The combined effects of light and acute ischemia on the structure of the rabbit retina: a light and electron microscopic study

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The ultrastructure of the rabbit retina has been investigated to determine the combined effects of light and ischemia. Both eyes of 16 adult Dutch rabbits were exposed to light of an intensity known to be near or below the threshold for ultrastructural changes in the retina. In addition, one eye of each animal was subjected to one of four periods of pressure-induced total acute ischemia. Exposure to light alone resulted in only minor disturbances of the receptor cell outer segments. The remainder of the retina was of normal morphologic appearance. Exposure to the combined insults of light exposure and ischemia produced considerably more damage to the inner and outer retina. Light exposure combined with short periods of ischemia (15 to 30 min) resulted in edema of the pigment epithelium and disturbances of the receptor cell inner and outer segments. Light exposure combined with longer ischemic periods (45 to 60 min) resulted in severe disturbances in the structure of the pigment epithelium, breakdown of the receptor cell outer segments, rupture of the inner segment mitochondria, and severe edema of the neural retina. The implication of this addictive or synergistic action of light and ischemia is discussed.

Key words: retina, light damage, total acute ischemia

Noel et al. have shown that long-term exposure to light of even moderate intensity can result in damage to the photoreceptors and ultimately degeneration of the outer retina. Adverse changes in the retinas of monkeys have been produced after light exposure associated with diagnostic procedures such as indirect ophthalmoscopy, intraocular fiber optic light as used in vitrectomy, and the use of operating microscopes and slit lamps. Although such intensity levels may not cause damage in the normal healthy human retina, the effect of similar levels of illumination on the pathologic fundus is unknown. Because light damage to the retina mainly affects the retinal receptor cells and because pressure-induced ocular ischemia in rabbits also results in structural damage to the outer retina, it is possible that ocular ischemia may lower the threshold to light damage in this species.

This article describes an investigation to determine whether the ischemic retina is more sensitive than the nonischemic retina to light-induced damage.
Fig. 1. Diagram of the experimental procedure. Light of a known intensity, 100 mW/cm² maximum, beam center at 2.5 cm from the end of the light guide (measured with a Rank Hilger FT 32 Thermopile). The light was directed into both eyes by means of the fiber optic system. In addition, the anterior chamber of one eye was cannulated and connected to a manometer and pump. This facilitated raising of the IOP to abolish the intraocular circulation.

Fig. 2. Both eyes of 16 adult Dutch rabbits, four in each group, were exposed to light. In addition, one eye of each animal was rendered ischemic for 15, 30, 45, or 60 min. The light exposure and the ischemic period were timed, as shown, to end simultaneously.

Materials and methods

For this study, 16 adult Dutch rabbits weighing 1.6 to 2.3 kg were used. They were anesthetized with intravenous urethane (5 ml/kg body weight of a 40% solution), the pupils of both eyes were maximally dilated with 1.0% cyclopentolate and 10% phenylephrine, and a tracheostomy was performed. The animals were placed in a prone position, with their heads secured in an upright position. The lids were retracted with specula. The anterior chamber of one eye was cannulated with a heparinized 23-gauge needle connected to a reservoir of heparinized saline (1000 IU/500 ml) and a mercury-filled manometer and pump with which it was possible to raise and maintain the intraocular pressure (IOP) at any required level (Fig. 1).

The light source and fiber optic light guides were placed in position as previously described.9'10 Both eyes of each animal were exposed to similar levels of illumination for 1 hr by means of the fiber optic light guides. The illumination level employed has been previously determined to produce little or no retinal damage.9

From direct measurement of the beam intensity with a Rank Hilger FT32 thermopile and theoretical calculations of the area of retinal illumination, the light level incident on the retina has been estimated to be 23 mW/cm². The wavelengths incident on the retina range from 400 to 1400 nm.

The IOP in the cannulated eye was raised from an initial level of 18 mm Hg to 120 mm Hg to abolish the intraocular circulation for periods of 15, 30, 45, or 60 min. Each period of pressure-induced ischemia was timed so that it finished simultaneously with the end of the period of light.
exposure (Fig. 2). At the end of the experimental period the IOP was returned to 18 mm Hg, the animals were killed with an overdose of anesthetic, and both eyes were enucleated. The eyes were bisected in the equatorial plane and the vitreous was removed. The posterior halves were immersed in 3% glutaraldehyde buffered with 0.2M sodium cacodylate (pH 7.2 to 7.4) at room temperature.

A minimum of five tissue blocks of approximately 2 by 2 mm were taken. Four blocks were taken from the region of the visual streak: two centrally, one nasally, and one temporally. A fifth block was taken slightly inferior to the visual streak. These blocks were processed conventionally for electron microscopy and embedded in Spurr's resin.11

Sections 1 to 2 μm thick were stained with toluidine blue for light microscopy. Sections 50 to 80 nm thick stained with uranyl acetate and lead citrate were examined with a Philips 301 electron microscope.

