Effect of Light on Oxygen-Induced Retinopathy in the Mouse

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Purpose. To examine the effect of light on retinal neovascularization and vasculogenesis in a reproducible and quantifiable model of oxygen-induced proliferative retinopathy in the mouse.

Methods. C57Bl/6J mice were reared in room air, 68% oxygen, or 75% oxygen and were exposed to darkness, low cyclical light (200–350 lux), or high-intensity continuous light (3000–4500 lux). The entire retinal vascular pattern was visualized in fluorescein-dextran perfused flat-mount preparations. Proliferative retinopathy was quantified by counting neovascular nuclei in 6 μm cross-sections of whole eyes.

Results. Light exposure did not exacerbate the proliferative retinopathy that was seen after 68% oxygen exposure, which induced a meager proliferative response, nor after 75% oxygen exposure, which induced an exuberant proliferative response. In room air, retinas from all three illumination groups had normal vascular patterns.

Conclusions. In this model of oxygen-induced retinopathy, under the conditions tested, light neither exacerbated the hyperoxia-induced neovascularization nor affected normal retinal vascular development. Invest Ophthalmol Vis Sci. 1994;35:112–119

The incidence of retinopathy of prematurity (ROP) is increasing, and the factors influencing its pathogenesis need further exploration. Retinopathy of prematurity is correlated with the degree of retinal vascular immaturity and postconceptional age, as well as with the level and duration of hyperoxia exposure. However, many other factors, including the infant’s clinical status, may affect the incidence and severity of the disease. The effect of light exposure on ROP in newborns in nurseries has been debated for 50 years and has yet to be resolved. Clinical studies have yielded conflicting results. There is a need for prospective, randomized, tightly controlled clinical trials, but such trials are difficult to perform. The use of animal models with oxygen-induced retinopathy, in which variables can be more easily controlled, may help to evaluate the contribution of light to the pathogenesis of ROP.

We examined the effect of light on oxygen-induced proliferative retinopathy in a reproducible and quantifiable mouse model that is described in the preceding article by Smith et al in this issue. In this study, we found that light neither aggravated the hyperoxia-induced neovascularization nor altered the normal retinal vascular development.

MATERIALS AND METHODS

Light Exposure

This study adhered to the ARVO Statement on the Use of Animals in Ophthalmic and Vision Research. In total, 132 newborn mice (C57BL/6J) from 48 litters were randomly assigned to one of three groups until sacrifice on postnatal day 17 (P17) or 21 (P21):

- group 1, light deprivation from birth (P0);
- group 2, incandescent light, 200–350 lux (lumens/meter²), 12 hours on/12 hours off, from P0;
- group 3, broad-spectrum fluorescent light, 3000–4500 lux, 24 hours/day, from P2.

Before P2, pups in group 3 received low cyclical light to avoid cannibalism by the dams. Illumination...
was measured near the pups' eyes with a General Electric (Cleveland, OH) light meter, model #213. Group 1 was kept in a ventilated, light-excluding plywood chamber. When cages were inspected or changed twice a week, a red light (< 50 lux) lit the interior of the chamber for several seconds. Group 3 was exposed to four 20-watt lamps (Designer 830, Royal White, OSRAM Sylvania Inc., Danvers, MA) with spectral characteristics similar to the Cool White lamp often used in nurseries. Clear polycarbonate cages without bedding allowed light penetration from all sides. Clear acrylic shields separated the lamps from the cages. Heads of the group 3 pups were observed to be exposed to light >90% of the time. Ambient temperature was 23 ± 2°C to control for the possible influence of body temperature on the threshold for retinal light damage.7

Hydroxic Exposure

Proliferative retinopathy was induced as described in the preceding article by Smith et al.6 except that a clear acrylic hyperoxia chamber was used for maximum light penetration and oxygen levels were at 68% ± 2% or 75% ± 3%. Carbon dioxide, measured with a Bacharach Fyrite gas analyzer (Bacharach Inc., Pittsburgh, PA), was undetectable in the chamber. Control groups were exposed to room air from P0 until sacrifice.

