Retinal Oxygen Delivery and Metabolism Response to Hyperoxia During Bilateral Common Carotid Artery Occlusion in Rats

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nadequate oxygen supply is detrimental to the retinal tissue, which is extremely vulnerable due to its high metabolic activity.1 It is well established that hypoxia plays a central role in the events leading to neuronal cell damage and death due to ischemia.2–4 Retinal hypoxia has been implicated in retinal vascular occlusions and in late stages of diabetic retinopathy.5–10 In central retinal vein occlusion, hypoxia has been confirmed by oxygen tension (PO2) measurements during vitrectomy.11 The severe reduction in blood flow and attempts by the tissue to extract more oxygen has been shown to reduce venous oxygen saturation (SO2).12–14 Moreover, reduction in arterial SO2 was detected in branch and central retinal artery occlusions.15,16 In diabetic subjects, decreased intraocular PO2 was measured during vitrectomy.17 Moreover, in advanced diabetic retinopathy stages, obliterated capillaries reduce oxygen supply to regions of the retina, such that less oxygen is extracted, leading to increased retinal venous SO2, as reported in several studies.18–21 Although there are no available techniques for direct measurement of retinal tissue oxygenation in humans, experimental animal models have shown retinal hypoxia in retinal branch vein occlusion in miniature pigs,22 retinal artery occlusion in cats and rats,23,24 and diabetic cats.25

One treatment approach used to minimize retinal hypoxic damage is to increase oxygen supply by hyperoxia (inspiration of 100% O2). The goal of hyperoxia therapy is to restore cellular oxygenation of damaged cells and stimulate them to function more normally. This method reduced vascular endothelial growth factor expression in mice,26,27 inhibited neovascularization in adult nonhuman primate and mouse retina,28,29 and increased retinal oxygen consumption in hypoxia induced by retinal detachment in felines.30,31 Additionally, supplemental oxygen via vitreoperfusion in ischemic feline retinas has been shown to be protective of retinal damage when used with ischemia durations of less than 30 minutes.32,33 In patients with central retinal artery occlusion, hyperbaric oxygen treatment improved visual acuity, as long as irreversible infarction had not developed.34–36 Additionally, studies using normobaric hyperoxia treatment in patients reported better potential for improving visual acuity when administered
24 hours after occlusion compared to occlusions over 2 days.\textsuperscript{36,37} Another study that introduced hyperoxia up to 24 hours after occlusion was unsuccessful in improving visual acuity.\textsuperscript{38} Moreover, during brain ischemia, animal studies have demonstrated the ability of hyperoxia to provide beneficial effects such as an improvement of blood flow,\textsuperscript{39–41} an increase in oxygen delivery,\textsuperscript{42} and a decrease in infarct size\textsuperscript{43–45} when provided promptly during ischemic injury. Notably, it was found that improvement from hyperoxia in a middle cerebral artery occlusion stroke model was observed in occlusions shorter than 30 to 45 minutes.\textsuperscript{46,47}

We have previously reported reduced total retinal blood flow (TRBF), inner retinal oxygen delivery (DO\textsubscript{2}), and oxygen metabolism (MO\textsubscript{2}), and increased oxygen extraction fraction (OEF) both 30 minutes\textsuperscript{46} and 3 days\textsuperscript{47,48} after ischemia in rats induced by permanent bilateral common carotid artery occlusion (BCCAO). However, there is limited knowledge of these measurements in response to hyperoxia after the induction of retinal ischemia and specifically how responses to hyperoxia may change dependent on ischemia duration. This may be helpful to assess any potential benefit to damaged tissue. For example, improvements in these metrics in response to hyperoxia may indicate that the tissue has the potential for recovery after different durations of ischemia, whereas a lack of improvement may be suggestive of irreversible damage. The purpose of the current study was to test the hypothesis that the responses of TRBF, DO\textsubscript{2}, MO\textsubscript{2}, and OEF to hyperoxia are higher after minutes of BCCAO as compared to days of BCCAO.