Results

The appearance of the tissues varied little with location, and the following descriptions

Fig. 3. Electron micrograph of rod outer segments in a control eye exposed solely to light. Small irregularities of the disc stacking and pockets of flocculate material were present (arrows). (Bar = 2 μm.)

Fig. 4. Electron micrograph of the RPE after 60 min of light exposure and 15 min of ischemia. Proteinaceous detachments (PD) of the RPE were a frequent finding. Occasionally vacuoles containing material of a similar appearance to that seen below the detached RPE were present. V, Vacuoles; CC, choriocapillaris; BM, Bruch’s membrane (Bar = 10 μm.)
relate to the four areas of retina studied.

**Animals exposed to light alone.** In the 16 eyes exposed exclusively to light, the retina appeared similar to previously published descriptions of normal rabbit retina, with the exception of minor irregularities in the outer segments, consisting of focal disturbances in the ordered stacking of the outer segment discs and the presence of discrete areas of flocculate material within the outer segment (Fig. 3).

**Animals exposed to combined light and ocular ischemia.** Light exposure for 1 hr combined with 15 min of total ocular ischemia occasionally resulted in focal detachment of the retinal pigment epithelium (RPE) from Bruch’s membrane. The RPE, whether attached or detached, often showed distension of its mitochondria. The receptor cell outer segments frequently showed disturbances in the stacking of their discs. The cytoplasm and nuclei of receptor cells occasionally showed changes in their staining characteristics, but this was a fairly infrequent finding in this group.

When similar light exposure was combined with 30 min of ischemia, severe damage to the outer segments and gross changes in the appearance of the RPE resulted. The inner layers of the retina showed slight edema, which tended to be more severe in the inner nuclear layer.

Combination of light exposure with either 45 or 60 min of ischemia produced severe damage to the outer retina and the RPE and moderate-to-severe changes within the inner retina. In each case the severity of the damage was more marked than has previously been shown to result from the ischemic insult alone.

**Animals exposed to light and 15 min ischemia.** The most pronounced effect of combining 15 min of ischemia with 1 hr of light exposure was the appearance of areas of detachment of the RPE from the underlying Bruch’s membrane. The detached RPE was separated from Bruch’s membrane by a proteinaceous exudate, and RPE cells in or near these areas of detachment frequently contained vacuoles of proteinaceous fluid similar in appearance to that under the detached RPE and to the plasma in the related choriocapillaris (Fig. 4).

Retinal abnormality was largely restricted to those areas of retina related to the previously described RPE detachments. In other areas the only departure from normal was irregularity of disc stacking in the outer segments, which was marginally more marked than that seen in control light-exposed tissue. In areas where the RPE was detached the related retinal receptors showed swollen outer segments and considerable disorganization of disc stacking. The inner segments, however, were of near normal appearance. In the outer nuclear layer the receptor cell cytoplasm was shrunken and densely stained. The shrinkage of receptor cell cytoplasm affected the inner and outer receptor fibers as well as the perinuclear cytoplasm. This shrinkage and dense staining also affected the...
rod and cone synaptic terminals (Fig. 5). As if in compensation, the cytoplasm of Müller cells in this layer was relatively increased and showed an abnormal pale staining pattern. The abnormal staining characteristics of the Müller cell cytoplasm was present only within the outer nuclear layer, and the normal staining pattern of these cells in other retinal layers gave some Müller cells a two-toned appearance (Fig. 5).

The inner retinas of all animals in this group appeared normal.

**Animals exposed to light and 30 min ischemia.** In this group, detachment of the RPE
was a more prominent and more frequently encountered phenomenon than in the previous group. Vacuolation of the RPE was also more marked. Even in areas where the RPE remained attached to Bruch’s membrane the cells showed vacuolation, which at times involved a considerable proportion of the RPE cytoplasm (Fig. 6, a). In some cells the vacuoles appeared to result from massive distension of the basal infoldings (Fig. 6, b).

Again, retinal damage was more marked in areas overlying or immediately adjacent to RPE detachments and took the form of disruption of receptor cell outer segments. In these areas the related RPE cells contained numerous phagosomes (Fig. 7) and were abnormal in other respects also, containing numerous electron-lucent vesicles and slightly distended mitochondria (Fig. 7). The apical microvilli and basal infoldings of the RPE were also of a highly unusual appearance (Fig. 7).

Shrinkage and increased staining of the receptor cell cytoplasm were also seen in this group. However, apart from slight edema of the cytoplasm of cells in the inner nuclear layer in a few sections, the inner retinas all retained normal appearances (Fig. 8).

In these groups of animals little difference could be detected between the effects of the two periods of ischemia.

Fig. 7. Electron micrograph of the RPE after 60 min of light exposure coupled with 30 min of ischemia from an area showing marked outer segment damage. The pigment epithelial cytoplasm contained numerous phagosomes and electron-lucent vesicles. The apical microvilli and the basal infoldings were highly unusual in appearance. F, Phagosomes; V, vesicles; B1, basal infoldings. (Bar = 5 μm.)

Animals exposed to light and 45 or 60 min ischemia. In these groups of animals little difference could be detected between the effects of the two periods of ischemia.