Preparation of Retinas

Mice (P17 or P21) were anesthetized and perfused with fluorescein-dextran or paraformaldehyde. The eyes were removed and processed for retinal flat mounts, paraffin cross-sections, and glial fibrillary acidic protein (GFAP) staining as described by Smith et al.6

Quantification of Proliferative Retinopathy

This method is described by Smith et al.6 Six sections per eye were counted for neovascular nuclei. Groups were compared by analysis of variance. For groups that were different, adjusted Student's t-tests were performed with the Instat program (GraphPad Software, San Diego, CA). To assess the fidelity of the counting method, double-masked determinations were made by two observers, and linear regression showed high correlation (r = 0.95, n = 60 cross-sections from 20 eyes, P < 0.001).

Morphometric and Light Microscopic Analysis of Retinal Light Damage

The retinal outer nuclear layer (ONL) thickness was measured with an ocular micrometer in 6 µm paraffin cross-sections under light microscopy (X400). Values were compared for groups 1 and 3 with the unpaired two-tailed Student's t-test. The total ONL area per section was calculated8 and normalized for retinal length. Cross-sections were examined by light microscopy for signs of degenerative changes in photoreceptor cells with continuous light exposure.

RESULTS

Histology of the Retinal Vasculature After Room Air and Light Exposure

In room air, the vascular pattern delineated in retinal flat mounts with fluorescein-dextran perfusion was the same in the absence (Fig. 1A) or in the presence of intense continuous light (Fig. 1B). A superficial radial branching plexus was seen connected to a deeper polygonal plexus. Low cyclical light exposure of 200–350 lux (data not shown) produced the same pattern as exposure to darkness or 3000–4500 lux. Similar patterns were observed in P17 mice (Fig. 1A, 1B) and P21 mice (data not shown). In room air, the retinal vascular pattern seen in paraffin cross-sections was the same without (Fig. 1C) and with light exposure (Fig. 1D). Small PAS-positive blood vessels were found in the nerve fiber and in the inner and outer plexiform layers. No vascular nuclei extended from the nerve fiber layer into the vitreous.

Histology of the Retinal Vasculature After Hyperoxia and Light Exposure

After exposure to hyperoxia, there was virtually no fluorescein-dextran perfusion of the central capillary network in the posterior pole except for a few tortuous larger radial vessels. In the midperiphery of all the retinal quadrants (without predilection for any quadrant), there were clusters of abnormal new vessels or “tufts.” The same pattern was seen in low cyclical light, 200–350 lux (data not shown), darkness (Fig. 2A), and 3000–4500 lux (Fig. 2B). There was less central hypoperfusion after 68% (data not shown) than after 75% oxygen. Similar patterns were observed in P17 mice (Fig. 2, A and B) and P21 mice (data not shown). In paraffin cross-sections, the vascular pattern following hyperoxic exposure was the same without (Fig. 2C) and with light exposure (Fig. 2D). Tufts of vascular nuclei extended from the internal limiting membrane into the vitreous. After exposure to 68% oxygen (data not shown), there were fewer neovascular tufts and smaller intraretinal vessel profiles than after 75% oxygen (Fig. 2 C and D).

Quantification of Proliferative Retinopathy

Mice reared in room air had no proliferative retinopathy after exposure to either 3000–4500 lux of light or to darkness (Fig. 3). There were 0.6 ± 0.9 (SD; SEM = 0.1; n = 9) nuclei in the light and 0.7 ± 1 (SD; SEM = 0.1; n = 10) nuclei in the dark (P > 0.05, dark versus intense light). Light exposure (3000–4500 lux) did not
affect the proliferative response to 68% oxygen ($P > 0.05$), even though 68% oxygen induced minimal proliferative retinopathy (Fig. 3). After exposure to 75% oxygen, a treatment that induced more robust proliferative retinopathy, there was less neovascularization with exposure to 3000–4500 lux of light than with exposure to darkness ($P < 0.001$; Fig. 3). The SEMs (not shown) for the hyperoxia-treated groups were 4% to 11% of the mean value. Light, hyperoxia exposure, or both did not affect the number of intraretinal blood vessel profiles ($P > 0.05$; Fig. 4).

There was no relationship between the number of new vessels and the weight or sex of the mice. All treatment groups weighed 15% to 36% less than did the mice exposed to darkness and room air. Intense light or hyperoxia may have caused maternal stress that interfered with nursing because the weight gain of the litters improved when the dams were rotated with surrogates every few days.