**Bilateral Common Carotid Artery Occlusion Procedure**

During BCCAO procedures, rats were anesthetized by 1.5% to 2.5% isoflurane. The common carotid arteries were accessed via a midline prelaryngeal incision, dissected from the sympathetic and vagus nerves and ligated, as previously described.\textsuperscript{47,48}

**Imaging**

Prior to imaging, rats were anesthetized by injection of ketamine (90 mg/kg) and xylazine (5 mg/kg). Maintenance doses (45 mg/kg ketamine and 2.5 mg/kg xylazine) were administered during imaging, as needed. As previously described,\textsuperscript{47,48,50} a catheter was placed in the femoral artery for administration of polystyrene fluorescent microspheres (Life Technologies, Eugene, OR, USA) and Pd-porphine (Frontier Science, Boston, MA, USA). Blood velocity was measured by imaging the movement of the microspheres in the vasculature over time, and PO\textsubscript{2} was measured based on the phosphorescence lifetime of Pd-porphine, an oxygen-sensitive molecular probe.\textsuperscript{47,48,50} The Pd-porphine was administered via the catheter at a dosage of 20 mg/kg. Pups were diluted with 2.5% phenylephrine (Paragon, Portland, OR, USA) and 1% tropicamide (Bausch and Lomb, Tampa, FL, USA). A glass cover slip with 2.5% hypromellose ophthalmic demulcent solution (HUB Pharmaceuticals, Plymouth, MI, USA) was applied to the eye for hydration and elimination of refractive power. During imaging, anesthesia was sustained and an animal holder with copper tubing that perfused warm water that maintained body temperature. Systemic SO\textsubscript{2} was measured with a pulse oximeter (SurgiVet V3395 TPR Monitor; Smiths Medical, Waukesha, WI, USA). Saline injections (0.9%) were administered throughout imaging to maintain hydration. Imaging was performed in one eye of each rat.

Retinal venous diameter (D) and blood velocity (V) were measured by our previously described imaging system and used to calculate TRBF.\textsuperscript{50,51} Measurements of individual vessels on red-free images were averaged to obtain mean arterial and venous D (D\textsubscript{A}, D\textsubscript{V}). Displacement of microspheres over time was determined from analysis of fluorescence images acquired at a high rate to obtain multiple V measurements for each vein and averaged to calculate venous velocity (V\textsubscript{V}).\textsuperscript{50,51} Blood flow was calculated in each vein as V\textsubscript{V}τDV\textsubscript{2}/4 and then summed over all the veins to calculate TRBF. Retinal vascular oxygen tension (PO\textsubscript{2}) was measured using our established optical section phosphorescence lifetime imaging system.\textsuperscript{47,48,50,51} Phosphorescence lifetimes of Pd-porphine in the retinal arteries and veins were converted to PO\textsubscript{2} measurements using the Stern–Volmer equation.\textsuperscript{52,53} Vascular O\textsubscript{2} content was calculated as the sum of oxygen bound to hemoglobin and dissolved in blood.\textsuperscript{54} O\textsubscript{2} content = SO\textsubscript{2} × HgB × C + k × PO\textsubscript{2}. SO\textsubscript{2} is the oxygen saturation calculated from PO\textsubscript{2} and the rat hemoglobin dissociation curve.\textsuperscript{55} Hgb is the rat hemoglobin concentration value (13.8 g/dL).\textsuperscript{56} C is the maximum oxygen-carrying capacity of hemoglobin (1.39 mL O\textsubscript{2}/g).\textsuperscript{57} and k is the solubility of oxygen in blood (0.0032 mL O\textsubscript{2}/DL mm Hg). Arterial (O\textsubscript{2A}) and venous (O\textsubscript{2V}) oxygen contents were calculated by averaging values in retinal arteries and veins, respectively. Arteriovenous oxygen content difference (O\textsubscript{2AV}) was calculated as the difference between O\textsubscript{2A} and O\textsubscript{2V}. DO\textsubscript{2}, MO\textsubscript{2}, and OEF were calculated as TRBF × O\textsubscript{2A}, TRBF × O\textsubscript{2AV}, and...
**RESULTS**

**Systemic Condition**

Systemic SO2 and O2A measurements are presented in Table 1. Under RA, the systemic SO2 was 75% ± 9%, 74% ± 5%, and 75% ± 4% in the 30 minutes, 1 day, and 3 days BCCAO groups, respectively. Under hyperoxia, the systemic SO2 was 93% ± 1%, 93% ± 4%, and 92% ± 4% in the 30 minutes, 1 day, and 3 days BCCAO groups, respectively. SO2 was significantly higher under hyperoxia than that under RA in all BCCAO groups (P < 0.001). Under RA, O2A was 9.3 ± 2.1 mLO2/dL, 11.7 ± 2.1 mLO2/dL, and 11.5 ± 1.4 mLO2/dL in the 30 minutes, 1 day, and 3 days BCCAO groups, respectively. Under hyperoxia, O2A was 15.8 ± 2.1 mLO2/dL, 17.4 ± 2.7 mLO2/dL, and 17.9 ± 2.2 mLO2/dL in the 30 minutes, 1 day, and 3 days BCCAO groups, respectively. O2A was significantly higher under hyperoxia than that under RA in all BCCAO groups (P < 0.001).

