The Use of Hyperbaric Oxygen Therapy in the Treatment of Sickle Cell Hyphema

Christopher R. Wallyn,* Lee M. Jampol,+ Morton F. Goldberg,§ and Claude L. Zanetti§

Eyes of adult albino rabbits were injected intracamerally with either normal saline or anticoagulated human sickle cell blood to study the effect of hyperbaric oxygen administration on sickle cell hyphema. The administration of two atmospheres of 100% oxygen for 2 hr to the rabbits raised the pO₂ of the aqueous humor from a baseline value of 63.5 ± 12.3 mmHg (mean ± SD) to 620 ± 133 mmHg in saline-injected eyes and 503.7 ± 89.1 mmHg in eyes injected with human sickle cell blood. This rise in pO₂ was highly significant (P < 0.0001). The percentage of sickled cells in the anterior chamber 2 hr after injection decreased from 35.7 ± 32.4% in rabbits breathing room air to 4.1 ± 2.8% in rabbits exposed to hyperbaric oxygen for 2 hr. Hyperbaric oxygen can thus significantly raise aqueous humor pO₂ values and decrease the sickling of erythrocytes in the anterior chamber and may be of value in patients with sickle cell hyphema. Invest Ophthalmol Vis Sci 26:1155-1158, 1985

Patients with sickling hemoglobinopathies, including sickle cell trait, have an increased risk for permanent loss of vision following hyphema caused by blunt trauma, penetrating ocular injury, or intraocular surgery. This group of patients composes about 10% of the black population in the United States.1 Several factors in the anterior chamber enhance intracameral sickling, including a high ascorbate level, low pH, high pCO₂, and low pO₂.2,3 The sickled erythrocytes cause a logjam in the trabecular meshwork, which decreases aqueous humor drainage through the trabecular meshwork; this may result in a severe secondary glaucoma. An elevated intraocular pressure and a tendency for sickling in the ocular microcirculation increase the risk for ischemic damage to the optic nerve and retina.4

The present protocol was designed to evaluate if hyperbaric oxygen therapy can raise aqueous humor pO₂ levels, and if high partial pressures of oxygen in aqueous humor can prevent or reverse sickling of intracameral erythrocytes.

**Materials and Methods.** Experimental hyphema was induced in 2- to 3-kg adult albino rabbits using anticoagulated human sickle cell blood obtained from a donor with SB-thalassemia (sickle cell thalassemia). After collection by venipuncture, the donor blood was immediately anticoagulated with EDTA and maintained at room temperature until being injected into rabbit anterior chambers within 1 hr.

All rabbits were initially anesthetized using a 1-ml intramuscular injection of a 1:5 mixture of acepromazine (10 mg/ml) and ketamine (100 mg/ml). This was supplemented as necessary. Additional anesthesia was achieved using topical proparacaine prior to paracentesis.

Paracentesis was performed on both eyes of each rabbit to determine the baseline level of aqueous humor pO₂. The paracentesis was accomplished using a 0.165-ml heparinized capillary tube with a fixed 25-gauge needle. The needle was inserted directly through the corneal limbus and into the anterior chamber, where the capillary tube was allowed to fill completely. After this initial aspiration of primary aqueous humor, 0.15 ml of anticoagulated human SB-thalassemia blood was injected into the right eye of each rabbit through the corneal limbus using a 30-gauge needle and a tuberculin syringe. The paracentesis tracts were tamponaded with minimal pressure with a cotton-tipped applicator; slight occasional leakage occurred but could not be quantitated. In the left eye of each rabbit, 0.15 ml of saline was injected in an identical manner to serve as a control for the paracentesis. Following the establishment of a hyphema in the right eye and a saline control in the left eye, half of the rabbits were randomly selected for a 2-hr trial of hyperbaric oxygen therapy and half were kept in room air as controls.

The experiment was conducted in a large, "walk-in" hyperbaric chamber (Vacudyne; Chicago Heights, IL) at 2 atm pressure absolute, 14.7 lbs/in² gauge pressure (PSIG). The calibration of the four individual chamber pressure gauges (Crosby 0-90 PSIG, Crosby Manufacturers; Boston, MA) was checked against a mercury manometer at 5 PSIG (259 mmHg). All gauges read within 0.5 PSI of one another at 14.7 PSIG.