**Morphometric and Light Microscopic Analysis of Retinal Light Damage**

Mice reared under hyperoxic conditions and exposed to either darkness or to 3000–4500 lux of light had the same ONL thickness (except for one zone in the light-exposed group in which the ONL was significantly thicker, $P < 0.04$; Fig. 5). The ONL areas per retinal length were not significantly different ($0.0469 \pm 0.0035 \text{mm}^2$/mm in the dark versus $0.0496 \pm 0.0031 \text{mm}^2$/mm in intense light, $P > 0.05$). ONL areas were normalized for retinal length to offset disparity in area due to differences in length among groups (21%). Within the groups, the measured lengths were within 10% of the mean length. No degenerative changes in photoreceptor cells were noted after exposure of the mice to 3000–4500 lux of continuous light for 15 to 19 days. In P17 and P21 mice reared in room air, GFAP localized to the astrocytes in the inner layer of...
FIGURE 2. Comparison of retinas from dark- and light-exposed mice reared in 75% hyperoxia.
Flat-mount preparations of fluorescein-dextran perfused retinas from P17 mice exposed to
(A) darkness or to (B) 3000-4500 lux of continuous light. Paraffin cross-sections, 6 µm,
stained with PAS and hematoxylin, from P21 mice exposed to (C) darkness or to (D) 3000-4500 lux of continuous light. Original magnification ×6 for A and B, ×100 for C and D. V, vitreous; ILM, internal limiting membrane; GCL, ganglion cell layer; IPL, inner plexiform layer; ONL, outer nuclear layer; H, extravasated red blood cells in the vitreous. In A and B, arrows indicate neovascular tufts. In C and D, thin arrows indicate neovascular tufts, and intraretinal blood vessels are indicated by thick arrows.

the retina regardless of light history (data not shown). After hyperoxic exposure, the increased GFAP staining in astrocyte and Müller's cell processes was the same in the absence or the presence of 3000-4500 lux of light (data not shown).

DISCUSSION
In 1943, Terry⁹ first suggested that exposure of premature infants to light might be one of the factors causing ROP. Since then, a number of clinical trials have yielded conflicting reports on the influence of light on ROP.¹⁰⁻¹⁴ Many of these studies were nonrandomized, lacked controls for pertinent variables, and had small numbers of patients. Such studies are difficult to interpret because gestational age, medical problems, genetic characteristics, and a myriad of other variables, including hyperoxic exposure³ and intensity of light exposure,⁹ differ from infant to infant and nursery to nursery.

We examined the effect of light exposure on a reproducible, quantifiable, and genetically defined mouse model of oxygen-induced retinopathy, described by Smith et al.⁶ No adverse effect of light on oxygen-induced proliferative retinopathy was seen under conditions producing either scant neovascularization (68% oxygen) or more exuberant neovascularization (75% oxygen). Interestingly, the retinas had more neovascular nuclei after 75% oxygen exposure in the dark than in the light. The reason for this is unclear. Perhaps the mouse retina, like cat¹⁵ and human retinas,¹⁶ has a higher metabolic demand in the
dark than in the light. The vasoproliferative response might reflect the retina's attempt to meet its metabolic needs. However, the new vessels extended away from the photoreceptors into the vitreous, and the number of vascular profiles in the deeper retina was unchanged by light.

We observed biologic variability in the neovascular response among mice within a group and among littermates from the same group, accounting for the high standard deviation. Despite the variability, however, a sufficient number of animals were assessed to minimize the error and to establish statistical significance. The large change in proliferative response seen with a small change in hyperoxia levels may reflect a "threshold" for this model and highlights the difficulty of designing and executing meaningful clinical trials in which variables are difficult to control.3

A preliminary study with a small sample of animals appeared to show more neovascularization with light exposure than dark exposure.17 In these initial studies, the data from mice exposed to 68% and to 75% oxygen were pooled. In this report, when the data obtained from 68% and 75% oxygen were assessed separately and the sample size was tripled, the difference between light and dark was insignificant. Additionally, by increasing the sample size, an initial finding of light-induced neovascularization in room air was shown to be invalid.
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FIGURE 5. Distribution of retinal ONL thickness of P21 mice reared in darkness or in 3000–4500 lux of light and exposed to 68% oxygen. Eyes were oriented in the superior-inferior axis. Error bars indicate SD, n = 6, mice per group (* P < 0.04).

