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The Effect of Light on Oxygen-Induced Vasoproliferative Retinopathy in Newborn Kittens

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The effect of bright fluorescent light (115 fc) on oxygen-induced retinopathy in newborn kittens was compared with that of complete darkness and cyclic illumination of 12 hr of bright light (115 fc) and 12 hr of complete darkness. No significant difference was found in the extent of preretinal vasoproliferation and retinal ultrastructural findings among the three groups of kittens reared in different levels of illumination and a control group raised in a standard laboratory illumination level of 40 fc. These results confirmed that light is not a required factor for the development of oxygen-induced retinopathy in kittens. Invest Ophthalmol Vis Sci 33:1595–1598, 1992

Extensive experimental evidence, both in vitro and in vivo, has demonstrated photoreceptor damage and electroretinographic disturbances as a consequence of lipid peroxidation induced either by oxygen[1] or light.[2,3] The preterm infant is subjected to prolonged exposure to ambient nursery illumination at levels that have been found to produce retinal damage in animals.[4–8] It has long been hypothesized that bright neonatal nursery illumination may be implicated as a cause of retinopathy of prematurity (ROP). Two recent clinical studies presented contradictory results about the effects of light on the incidence and development of ROP in low birth weight premature infants.[9,10] One study reported that there was a higher incidence of ROP in the group of infants weighing less than 1000 g who had been exposed to brighter nursery lights.[9] In the other study,[10] no difference was found in the incidence and severity of ROP between a group of 161 premature babies who were exposed to reduced-intensity nursery lighting (15 fc) and a similar group of premature infants exposed to standard bright nursery lighting (55 fc). To contribute to our understanding of the role of light toxicity in the pathogenesis of ROP, we did an animal study in which we examined the effect of bright light versus complete darkness and cyclic illumination on oxygen-induced retinopathy in newborn kittens.

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Materials and Methods

Twenty-four kittens were divided into four groups designated A, B, C, and D. These experiments adhered to the ARVO Resolution on the Use of Animals in Research. Group A included six kittens, aged 4 to 6 days, that were reared with their mothers in an atmosphere of oxygen 80% in a large transparent polycarbonate incubator. All eyes were open at this age. The incubator was designed especially for this kind of experiment; the CO_2 concentration, humidity, and temperature were controlled. The illuminance level measured in the incubator was maintained at 115 fc 24 hr a day. This illumination was obtained from two 40-W General Electric (Los Angeles, CA) "cool white" fluorescent tubes. The spectral irradiance of this illumination, measured near the kittens' eyelids, was as follows: at 290–320 nm, 1; at 320–400 nm, 16; at 400–700 nm, 500; and at 700–800 nm, 18 $\mu W/cm^2/nm$. The kittens were reared in these conditions for 72 hr. After this, they were removed from the incubator and returned with their mothers to a normal oxygen concentration with laboratory room air. The illuminance level measured at the kittens' location in the laboratory was maintained at 115 fc. The kittens were reared with their mothers in room air under this illumination for 3 weeks. At the end of this period, they were killed. Their eyes were enucleated and divided in two callots, superior and inferior, cut horizontally through the optic nerve head. One half of each callot, cut horizontally again, was fixed in buffered formaldehyde 10% and processed conventionally for hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) staining. The other half of each callot was fixed in glutaraldehyde 3% and processed for transmission electron microscopy (TEM). Just before they were killed, funduscopic examination was done using a

Heine indirect funduscope after pupillary dilation with phenylephrine 10% and cyclopentolate 1% drops.

Group B included 6 kittens, aged 4 to 6 days, who were reared with their mothers in the same manner as the kittens in Group A except that the environmental illumination level was different. Group B kittens were maintained in complete darkness (0 fc) while being oxygenized in the incubator for 3 days and also during the period of 3 weeks of normal air breathing. All kittens' eyes were completely open before entering the incubator. Funduscopic examination was done before they were killed. After death, their eyes were enucleated, cut into two callots as described, and processed conventionally for H&E and PAS staining and TEM.

