Retinal Oxygen Tension and Oxygen Reactivity in Retinopathy of Prematurity in Kittens

J. Terry Ernest* and Thomas K. Goldsrick†

In the first series of experiments, preretinal oxygen tensions were measured using microelectrodes in newborn kittens, at 6 ± 2 days of age, following their placement in an atmosphere of 80 to 90% oxygen for 5 ± 1 day. The oxygen exposure caused an obliteration of the normally developing retinal vasculature. Preretinal oxygen tensions in the resultant avascular retina were close to zero while those in the vascular retina were near normal. The avascular retina was a “sink” for ocular oxygen. Oxygen breathing resulted in expected increases in preretinal oxygen tension, but, surprisingly, the preretinal oxygen tension decreased with continued oxygen administration. Because the oxygen tension in the avascular retinal area is determined primarily by the choroidal circulation, we speculated that increased oxygen caused a decreased choroidal blood flow. In the second series of experiments, however, choroidal blood flow was measured in the kittens by applying a temperature probe to the sclera and oxygen breathing did not appear to have an effect. These results did not support the initial speculation. It may be that the decrease in preretinal oxygen tension observed with continued oxygen administration resulted from progressive increase in utilization. Invest Ophthalmol Vis Sci 25:1129–1134, 1984

Immature retinal vessels exposed to high oxygen levels may become permanently obliterated and then, on return to air breathing, optic disk, retinal, and vitreal neovascularization occurs.1,2 It has been hypothesized that under conditions of hyperoxia, the choroidal circulation may furnish abnormally high concentrations of oxygen to the retina, which causes an obliteration of its vasculature.3,4 A return to room air then results in hypoxia, which induces neovascularization.

In the studies herein reported, we compared preretinal oxygen tensions in vascular and avascular retinal areas of kittens, which previously had been exposed to a high oxygen atmosphere. We also measured preretinal oxygen tensions and relative choroidal blood flow in response to 100% oxygen breathing.

Materials and Methods

We used 43 kittens from 14 litters in our study and the investigations conformed to the ARVO Resolution on the Use of Animals in Research. We oxygenated 38 kittens from 10 litters and successfully measured preretinal oxygen tension in 24 kittens from 8 litters and scleral temperature in 7 kittens from 2 litters. The intraocular pressure was measured in one kitten. Because of their low body weight, only a very few blood gas measurements could be made on each kitten but serial blood gases were measured in two kittens, one of which was awake. Vascular cast studies5 were carried out on four oxygen-treated kittens and on five normal kittens. The animals were oxygenated by placing them in an atmosphere of 80–90% oxygen for 5 ± 1 day beginning at ages 4–8 days. The animals, ranging in age from 12–56 days and in weight from 200–600 g, were anesthetized with 50 mg/kg of alpha chloralose administered intravenously as a 10% solution in polyethylene glycol. A tracheostomy was carried out and the animals placed on a respirator (Model 662, Harvard Apparatus Company) and paralyzed with tubocurarine chloride (0.1 mg/kg) and gallamine triethiodide (0.5 mg/kg). The inspiratory pressure was monitored by a pressure transducer (Model 1280C, Hewlett-Packard) and was maintained at 10 mmHg by adjusting the tidal volume. The arterial blood pressure was monitored from a cannulated femoral artery with a pressure transducer and ranged between 50 and 90 mmHg with pulse
rates ranging between 200 and 250 per min. The animal’s temperature was measured and recorded with a rectal thermometer and maintained constant at approximately 38°C with a thermoblocking and temperature control unit (Model TCU 731, EKEG Electronics Co. Ltd.). Arterial blood samples were analyzed for pHi, PCO₂ and PO₂ with a blood pH and gas analyzer (Model 168, Corning). The animal’s pH was maintained at 7.36 ± 0.12 (mean ± SD) by adjusting the respiratory rate and, with the tidal volumes used, this resulted in arterial PCO₂ levels of 28.8 ± 9.3 mmHg and PO₂ levels of 94.5 ± 19.9 mmHg. In the kittens too small to obtain multiple blood samples, the end-tidal PCO₂ was monitored with a medical gas analyzer (Model LB-1, Beckman Instruments). The above values are similar to those obtained from the unanesthetized kitten of comparable age and weight.