Half of the anesthetized rabbits were transported to the hyperbaric chamber, placed inside a sealed...
The first few drops taken from each hyphema eye were immediately fixed in 2% buffered glutaraldehyde and prepared in the same fashion as the hyphema samples. The mean value for the baseline pO2 of rabbit primary aqueous humor was 63.5 ± 12.3 mmHg (n = 12) (Table 1). Two hours after establishing an experimental hyphema, the pO2 values were statistically no different from the baseline value, with a mean pO2 of 58.9 ± 11.8 mmHg (n = 6). After two hours of hyperbaric oxygen therapy, we found a dramatic increase in the pO2 of aqueous humor in both the hyphema eye and the saline control eye. The mean pO2 for the hyphema eye was 503.7 ± 89.1 mmHg (n = 11), while the saline control eye was significantly higher at 620.3 ± 133 mmHg (n = 14). Both values are obviously increased over the baseline pO2, but statistical analysis showed the hyphema eyes to have significantly lower values than the saline eyes (P < 0.02).

Sickle cell counts: A count of sickled erythrocytes was done on the donor venous blood prior to injection into the anterior chamber. The blood was fixed with 2% buffered glutaraldehyde and prepared in the same fashion as the hyphema samples. The mean value for the percentage of sickled cells was found to be 44.75 ± 15.9 (n = 4) (Table 2).

Two hours after injection into the anterior chamber the percentage of cells found to be sickled in aqueous humor was 35.7 ± 32.4 (n = 9). This value corresponds to the animals kept at ground level, breathing room air for 2 hr, and it does not differ statistically from the venous blood sample. The rabbits exposed from the oxygen tent. The capillary tubes were then sealed, placed on ice, and passed out of the chamber through a rapid transfer compartment for analysis of the pO2. All aqueous humor samples were analyzed at ground level pressure using a pH/blood gas analyzer (IL System 1303, Instrument Lab System; Lexington, MA).

The hyphema samples were collected and fixed in 2% buffered glutaraldehyde, coded, and prepared using the Cytospin technique. Counts of the percentage of cells in the sickled configuration were done in a controlled fashion by a masked observer (M.F.G.).

Statistical analysis was used to determine the significance of certain mean values in analyzing the results. A paired t-test was done on all data for which the variances were equal. A two-sample t-test was done on all data for which the variances were not equal. This was done to ensure the validity of the basic assumption that all the data are independent, identically distributed, and normal. Data are expressed as mean ± 1 standard deviation (SD).

All procedures involving animals were performed in adherence to the ARVO Resolution on the Use of Animals in Research.

**Results. pO2 determinations:** The aqueous samples obtained by the initial paracentesis were used to establish a baseline value for the pO2 of rabbit primary aqueous humor. The mean value for the baseline pO2 was 63.5 ± 12.3 mmHg (n = 12) (Table 1). Two hours after hyperbaric treatment, we found a dramatic increase in the pO2 of aqueous humor in both the hyphema eye and the saline control eye. The mean pO2 for the hyphema eye was 503.7 ± 89.1 mmHg (n = 11), while the saline control eye was significantly higher at 620.3 ± 133 mmHg (n = 14). Both values are obviously increased over the baseline pO2, but statistical analysis showed the hyphema eyes to have significantly lower values than the saline eyes (P < 0.02).

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**Table 1. Aqueous humor pO2 values**

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<thead>
<tr>
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<th>Mean pO2 (mmHg)</th>
<th>SD</th>
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<tbody>
<tr>
<td>Baseline (primary aqueous humor, n = 12)</td>
<td>63.5*</td>
<td>12.3</td>
</tr>
<tr>
<td>Without hyperbaric oxygen therapy After 2 hr, OD (hyphema, n = 6)</td>
<td>58.9*</td>
<td>11.8</td>
</tr>
<tr>
<td>After 2 hr, OS (saline, n = 6)</td>
<td>64.2*</td>
<td>14.4</td>
</tr>
<tr>
<td>With hyperbaric oxygen therapy After 2 hr, OD (hyphema, n = 11)</td>
<td>503.7†</td>
<td>89.1</td>
</tr>
<tr>
<td>After 2 hr, OS (saline, n = 14)</td>
<td>620.3†</td>
<td>133.0</td>
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* The differences among these three values was not significant.
† The difference between these two values was significant with P < 0.02.