Group C included six kittens, aged 4 to 6 days, with open eyes, who were reared with their mothers in the same manner as those in Groups A and B, except for the environmental illumination level. They were maintained in a cyclic illumination of alternative 12 hr bright fluorescent light (115 fc) and 12 hr of complete darkness each day. This cyclic illumination was continued through the entire experiment, including 3 days of oxygenation and 3 weeks of normal air breathing.

Group D included six kittens, aged 4 to 6 days, who were reared with their mothers in the same manner as those in Groups A, B, and C. However, they were kept under the normal environmental illumination in our laboratory. The mean constant illumination level measured at the incubator was 40 fc. These kittens served as the control group, and after death, their eyes were enucleated and processed in the same way as those in the other three groups. The degree of retinal toxic changes was determined by qualitative assessment of the cytopathologic changes in the superior and inferior callots of the retina. The degree of preretinal neovascularization was assessed by comparing the retinal sections in Groups A, B, and C with those in Group D, with the help of an image analyzer.

Results

Identical funduscopic and histologic findings of vasoproliferative retinopathy, as described in a previous study,[11] were found in all eyes. No difference could be found between the four groups with respect to cytologic retinal findings and preretinal neovascularization. The main retinal vessels of the vascularized posterior retina were dilated greatly. The border between the avascular and vascularized retina was located at the equator and characterized by dilated brush-like vascular endings. Arborizing glomerular tufts of new vessels were seen growing in a diffuse pattern toward the vitreous from the retina of the posterior pole and from the optic disc itself. The delicate preretinal new vessels were seen growing by budding from preexisting intraretinal vessels and spreading into the vitreous without accompanying glial or fibrous tissue. These vitreoretinal findings were identical to those we described earlier in a study that revised oxygen-induced retinopathy in newborn kittens as a model for ischemic vasoproliferative retinopathy.[11] The same histologic and clinical findings of dilated and irregular iris vessels resembling rubeosis iridis also were seen.

No specific cytopathologic changes were noticed in the retinal layers, retinal pigment epithelium, and choroid of all kittens in Groups A, B, C, and D, either by light or by electron microscopy. There was no significant difference between the extent of preretinal new vessels found in the kittens reared in standard illumination, bright illumination, or complete darkness, and the extent of new vessels found in those kittens that were reared under circadian illumination (12 hr of bright light and 12 hr of darkness).

Discussion

In 1952, it was reported that light, mydriatics, and retinal exposure to ophthalmoscopic examination did not contribute to ROP.[12] According to these data, short- and long-term monocular and binocular occlusion showed no significant decrease in the incidence of disease. Others,[13] in 1948, reached the same conclusion that light was not a causative factor in low birth weight neonates with ROP. When a prospective study investigated the effect of infant exposure to light in two neonatal intensive care units, the incidence of ROP was 86% among 74 infants maintained in the standard bright nursery environment (median light level, 60 fc) compared with 54% among 154 infants of a similar birth weight (less than 1000 g) for whom the light levels were reduced to an average of 25 fc.[9] These authors concluded that safety standards regarding current nursery lighting practices should be reassessed with respect to neonates weighing less that 1000 g. Recently, a similar study was done on two larger groups of premature babies, one group exposed to 15 fc of nursery illumination and the other, to 55 fc, the standard level of illumination in nurseries.[10] No difference was found in either the incidence or severity of ROP between these two groups.

