2, and 11 ± 4 mm Hg in combined groups 1 and 2. The retinal arteriovenous PO₂ 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 PO₂ 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 PO₂ and intravascular chorioretinal PO₂ was measured in spontaneously breathing anesthetized rats. The systemic arterial PO₂ 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 PO_2 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 PO_2 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 PO_2 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 PO_2 and establish the physiologic condition 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 PO_2 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 chorioretinal PO_2 .

Retinal arterial PO_2 measurements were similar to previously reported measurements using a fluorescence quenching technique that measured juxta-arteriolar PO_2 in spontaneously breathing anesthetized rats [34]. However, the retinal arterial and venous PO_2 measurements in the current study were different compared to previously published values obtained in rats with similar systemic arterial PO_2 , but using a phosphorescence imaging system that lacked depth discrimination [21]. The difference in the PO_2 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 PO_2 measurements can be significantly influenced by the underlying choroid and choriocapillaris that have higher PO_2 values.

As anticipated, the choroidal PO_2 was comparable to the systemic arterial PO_2 , since the choroid is a high-flow vascular system. However, the choroidal PO_2 values measured in the current study were 20% higher than mea-

surements obtained in a previous study using oxygen-sensitive microelectrodes in hypoxic cats with comparable systemic arterial PO_2 [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 PO_2 has not been previously measured, the PO_2 is expected to be intermediate between the arterial and venous PO_2 . However, in the current study, the PO_2 measured in the retinal capillaries was not statistically different from measurements in the retinal artery PO_2 ($p > 0.1$), which seems to suggest that PO_2 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 PO_2 . 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 PO_2 , hence resulting in similar retinal capillary and artery PO_2 measurements. The intrasubject variability in PO_2 measurements was 3 mm Hg, indicating that changes of about 6 mm Hg ($2 \times SD$) in the chorioretinal PO_2 can be detected over time with 95% confidence. The intersubject variability measurements imply that pathologic PO_2 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 PO_2 in experimental animal models of retinal diseases may help elucidate the role of hypoxia in the development of retinal vascular pathologies. Overall, chorioretinal PO_2 measurement has potential value for assessment of changes due to various physiological alterations.

Acknowledgements

This study was supported by the National Eye Institute, Bethesda, Md., USA, EY14917 (M.S.) and EY1792 (UIC), and an unrestricted fund from Research to Prevent Blindness, New York, N.Y. (University of Illinois at Chicago).