In the first series of experiments, preretinal oxygen tension measurements were obtained from 24 kittens from 8 litters using a modification of the technique we have previously described. The pupils were dilated with 10% phenylephrine hydrochloride and 1% cyclopentolate hydrochloride, and the oxygen microelectrode was passed through the pars plana into the vitreous cavity. The microelectrode was controlled with a micromanipulator, and the ocular fundus was observed by an indirect ophthalmoscope. We used selected, glass insulated, gold plated, recessed tip microelectrodes (Model 723, Transidyne General Corp.) modified by greatly reducing the depth of the recess to improve visualization and measurement. The microelectrodes were calibrated in isotonic saline buffered to pH 6.75 equilibrated at 35°C with both 100% nitrogen and 5% oxygen in nitrogen before insertion into the eye and after withdrawal. These two calibrations were reasonably close but because the calibration after withdrawal was usually done within 30 min of the measurements of interest, this last calibration usually was employed. In the eye, the potential of the oxygen microelectrode was adjusted to be the same as that in the calibration bath, −0.800 volts with respect to the potential of a reversible Ag/AgCl electrode in isotonic saline (−0.284 volts). The modification of our previously described procedure, buffering the calibration solution and adjusting the microelectrode potential after its insertion into the eye, was necessary in order to obtain accurate values of the preretinal oxygen tensions close to zero mmHg. The selected microelectrode currents were always less than 2 × 10⁻¹¹ amp in nitrogen and the sensitivities were of the order of 10⁻¹¹ amp/mmHg. The measurements were made in the vitreous humor facing the two different areas of the retina, one having blood vessels and the other, more peripheral to the optic disk, having none. The position of the tip of the microelectrode was unusually difficult to determine optically in the kittens. The microelectrode therefore was advanced until the derivative of the measured electrical current changed sign (ie, if it had been increasing it decreased) perhaps because of bending of the microelectrode. At this point, we also observed relatively large fluctuations in the measured electrical current signaling contact with the retina. The microelectrode was then withdrawn about 200 µm. Since this was at an angle, the tip of the microelectrode was then about 100 µm from the retinal surface.

Preretinal oxygen tensions were obtained from vascular and avascular retinal areas and from the avascular retina either during continuous oxygen breathing or during 1 min episodes of 100% oxygen breathing followed by 4 min episodes on room air. The preretinal oxygen tension transients following continuous oxygen breathing consisted of an exponential increase and then a plateau, while those following intermittent oxygen breathing consisted of a sharp increase to a peak and then a decrease to baseline. These latter were considered to be characterized by the peak values. We were not able to measure avascular preretinal oxygen tensions in normal kittens of comparable age. The retinal vasculature was virtually complete by the time the hyaloid system had atrophied and microelectrodes could be inserted.

To confirm that any changes observed in the peak values were, in fact, caused by changes in the preretinal oxygen tension, a number of separate experiments were conducted to test for artifacts. We measured serial arterial blood oxygen and carbon dioxide tensions and pH from a normal kitten during continuous 100% oxygen breathing who had undergone all of the surgery required for the usual experiment. The values were similar to those from the experimental kittens under similar conditions. We measured the following during the intermittent oxygen breathing protocol: the intraocular pressure, the arterial oxygen tension at the peak value of the preretinal oxygen tension, and the preretinal oxygen tension transients after repositioning the oxygen microelectrode.

In the second series of experiments, we used local scleral temperature to measure choroidal blood flow in seven kittens from two litters. One of us previously used temperature to measure optic nerve head blood flow, and others have used the technique to estimate changes in choroidal blood flow. In the studies herein reported, we used a flat thermistor probe with a diameter of 5 mm (Model 44018, Omega Engineering, Inc.). The current in the circuit was approximately 0.1 mA and the sensitivity was approximately
Table 1. Preretinal oxygen tensions, air breathing