Disturbances on light on two degrees of retinopathy: one with mild and the other with more exuberant vasoproliferation. Despite the differences in experimental design and choice of animal species, our findings concur with the two original reports.

The light intensity of 3000–4500 lux used in this study is comparable to that experienced intermittently by premature infants from heat lamps and phototherapy lights in the nursery. However, the mouse has fused lids until PI4, decreasing to some degree the light reaching the retina. In this study, the eyes were open for 48 hours before the onset of proliferative retinopathy at PI6, and yet light did not aggravate the vascular proliferation. In premature infants, the closed eyelids act as a red-pass filter, and, unlike neonatal rodents, infants can open their eyes for significant periods of time. Therefore, the premature infant retina, despite protective eye shields during phototherapy, might receive a higher light dose and at shorter wavelengths than can be simulated in rodent models. We did not examine the effect of short cycles of intense light exposure on three parameters that reflect light toxicity to the rodent retina: ONL thinning, retinal histology, and increased GFAP staining. The ONL was slightly thicker in one zone of the light-exposed retinas, but the ONL area, related to the number of photoreceptors present, was the same after exposure to light or darkness. This apparent ONL thickening might have been due to differences in retinal length rather than to light exposure. Although no histopathologic changes were seen in the retina after light exposure, the damage may be subtle and may require ultrastructural analyses. Another study showed increased GFAP staining with intense light exposure in rats. These results differ from ours, possibly because of the difference in animal species used, the duration and intensity of light exposure, or both.

A link between light-induced retinal damage and proliferative retinopathy has not been established. It has been postulated that light of sufficient energy might generate oxygen radicals that have also been implicated in the pathogenesis of ROP. Intense light exposure is known to induce oxidation of rod outer segment membrane lipids. However, there is circumstantial evidence that light-induced photoreceptor damage may be unrelated to ROP. We see oxygen-induced retinopathy in mice without evidence of photoreceptor injury after exposure to hyperoxia and darkness or intense light. Despite light-induced photoreceptor injury in rats, the same pattern of oxygen-induced retinopathy is seen with dark and intense light exposure. Furthermore, in room air, intense light can damage photoreceptors in the rat, pig, monkey, and in the human without inducing retinal vasoproliferation.

Although light damage to photoreceptors may be unrelated to ROP, shorter wavelengths of light might be phototoxic to immature blood vessels. Blue light inhibits the growth of bovine aortic endothelial cells, RPE cells, and fibroblasts in vitro. This effect is prevented by adding antioxidant enzymes, suggesting a photooxidative mechanism of light damage. However, it is unclear whether these cells contribute to the pathogenesis of ROP. Many phototherapy lamps emit nearly all their energy between 400 and 500 nm, possibly adding to oxidative stress on the retina. In our study, of the total energy emitted by the fluorescent lamps, only 5% was ultraviolet (<380 nm) and 15% was violet and blue light. Perhaps a greater intensity of blue light might have affected the degree of proliferative retinopathy in our model.

Genetic factors may affect the susceptibility to retinal phototoxicity. Adult C57Bl/6J mice are more resistant to light-induced retinal damage than other mouse strains. This may explain, at least in part, why we saw no evidence of ONL thinning with intense light exposure. In addition, neonatal mice, like young rats, might be more resistant to light damage than older animals. Infants also differ in their genetic susceptibility to ROP. Black people are less susceptible than is the general population to ROP, and it has been postulated that ocular melanin may have a protective role.
In conclusion, light exposure did not affect normal retinal vascular development or exacerbate oxygen-induced retinopathy in our mouse model under controlled experimental conditions. However, one should be cautious in extrapolating from animal models of oxygen-induced retinopathy to human ROP. At present, little is known about the mechanisms of the developing retinal vasculature, and further basic and clinical research are needed to address this important issue.

Key Words
light, oxygen, mice, oxygen-induced retinopathy, retinopathy of prematurity

Acknowledgments
The authors thank Richard Sullivan for technical assistance and Angela McLellan for assisting in quantifying the neovascularization; Erica Wheeler for performing the GFAP staining; and Diane Medeiros for measuring the CO₂ concentrations.

References