| Characteristic | Baseline | 30 Minutes | 1 Day | 3 Days |
|----------------|----------|------------|-------|--------|
| Systemic SO2  | RA, %    | 75 ± 9     | 74 ± 5| 75 ± 4 |
|               | Hyperoxia, % | 95 ± 1    | 95 ± 4| 92 ± 4 |
| P value       | <0.0001  | <0.0001    | <0.0001|        |
| O2A (mLO2/dL) | RA       | 9.3 ± 2.1  | 11.7 ± 2.1| 11.5 ± 1.4 |
|               | Hyperoxia| 15.8 ± 2.1 | 17.4 ± 2.7| 17.9 ± 2.2 |
| P value       | <0.0001  | <0.0001    | <0.0001|        |

Below the SO2 and O2A values are the P values from paired t-tests between RA and hyperoxia in each BCCAO group. Bold indicates significant difference (P ≤ 0.05). SO2 and O2A were significantly higher after hyperoxia compared to RA in the 30 minutes, 1 day, and 3 days BCCAO groups.

**Total Retinal Blood Flow**

TRBF measurements are presented in Table 2. TRBF at baseline was 6.5 ± 1.1 μL/min. Measurements in the 30 minutes, 1 day, and 3 days BCCAO groups under RA were 1.5 ± 0.8 μL/min, 3.0 ± 1.8 μL/min, and 2.6 ± 0.9 μL/min, respectively. Measurements in the 30 minutes, 1 day, and 3 days BCCAO groups under hyperoxia were 1.9 ± 0.7 μL/min, 1.9 ± 1.2 μL/min, and 2.4 ± 1.4 μL/min, respectively. FIO2 condition and BCCAO duration had a significant interaction effect on TRBF (P = 0.005). TRBF did not differ between RA and hyperoxia after 30 minutes or 3 days of BCCAO (P ≥ 0.18) but was decreased under hyperoxia as compared to RA after 3 days of BCCAO.

| Characteristic | Baseline | 30 Minutes | 1 Day | 3 Days |
|----------------|----------|------------|-------|--------|
| TRBF (μL/min)  | RA       | 6.5 ± 1.1  | 1.5 ± 0.8| 3.0 ± 1.8| 2.6 ± 0.9 |
|               | Hyperoxia| 1.9 ± 0.7  | 1.9 ± 1.2| 2.4 ± 1.4|        |
| P value        | 0.18     | 0.008      | 0.41  |        |
| DO2 (nLO2/min) | RA       | 937 ± 168  | 147 ± 97| 381 ± 284| 303 ± 114 |
|               | Hyperoxia| 311 ± 139  | 352 ± 254| 450 ± 284|        |
| P value        | 0.006    | 0.46       | 0.07  |        |
| MO2 (nLO2/min) | RA       | 267 ± 95   | 143 ± 95| 182 ± 102| 214 ± 89 |
|               | Hyperoxia| 241 ± 75   | 104 ± 75| 152 ± 44|        |
| P value        | 0.02     | 0.04       | 0.06  |        |

Below the TRBF, DO2, and MO2 values are the P values from paired t-tests between RA and hyperoxia in each BCCAO group. Bold indicates significant difference (P ≤ 0.05). In response to hyperoxia, only MO2 after 30 minutes of BCCAO approximated baseline values.
1 day of BCCAO ($P = 0.008$). Under RA, TRBF was lower after 30 minutes of BCCAO compared to 1 day of BCCAO ($P = 0.03$), whereas under hyperoxia, there were no differences in TRBF among BCCAO groups ($P = 1.00$). Compared to baseline, TRBF was reduced in all BCCAO groups under hyperoxia ($P < 0.001$).

**Oxygen Delivery**

$DO_2$ measurements are presented in Table 2. $DO_2$ at baseline was $937 \pm 168 \text{nLO}_2/\text{min}$. Measurements in the 30 minutes, 1 day, and 3 days BCCAO groups under RA were $147 \pm 97 \text{nLO}_2/\text{min}$, $381 \pm 284 \text{nLO}_2/\text{min}$, and $303 \pm 114 \text{nLO}_2/\text{min}$, respectively. Measurements in the 30 minutes, 1 day, and 3 days BCCAO groups under hyperoxia were $311 \pm 139 \text{nLO}_2/\text{min}$, $352 \pm 254 \text{nLO}_2/\text{min}$, and $450 \pm 284 \text{nLO}_2/\text{min}$, respectively. $FiO_2$ condition and BCCAO duration had a significant interaction effect on $DO_2$ ($P = 0.03$). $DO_2$ was increased under hyperoxia as compared to RA after 30 minutes of BCCAO ($P = 0.006$). There was no statistically significant change in $DO_2$ under hyperoxia after 1 or 3 days of BCCAO ($P \geq 0.07$). Under RA, $DO_2$ was lower after 30 minutes of BCCAO compared to 1 day of BCCAO ($P = 0.02$), while under hyperoxia, $DO_2$ was not significantly different among BCCAO groups ($P \geq 0.54$). Compared to baseline, $DO_2$ was reduced in all BCCAO groups under hyperoxia ($P \leq 0.04$).