<table>
<thead>
<tr>
<th>Kitten</th>
<th>Age (days)</th>
<th>Weight (g)</th>
<th>Blood pressure mean (mmHg)</th>
<th>Preretinal PO2 (mmHg)</th>
<th>Avascular PO2 (mmHg)</th>
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<td></td>
<td></td>
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<td>453</td>
<td>85</td>
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<td>488</td>
<td>54</td>
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<tr>
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<td>33</td>
<td>575</td>
<td>68</td>
<td>11.2</td>
<td>3.6</td>
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<tr>
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<td>33</td>
<td>564</td>
<td>65</td>
<td>12.8</td>
<td>2.4</td>
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<tr>
<td>Mean</td>
<td>30</td>
<td>520</td>
<td>68</td>
<td>15.6</td>
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6 mV per degree centigrade. The temperature of the sclera could be measured within ±0.01°C. It is difficult to convert the temperature of the sclera into a choroidal blood flow. However, an increase in the intraocular pressure of three times, known to decrease choroidal blood flow by approximately 25%, resulted in a decrease in scleral temperature of approximately 1°C. In this series of experiments, the protocol was the same as that utilized for the intermittent oxygen breathing measurements. Scleral temperature was monitored during 1 min episodes of 100% oxygen breathing followed by 4 min episodes on room air. In this series of experiments, the intraocular pressure was maintained at 15 mmHg by adjusting the height of an infusion bottle connected to an anterior chamber cannula through a pressure transducer.

Results

Examination of normal kittens by ophthalmoscopy and vascular casts demonstrated that at 4–8 days of age, while there was some variability, the retina was usually almost completely vascularized and the choroidal blood vessels appeared normal. Following 5 ± 1 day of 80–90% oxygen exposure, however, the retinal vasculature was obliterated except for minimal vascularization of the optic disc. At this time, the choroidal vessels appeared grossly normal. Within 1–2 weeks after the kittens had been removed from the oxygen atmosphere, there was neovascularization of the optic disc, retina, and vitreous. Up to several weeks, this extended out from the optic disc only a few millimeters.

The air breathing measurements of oxygen tension near retinal areas with and without vascularization are shown in Table 1 from the four kittens with the most accurate preretinal oxygen tension measurements. The measurements obtained from the vitreous are not recorded in the table but they were lower than those obtained from the vascularized retina and higher than those from the avascular retina. As a result, when the microelectrode was moved from the vascularized retina out into the vitreous and then towards the avascular retina, the oxygen tension continuously decreased.

Measurements during oxygen breathing were made from the avascular retinal areas. Figure 1 is a photograph of a typical record obtained during intermittent oxygen breathing. Figure 2 shows the peak values of preretinal oxygen tension, from Figure 1, for each succeeding oxygen administration. Upon return to room air, the oxygen effect lasted a minimum of 30 min. The effect probably lasted longer, but we did not measure it. There was an early effect on the blood pressure, but it then stabilized while the avascular retinal oxygen tensions continued to decrease.

In separate experiments designed to confirm the validity of the experimental oxygen measurements,
the following results were obtained. Intraocular pressure did not change with oxygen breathing. The arterial blood oxygen tension was approximately 400 mmHg at the peak of the preretinal oxygen tension and did not decrease with subsequent oxygen administrations. When the microelectrode was repositioned, there was usually no indication that its position had changed (ie, the succeeding intermittent peaks showed the same trend) although on occasion there did seem to be some movement away from the retina. These latter findings were always accompanied by an inconsistent trend in the peak values, and these were considered to be movement artifacts.

The scleral temperature, reflecting choroidal blood flow, changed with large changes in body temperature but not with the usual ±0.1°C fluctuation in body temperature after the kitten’s body temperature had stabilized. Scleral temperature did change, however, with changes in the systemic blood pressure even over a respiratory cycle. With oxygen breathing systemic blood pressure changed, usually decreasing. Although this caused a small, initial decrease in scleral temperature, there was not a continuous decrease with oxygen breathing (Fig. 3). We interpret this to mean that changes in choroidal blood flow do not explain the decreases in preretinal oxygen tension we observed with oxygen breathing (Figs. 1, 2).

![Fig. 2. Mean systemic blood pressure (top) and preretinal oxygen tension (bottom) at the peak preretinal oxygen tensions shown in Figure 1.](image)

![Fig. 3. Photograph of a representative record of scleral temperature (top) and mean systemic blood pressure (bottom) from a 46-day-old kitten. Oxygen was repeatedly administered for 1 min followed by 4 min of air breathing. The intraocular pressure was maintained at 15 mmHg. The temperature change corresponding to a change in blood flow of approximately 25% was approximately 1 degree centigrade (see text).](image)
Discussion