According to other studies, healthy preterm and term infants showed no gross retinal damage when exposed to bright light in the nursery. This was determined by electroretinographic, light threshold, and visual acuity screening.[14,15]

The model of oxygen-induced retinopathy, first produced in the newborn kitten by exposure to hyperoxic conditions,[16,17] was revised by us previously.[11] In

this article, we supported the view of others[16,17] that this disorder is basically an acute vasoproliferative retinopathy induced by retinal ischemia caused by extensive oxygen-induced vasoconstriction, probably with the subsequent secretion of a vasoproliferative factor from the ischemic retina.[11] Other researchers also have studied the pathogenesis of this oxygen-induced retinopathy and its prevention.[18-20] Despite the differences between this experimental model of ROP and human ROP, mainly with respect to the absence of spindle cells in the avascular retina of the kitten and mode of new-vessel development,[11,21] both are related etiologically to the toxic effects of high concentrations of ambient oxygen on the retinal vasculature and the retinal tissue itself. Besides oxygen, other causative factors, such as retinal immaturity with decreased availability of tissue antioxidants, have been suggested to play an important role in the pathogenesis of ROP.[22,23] Two agents that protect the retinal cell membrane from free radical-induced lipid peroxidation could be absent or ineffective in the undeveloped or immature retina of preterm and low birth weight infants (<1000 g). This situation could be exacerbated when these infants or newborn animals are placed in a hyperoxic environment as follows: (1) the retinal level and/or activity of the enzyme superoxide dismutase was shown in kittens to drop precipitously in oxygen atmosphere[23] and (2) the plasma levels of an antioxidant, vitamin E, were found to be relatively low in high-risk preterm infants treated with oxygen.[22]

Lipid peroxidation and subsequent photoreceptor damage also may be induced by light.[2,3] Some believe that bright neonatal nursery illumination may be a causative factor in the pathogenesis of ROP,[24] and it was found that the greatest eyelid opening in the incubator occurs in the youngest neonates known to be at greatest risk.[25] Others[26] discovered that these highest-risk immature infants are nursed under bright light, whose spectral power distribution is weighed toward the blue end of the spectrum.

We therefore decided to undertake our study to compare the effect of bright fluorescent light versus complete darkness or cyclic illumination on oxygen-induced retinopathy in newborn kittens. As mentioned, this is a traditional animal model of ROP, although somewhat different both clinically and histologically from human ROP.[11,21] This kitten model is defined better as an ischemic vasoproliferative retinopathy.[11] Our results showed no significant difference in the extent of preretinal vasoproliferation and retinal ultrastructure between kittens reared in constant 115-fc illumination and those reared in complete darkness or in cyclic conditions. The conclusion that can be drawn from the experiment with the dark-reared kittens is that light is not a required factor for the development of oxygen-induced retinopathy in kittens.

Unfortunately, we did not measure the time extent of the kittens' eyelid opening during the day and night because of technical problems. However, all eyes were open anatomically when the kittens were placed in the incubator for oxygenation. This is important because only 10% of visible light and 1% of ultraviolet light are transmitted through eyelid tissues. We also assumed that there was no difference between the extent of time the eyelids were open in the various groups.

There is a possibility that the retinal photoreceptors of these kittens were relatively insensitive to the level of illumination we used, a higher level than the maximum level of illumination measured by others[9,10] in neonatal intensive care units. In addition, it was found that phenotypically identical animal populations with different genetic constitutions can show markedly different sensitivities to light.[27] These authors concluded that genetic factors are a major determinant for the severity of light damage in different animal strains and human neonates with different genes.

We chose a maximal illumination intensity of 115 fc because the exposure time in our experiments was long in comparison with that in other studies[5] in which a 400-fc intensity was used for a relatively short time to test newborn primates. These authors found evidence of phototoxicity in the primate retina under this illumination. Nevertheless, our maximal level of illumination was approximately twice as high as the standard level of bright nursery light. Another point to be considered is the dense tunica vasculosa lentis posterioris in the kitten, which interferes with the passage of light toward the retina.

With regard to the spectrum of light we used, it can be concluded that the cool white fluorescent tubes emitted relatively little ultraviolet light. This low ultraviolet emission resulted from the low color temperature of this kind of fluorescent light source. The current clinical understanding of the relative safety of cool white fluorescent lighting is reflected in the use of this type of illumination in neonatal intensive care units and also in the use of opaque eye shades during phototherapy for neonatal jaundice.

Key words: light toxicity, oxygen therapy, oxygen-induced retinopathy, kittens

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