**Oxygen Metabolism**

$MO_2$ measurements are presented in Table 2. $MO_2$ at baseline was $267 \pm 95 \text{nLO}_2/\text{min}$. Measurements in the 30 minutes, 1 day, and 3 days BCCAO groups under RA were $143 \pm 95 \text{nLO}_2/\text{min}$, $182 \pm 102 \text{nLO}_2/\text{min}$, and $214 \pm 89 \text{nLO}_2/\text{min}$, respectively. Measurements in the 30 minutes, 1 day, and 3 days BCCAO groups under hyperoxia were $241 \pm 75 \text{nLO}_2/\text{min}$, $104 \pm 75 \text{nLO}_2/\text{min}$, and $152 \pm 44 \text{nLO}_2/\text{min}$, respectively. $FiO_2$ condition and BCCAO duration had a significant interaction effect on $MO_2$ ($P < 0.001$). $MO_2$ was increased under hyperoxia as compared to RA after 30 minutes of BCCAO ($P = 0.02$). Additionally, $MO_2$ was decreased under hyperoxia as compared to RA after 1 day of BCCAO ($P = 0.04$). There were no significant differences in $MO_2$ among BCCAO groups under RA ($P \geq 0.35$), while under hyperoxia, $MO_2$ was higher after 1 and 3 days of BCCAO compared to 30 minutes of BCCAO ($P \leq 0.02$). $MO_2$ was not significantly different than baseline after 30 minutes of BCCAO under hyperoxia ($P = 1.00$), but was lower than baseline after 1 and 3 days of BCCAO ($P \leq 0.02$).

**Oxygen Extraction Fraction**

$FiO_2$ condition and BCCAO duration did not have a significant interaction effect on OEF ($P = 0.41$). However, both BCCAO duration and $FiO_2$ condition had significant main effects on OEF ($P < 0.001$). OEF was lower under hyperoxia ($0.57 \pm 0.04$) compared to that under RA ($0.79 \pm 0.04$) ($P < 0.001$). OEF after 30 minutes of BCCAO ($0.90 \pm 0.05$) was higher than that after 1 and 3 days of BCCAO ($0.54 \pm 0.06$ and $0.59 \pm 0.06$, respectively) ($P \leq 0.003$). Under hyperoxia, OEF was higher than baseline after 30 minutes of BCCAO ($P < 0.001$) but was not different from baseline after 1 and 3 days of BCCAO ($P \geq 0.30$).

**DISCUSSION**

This is the first time, to our knowledge, that the effect of hyperoxia on physiologic retinal parameters after various durations of partial ischemia has been reported. It was also novel that we measured these parameters in the same animals at baseline, which is rarely possible clinically. The results confirmed our hypothesis that responses in oxygen metrics are dependent on BCCAO duration.

**Choroidal Oxygen Supply**

In numerous studies, the choroid has been shown to contribute an increased amount of oxygen to the retina during hyperoxia. In a normally perfused retina under RA, an increase in oxygen causes vasoconstriction. In addition, with the administration of hyperoxia, blood flow decreases to autoregulate $O_2$ delivery since there is increased oxygen from the choroid. Additionally, $MO_2$, which indicates the rate at which $O_2$ is extracted from the retinal circulation, cannot represent some of the $O_2$ consumed by the part of the inner retina close to the choroid. So hyperoxia could cause $MO_2$ to be reduced even if the total amount of oxygen consumed by the inner retina was the same. Therefore, without ischemia, hyperoxia should decrease TRBF, $DO_2$ (the product of blood flow and $O_2A$), and $MO_2$. However, if hyperoxia increases these factors, the increase could be falsely low because of the amount of oxygen being supplied by the choroid to part of the inner retina. Alternatively, if hyperoxia decreases these factors, it may indicate that the results are dominated by choroidal oxygen supply. Additionally, BCCAO causes choroidal blood flow to be decreased. This makes interpretation of our results more difficult.