**Table 2. Percent of sickled cells in aqueous humor**

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
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<tbody>
<tr>
<td>Venous sample before experiment (n = 4)</td>
<td>44.75*</td>
<td>15.9</td>
</tr>
<tr>
<td>% Sickled cells in rabbits without hyperbaric oxygen therapy (n = 9)</td>
<td>35.7†</td>
<td>32.4</td>
</tr>
<tr>
<td>% Sickled cells in rabbits with hyperbaric oxygen therapy (n = 17)</td>
<td>4.1†</td>
<td>2.8</td>
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* These two values are not significantly different.
† These two values are statistically different with P < 0.019.
to hyperbaric oxygen therapy for 2 hr had a significantly decreased percentage of sickled cells in aqueous humor with a mean value of 4.1 ± 2.8 (n = 17; P < 0.019).

Discussion. Ocular trauma with subsequent hyphema can result in visual loss through several mechanisms. Persistence of hemorrhage in the anterior chamber can lead to blood staining of the cornea. Alteration in filtration mechanics can cause a severe secondary glaucoma, which may lead to a permanent loss of vision due to optic nerve atrophy and retinal ischemia.

Patients with S-hemoglobin and hyphema are at an increased risk for these types of ocular damage because of the tendency for erythrocytes to sickle in the anterior chamber, thereby influencing filtration mechanics. Goldberg and co-workers found the anterior chamber and aqueous humor to be a very harsh environment for erythrocytes with S-hemoglobin. Erythrocytes with S-hemoglobin sickle much more readily in aqueous humor than in venous blood and become trapped in the trabecular meshwork, resulting in decreased aqueous humor outflow. This diminished outflow may result in persistence of hyphema and elevated intraocular pressure. Sickling of erythrocytes in the microcirculation of the optic nerve and retina predisposes these patients to visual loss, even at levels of intraocular pressure that are safe in patients with normal hemoglobin. For these reasons, a therapy to decrease sickling would be of great value in the treatment of sickle cell hyphema.

Hyperbaric oxygenation has been used during scleral buckling procedures to prevent anterior segment necrosis in patients with sickling hemoglobinopathies. Central retinal artery occlusion and other vascular insufficiencies of the retina and choroid have been treated experimentally with some apparent success in patients with normal hemoglobin. We have demonstrated that hyperbaric oxygenation can raise aqueous humor pO2, and that this higher pO2 reduces the amount of sickling present. It is acknowledged that underestimation of the aqueous humor pO2 measurements in the hyperbaric-treated animals may have occurred due to “off-gassing” of supersaturated oxygen with decompression of the samples to the surface. We believe, however, that the values measured represent the minimal pO2 values achievable in the hyperbaric chamber. Whether the oxygen enters the eye directly through the cornea or through the vasculature was not investigated in these experiments. Many investigators have studied the oxygen tension (pO2) of aqueous humor of various species, and the data have shown considerable variation. Recent data for rabbit aqueous humor pO2 values include a pO2 of 72 mmHg (see Kwan et al) and 30.5 mmHg (see Wegener and Moller). It has been well demonstrated that the pO2 within the anterior chamber varies according to location and the method of measurement. Our measurements represent an average pO2 of aqueous humor after it has been removed from the anterior chamber.

Our studies have clearly shown that the administration of 2 hr of hyperbaric oxygen therapy can reverse or prevent sickling of intracameral erythrocytes. We have not determined if sickling occurs upon discontinuation of the oxygen although it seems likely that it does. The time course of this further sickling would be of importance in determining the schedule of hyperbaric oxygen needed to treat human hyphemas. Hyperbaric oxygen can be toxic. We observed no clinical toxicity in the rabbits during our 2-hr experiment, but no histopathologic studies were done. It is certain that toxicity (including central nervous system and ocular toxicity) would be observed in both rabbits and patients if hyperbaric oxygen therapy were continued for prolonged periods of time. A pulsed exposure to hyperbaric oxygen is often used in patients with anaerobic infections, multiple sclerosis, and other conditions. It is this mode of therapy that would potentially be of value in sickle cell patients. Hopefully intermittent unsickling of the erythrocytes would facilitate resorption of the hyphema.