References

- Anderson DR: Is ischemia the villain in glaucomatous cupping and atrophy; in Brockhurst RJ, Boruchoff SA, Hutchinson BT, Lessell S (eds): *Controversy in Ophthalmology*. Philadelphia, Saunders, 1977.
- Bursell SE, Clermont AC, Shiba T, King GL: Evaluating retinal circulation using video fluorescein angiography in control and diabetic rats. *Curr Eye Res* 1992;11:287–295.
- Stefansson E: Oxygen and diabetic eye disease. *Graefes Arch Clin Exp Ophthalmol* 1990;228:120–123.
- Zarbin MA: Age-related macular degeneration: review of pathogenesis. *Eur J Ophthalmol* 1998;8:199–206.
- Mordes JP, Desemone J, Rossini AA: The BB rat. *Diabetes Metab Rev* 1987;3:725–750.
- Sima AA, Chakrabarti S, Garcia-Salinas R, Basu PK: The BB-rat – an authentic model of human diabetic retinopathy. *Curr Eye Res* 1985;4:1087–1092.
- Miyamoto K, Ogura Y, Nishiwaki H, et al: Evaluation of retinal microcirculatory alterations in the Goto-Kakizaki rat. A spontaneous model of non-insulin-dependent diabetes. *Invest Ophthalmol Vis Sci* 1996;37:898–905.
- Portha B, Blondel O, Serradas P, et al: The rat models of non-insulin dependent diabetes induced by neonatal streptozotocin. *Diabetes Metab* 1989;15:61–75.
- Morrison J, Farrell S, Johnson E, et al: Structure and composition of the rodent lamina cribrosa. *Exp Eye Res* 1995;60:127–135.
- Satoh T, Yamaguchi K: Ocular fundus abnormalities detected by fluorescein and indocyanine green angiography in the Royal College of Surgeons dystrophic rat. *Exp Anim* 2000;49:275–280.
- Matuk Y: Retinitis pigmentosa and retinal degeneration in animals: a review. *Can J Biochem Cell Biol* 1984;62:535–546.
- Linsenmeier RA, Braun RD, McRipley MA, et al: Retinal hypoxia in long-term diabetic cats. *Invest Ophthalmol Vis Sci* 1998;39:1647–1657.
- Alder VA, Su EN, Yu DY, et al: Diabetic retinopathy: early functional changes. *Clin Exp Pharmacol Physiol* 1997;24:785–788.
- Cringle S, Yu DY, Alder V, Su EN: Oxygen tension and blood flow in the retina of normal and diabetic rats. *Adv Exp Med Biol* 1992;317:787–791.
- Linsenmeier RA: Effects of light and darkness on oxygen distribution and consumption in the cat retina. *J Gen Physiol* 1986;88:521–542.
- Cringle SJ, Yu DY, Yu PK, Su EN: Intraretinal oxygen consumption in the rat in vivo. *Invest Ophthalmol Vis Sci* 2002;43:1922–1927.
- Yu DY, Cringle SJ, Su EN: Intraretinal oxygen distribution in the monkey retina and the response to systemic hyperoxia. *Invest Ophthalmol Vis Sci* 2005;46:4728–4733.
- Yu DY, Cringle SJ: Retinal degeneration and local oxygen metabolism. *Exp Eye Res* 2005;80:745–751.
- Berkowitz BA, Kowluru RA, Frank RN, et al: Subnormal retinal oxygenation response precedes diabetic-like retinopathy. *Invest Ophthalmol Vis Sci* 1999;40:2100–2105.
- Berkowitz BA, Roberts R, Luan H, et al: Drug intervention can correct subnormal retinal oxygenation response in experimental diabetic retinopathy. *Invest Ophthalmol Vis Sci* 2005;46:2954–2960.
- Shonat RD, Kight AC: Oxygen tension imaging in the mouse retina. *Ann Biomed Eng* 2003;31:1084–1096.
- Blumenroder S, Augustin AJ, Koch FH: The influence of intraocular pressure and systemic oxygen tension on the intravascular pO₂ of the pig retina as measured with phosphorescence imaging. *Surv Ophthalmol* 1997;42(suppl 1):S118–S126.
- Wilson DF, Vinogradov SA, Grosul P, et al: Oxygen distribution and vascular injury in the mouse eye measured by phosphorescence-lifetime imaging. *Appl Opt* 2005;44:5239–5248.
- Wilson DF, Vinogradov SA, Grosul P, et al: Imaging oxygen pressure in the retina of the mouse eye. *Adv Exp Med Biol* 2005;566:159–165.
- Shahidi M, Blair NP, Mori M, Zelkha R: Feasibility of noninvasive imaging of chorioretinal oxygenation. *Ophthalmic Surg Lasers Imaging* 2004;35:415–422.
- Shakoor A, Shahidi M, Blair NP, Mori M: Noninvasive assessment of chorioretinal oxygenation changes in experimental carotid occlusion. *Curr Eye Res* 2005;30:763–771.
- Shahidi M, Shakoor A, Shonat R, et al: A method for measurement of chorioretinal oxygen tension. *Curr Eye Res* 2006;31:357–366.
- ANSI: American National Standard for Safe Use of Lasers – ANSI Z136.1-1993. Orlando, Laser Institute of America, 1993.
- Sumitra M, Manikandan P, Rao KV, et al: Cardiorespiratory effects of diazepam-ketamine, xylazine-ketamine and thiopentone anesthesia in male Wistar rats – a comparative analysis. *Life Sci* 2004;75:1887–1896.
- Torbati D, Totapally BR, Camacho MT, Wolfsdorf J: Experimental critical care in ventilated rats: effect of hypercapnia on arterial oxygen-carrying capacity. *J Crit Care* 1999;14:191–197.
- Wyatt JD, Scott RA, Richardson ME: The effects of prolonged ketamine-xylazine intravenous infusion on arterial blood pH, blood gases, mean arterial blood pressure, heart and respiratory rates, rectal temperature and reflexes in the rabbit. *Lab Anim Sci* 1989;39:411–416.
- Oztas B, Akgul S, Arslan FB: Influence of surgical pain stress on the blood-brain barrier permeability in rats. *Life Sci* 2004;74:1973–1979.
- Phillips AS, Mirakhur RK, Glen JB, Hunter SC: Total intravenous anaesthesia with propofol or inhalational anaesthesia with isoflurane for major abdominal surgery. Recovery characteristics and postoperative oxygenation – an international multicentre study. *Anaesthesia* 1996;51:1055–1059.
- Zuckerman R, Cheasty JE, Wang Y: Optical mapping of inner retinal tissue PO₂. *Curr Eye Res* 1993;12:809–825.
- Linsenmeier RA, Braun RD: Oxygen distribution and consumption in the cat retina during normoxia and hypoxemia. *J Gen Physiol* 1992;99:177–197.