In this study, we made two different types of preretinal oxygen tension measurements. Air breathing measurements were made in areas of the retina with and without retinal vessels, and oxygen breathing measurements were made in the areas without retinal vessels. The oxygen was either administered continuously or measurements were made during transient episodes of oxygen breathing. The oxygen was administered intermittently to increase the reproducibility of the measurements. Each measurement has specific problems and sources of variability. In the first series of experiments, since the electrode was only in each position a few minutes, current drift and electrode movement were not a problem. It was difficult, however, to determine the precise position of the electrode tip. In previous studies, we have been able to see the indentation of the internal limiting membrane by the tip, and we then could withdraw the electrode 200 μm for the measurements. In the present studies, visual placement was less accurate but electrical methods were employed as described in Materials and Methods, and these permitted placement at about 100 μm from the retinal surface. Any microelectrode drift, either in position or current, would have generated inaccuracies, but as far as we could tell these did not cause the oxygen tension decreases seen in Figures 1 and 2.

The preretinal oxygen tensions in the vascular areas of the retina with the kittens breathing room air were similar to comparable values obtained from normal adult cats. Moving away from the retina into the vitreous, as expected, showed decreased oxygen tension. Movement of the microelectrode from the midvitreous toward the area of the retina without retinal vessels, however, resulted in even lower oxygen tensions (Table 1). It is evident that the avascular retinal area is a “sink” for oxygen from the vascularized areas of the retina. The avascular retina is relatively hypoxic and anaerobic metabolism may dominate, at least in the inner retina. It has been hypothesized that retinal neovascularization is due to hypoxia. It may be that either the products of, or the enzymes accounting for, the anaerobic metabolism diffuse through the vitreous and stimulate neovascularization. Intravitreal injections of lactic acid have been shown to produce retinopathy in kittens, but in a more recent study, lactic acid was not elevated in whole eyes with oxygen induced retinopathy. There may be, however, small but significant metabolic differences between vascular and hypoxic retinal areas within the same eye which could stimulate neovascularization.

Preretinal oxygen tensions were measured with oxygen breathing in avascular retinal areas (Figs. 1, 2). During oxygen breathing, preretinal oxygen tensions increased due to increased oxygen delivery by the choroidal circulation. We were surprised to find, however, that with continuous as well as repeated episodes of oxygen breathing, the oxygen response decreased. Moreover, after returning the kittens to room air breathing following episodes of oxygen breathing, the preretinal oxygen tensions in the avascular areas were below control levels. At first we believed that this oxygen effect was caused by a decrease in choroidal blood flow but, in the second series of experiments in which we measured scleral temperature, this was not borne out. Furthermore, in the normal adult cat, using our Indocyanine green dye clearance method to measure choroidal blood flow, we were not able to demonstrate a significant effect of 100% oxygen breathing on the circulation. The present findings may be explained by an increase in retinal oxygen utilization rather than a decrease in choroidal blood flow. Oxygen breathing may have stimulated dormant enzyme systems and/or “resting” cells might have become active.

It would appear that the choroidal circulation is necessary to produce high enough retinal oxygen tensions during oxygen breathing to cause retinal vascular obliteration since the vaso-obliterative effect of elevated oxygen on the kitten retinal vessels is prevented if the retina is detached and moved away from the choroid. In the normal kitten exposed to oxygen, the choroidal circulation furnishes excessive oxygen resulting in the obliteration of the retinal circulation. Indeed, it has been postulated that retinal vasoconstriction may be a normal physiologic mechanism to protect the immature retina from damaging effects of high blood oxygen levels. If a retinal detachment is induced in a kitten breathing room air, the apparently hypoxic retinal vessels proliferate throughout the retina and into the vitreous. Under these conditions, the retinal neovascularization is similar to that seen in the retinopathy of prematurity induced by oxygen exposure. This is evidence that without an adequate choroidal circulation, the neonatal retinal circulation will proliferate abnormally.

We hypothesize that in the retinopathy of prematurity, the choroidal circulation is initially deleterious because, during oxygen therapy, it furnishes excessive concentrations of oxygen to the inner retina resulting in constriction and obliteration of the retinal circulation. Later, on return to room air, the choroidal circulation is not adequate to provide the inner retinal metabolic needs initiating the process or processes that induce neovascularization. More research
needs to be done to determine the appropriate balance between oxygen therapy and retinal toxicity.

Key words: retinal oxygen tension, choroidal blood flow, kittens, retinopathy of prematurity, retrolental fibroplasia

Acknowledgments

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References