Although measurements were not done, the clinical correlations between intracameral sickling and secondary glaucoma would suggest that a decrease in sickling in patients would diminish the likelihood of a severe secondary glaucoma. Furthermore, the increased arterial pO2 may reverse sickling and prevent sludging of erythrocytes in the microcirculation of the retina and optic nerve. There is a risk that high arterial pO2, acting as a retinal vasoconstrictor, may promote ischemia, but the benefits of reduced sickling may outweigh the effects of possible vasoconstriction. In addition, the elevated level of pO2 in tissue may diminish the risk of tissue ischemia, retinal infarction, and/or optic nerve infarction.

These results suggest that hyperbaric oxygen therapy may be of benefit to patients with sickle cell hyphema. Prior to such use, further experiments will be necessary to document the feasibility of a safe dosage schedule of hyperbaric oxygen that would reverse or prevent sickling of erythrocytes without systemic or ocular oxygen toxicity.

Key words: hyphema, hyperbaric oxygen, sickle cell disease, aqueous humor, glaucoma

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From the Chicago College of Osteopathic Medicine,* the Department of Ophthalmology, Northwestern University Medical School,† the University of Illinois Hospital, Eye and Ear Infirmary,‡ and Edgewater Hospital, Pulmonary/Hyperbaric Medicine Division,§ Chicago, Illinois. Supported in part by the Comprehensive Sickle Cell Grant PHS-HL-151-68 from the National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland. Submitted for publication: October 17, 1984. Reprint requests: Lee M. Jampol, MD, Department of Ophthalmology, Northwestern University Medical School, 303 E. Chicago Avenue, Chicago, IL 60611.

References

The Acuity Card Procedure: A Rapid Test of Infant Acuity

MaryAlice McDonald,* Velma Dobson,* S. Lawson Sebris,* Lawrence Barch,† Denise Varner,* and Davida Y. Teller*

Forced-choice preferential looking (FPL) and operant preferential looking (OPL) procedures for testing infant acuity typically require 15–45 min to derive an acuity estimate. This article presents a new acuity assessment technique ("acuity cards") that combines FPL/OPL stimuli with an observer's subjective assessment of an infant's looking behavior. The infant is shown a series of gray cards that contain grating targets of various spatial frequencies. An observer watches the eye movement patterns and behavior of the infant and judges whether the infant can or cannot see the grating on each card in the series. Acuity is estimated as the highest spatial frequency that the observer judges the infant to be able to see. With this technique, the binocular acuity of normal children can be estimated with reasonable accuracy in the laboratory setting in 3–5 min. Invest Ophthalmol Vis Sci 26:1158–1162, 1985

The forced-choice preferential looking (FPL) and operant preferential looking (OPL) techniques developed in our laboratory1–3 have been valuable in documenting the development of visual acuity in normal infants and children. There is good agreement between our technique and other preferential looking (PL) techniques on acuity norms for infants and children of different ages.4 However, FPL/OPL techniques have been useful clinically only in the hands of a few experienced researchers, primarily because of the unavailability of simple, standardized equipment and because of the time constraints typically imposed by clinical settings.

A major reason that formal two-alternative forced-choice PL testing is time consuming is that a large number of trials must be used if standard errors are to be made acceptably small.3 At the same time, much of the information available in an infant's response to the grating test stimuli is not recorded. For example, although an observer in a PL procedure might judge the location of the grating correctly on both a trial with a relatively low spatial frequency grating and a trial with a near-threshold frequency grating, the infant's response to the two gratings would probably be quite different, with the infant showing a strong fixation preference for the low frequency grating but only a slight increase in fixation for the near-threshold grating. The goal of the present study was to test the possibility that an adult observer who was allowed to make a broadly integrative, subjective judgment about an infant's responses to grating stimuli could produce a rapid, accurate estimate of acuity. *

* A potential problem with the present approach is that reliance on an observer's integrated judgment, rather than formal two-alternative forced-choice procedures, increases the possibility of observer bias. The possibility that a measurement can be biased in principle should be distinguished from the question of whether it can be used in an unbiased way in practice. Continuing validation of the acuity card procedure will lie in further confirmation of the results of the present study—agreement with prior acuity norms and prior estimates of variability, and high interobserver reliability—across settings, observers and infants.