**Total Retinal Blood Flow**

TRBF showed no response to hyperoxia after 30 minutes of BCCAO. In a normally perfused rat retina, administration of supplemental $O_2$ causes a decrease in blood flow. However, in the current study, the lack of TRBF response to hyperoxia suggests a decreased autoregulatory vasoconstriction due to sustained oxygen demand under experimentally induced reduced blood flow for a short duration. In contrast, after 1 day of BCCAO, hyperoxia caused TRBF to decrease, which may be attributed to an increase in choroidal oxygen supply, consistent with a physiologic retinal vascular response. The reason for the finding of no significant change in TRBF in response to hyperoxia after 3 days of BCCAO is not clear, although we may speculate lower oxygen demand due to cell death after longer durations of BCCAO.

**Oxygen Delivery**

Since $DO_2$ is the product of TRBF and $O_2A$, both of these factors contribute to its response to hyperoxia. $DO_2$ increased in response to hyperoxia after 30 minutes of BCCAO primarily due to increased $O_2A$. However, after 1 day of BCCAO, no change to $DO_2$ was observed in response to hyperoxia, which can be attributed to decreased TRBF, as previously discussed, coupled with an increase in $O_2A$. Similarly, hyperoxia caused no change in $DO_2$ after 3 days of BCCAO. At longer durations of BCCAO, lower oxygen demand due to cell death and increased oxygen...
supply from the choroidal circulation contributed to maintaining DO2.

Oxygen Metabolism

We found that MO2 increased and approximated to normal levels in response to hyperoxia after 30 minutes of BCCAO. With such a short duration of reduced blood flow, very few retinal cells would have become unable to consume oxygen. Thus, with increased oxygen availability, retinal cells were able to extract enough oxygen to essentially normalize MO2. Additionally, because of the possible contribution of O2 from the choroid, the improvement in MO2 may be underestimated. In contrast, after 1 and 3 days of BCCAO, there was a decrease in MO2 in response to hyperoxia. Since it is unlikely that 15 minutes of hyperoxia would result in a significant loss in ability to metabolize oxygen, this decrease in MO2 appears to be attributable to an increase in oxygen availability from the choroid.

Oxygen Extraction Fraction

OEF was lower under hyperoxia compared to that under the RA condition, in which the tissue was metabolizing nearly all of the oxygen being delivered by the vasculature. Hyperoxia significantly increased oxygen availability to the retina, reducing OEF to a level that reflected healthier tissue. Furthermore, regardless of FiO2 condition, OEF was found to be higher after 30 minutes of BCCAO than that after 1 and 3 days. After such a short duration of BCCAO, the retina was extracting an average of 90% of the oxygen being delivered to the retina through the vasculature, which indicates marginally oxygenated tissue. On the other hand, after longer durations, choroidal flow presumably would have improved, causing a decrease in MO2 as discussed, which would have resulted in a lower OEF (0.55–0.60). Moreover, OEF under hyperoxia reverted to approximately normal values after 1 and 3 days of BCCAO.

Hyperoxia Treatment Window

Responses of DO2, MO2, and OEF to hyperoxia demonstrated the higher treatment potential after a short duration of ischemia compared to durations of a day or more. Other studies have similarly found that hyperoxia is most effective as a treatment when administered between 15 and 45 minutes after the onset of ischemia in neural and retinal tissue and that reducing the time between injury and treatment enhances the degree of improvement.4,9,40,64 Additionally, a study found that transient ischemia in neural tissue was more treatable with hyperoxia compared to permanent ischemia.9,40 Our results imply that decreasing the time interval between ischemic insult and hyperoxia treatment would optimize the potential for tissue to recover.

Limitations

The current study had several limitations. First, we did not measure systemic blood oxygen tension directly, although systemic SO2 was measured. Second, anesthesia used during imaging caused some degree of systemic hypoxia, as was confirmed by SO2 measurements, which may have limited our findings of responses to hyperoxia. Third, the small sample size may have increased the probability of making a type II error, such that some statistically significant differences may not have been found when they actually existed. Fourth, for calculations of O2A and O2V from the hemoglobin dissociation curve, a constant value for blood pH was used and variations due to hyperoxia66 were not accounted for. Fifth, it is possible that preconditioning by the ischemic insult may trigger endogenous neuroprotection, thus augmenting the effect of hyperoxia. Future studies are needed to investigate and differentiate the benefits of these two factors.

Conclusion

Our findings suggest that administration of supplemental oxygen minutes after ischemia has the potential to mitigate oxygen delivery impairments and revert oxygen metabolism approximately to normal values. Overall, the findings of the current study contribute to the knowledge of the effect of supplemental oxygen intervention on impaired retinal oxygen metrics due to experimental ischemia.

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