Again, frequent detachment of the RPE was seen, but damage to the retina was not confined to areas of RPE detachment, although the severity of retinal damage did correlate with the presence of a related detachment of the RPE.

Where the RPE remained attached, the RPE cells contained numerous vesicles of indeterminate origin. The apical microvilli and the basal infoldings were highly atypical (Fig. 9, a). In areas in which the RPE was detached, the cells contained numerous vesi-
icles filled with flocculate material. In these areas the basal infoldings were lost and the apical villi were highly disorganized, appearing to have lost their intimate association with the receptor outer segments (Fig. 9, b).

Retinal damage in areas where the RPE remained attached was similar to the severest damage seen after light combined with 15 min of ischemia. In more severely damaged areas associated with RPE detachment, the photoreceptor outer segments were disorganized and the inner segments showed marked swelling of the mitochondria. All the retinal components vitreal to the outer limiting membrane showed considerable intracellular edema. This was often marked in the horizontal and amacrine cells of the inner nuclear layer (Fig. 10). This pattern of damage was common to both groups exposed to 1 hr of light and 45 or 60 min of ischemia.

Discussion

In those tissues exposed solely to light some slight abnormalities of outer segment structure were seen. However, the outer segment has been shown by many investigators to exhibit considerable variation in the pattern of its disc stacking. It has been hypothesized that this variation stems from such factors as age,¹⁵ light exposure,¹⁶ trauma,¹⁷,¹⁸ and vitamin deficiency.¹⁹ In this respect slight outer segment irregularities must be considered physiologic variants of outer segment structure. The slight morphologic abnormalities seen in some of the eyes in this study that were exposed solely to light may have resulted from the light exposure but were probably not severe enough to impair the functioning of the receptor cell.

The RPE and photoreceptor cells appear to be highly susceptible to damage from a combined insult of light exposure and total acute ischemia.

When comparison is drawn between this study and studies of pressure-induced ischemia alone,⁷ ⁸ it is shown that the combined insult of light exposure and total ischemia is more damaging than an equivalent ischemic insult to an eye that was not exposed to light; the RPE, which is remarkably resistant to ischemic damage in the dark-adapted rabbit, showed gross abnormality when ischemia was combined with light exposure in this study. To facilitate comparison of the two insults, the shortest periods of acute ischemia that produced an identifiable cytologic manifestation of cellular damage were compared. The various features assessed are listed in Table I. The addition of light to the ischemic insult reduces the time of appearance of the cytologic features of damage by up to 50%. Although the time of appearance of the cytologic features of damage varied in the eyes subjected to the combined insult in comparison with the eyes previously reported for ischemic damage alone,⁷ ⁸ the order of appearance of the various features of damage was similar in the two results. It is apparent that light exposure considerably reduces the retina's ability to tolerate pressure-induced ischemia.

Whether the enhanced damage seen in
Fig. 9. a, Electron micrograph showing a region of attached RPE in an eye exposed to light and 60 min of ischemia. The basal infoldings and apical microvilli were abnormal. The cytoplasm contains numerous electron-lucent vesicles (V). The large electron-lucent vesicle is a lipid droplet (LD). These are characteristic of the rabbit retina. b, Electron micrograph showing a region of detached RPE in an eye exposed to light and 60 min of ischemia. The appearance of these cells was similar to those of the attached RPE, although vacuolation of the cytoplasm appeared to be more pronounced. V, Vesicles. (Bar = 5 μm.)

Eyes rendered ischemic while exposed to light should be considered potentiated light damage or potentiated ischemic damage is difficult to determine, since the retinal tissues exposed to light during ischemic episodes showed morphologies characteristics of light damage produced at higher levels of illumination and of ischemia. In addition, other features of damage such as proteinaceous detachment of the RPE, vacuolation...
Fig. 10. Electron micrograph showing the appearance of the retina after exposure to 60 min of light and 60 min of ischemia. The majority of the cells showed considerable intracellular edema. This was particularly marked in the horizontal (H) and amacrine (A) cells of the inner nuclear layer. Although the Müller cells (M) appeared normal in the region of the inner nuclear layer, their cytoplasm internal to the ganglion cell (G) layer was distended and of varying electron lucency. ONL, Outer nuclear layer; MC, Müller cell cytoplasm. (Bar = 10 μm.)

of the pigment epithelial cytoplasm, and severe Müller cell and inner retinal damage, appeared to be unique to the combined insult. Tso and Fine have demonstrated proteinaceous material within large vacuoles of the pigment epithelial cytoplasm 4 years after argon laser damage to the primate foveola. More recently Schmidt and Zuech have shown distension of the basal infoldings and vacuolation of the cytoplasm of the RPE, very
### Table 1. Effects of ischemia in dark-adapted and light-exposed eyes

<table>
<thead>
<tr>
<th>Feature</th>
<th>Time of appearance* (min)</th>
<th>Time of appearance in combined light and ischemia experiments (min)</th>
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<tr>
<td>RPE</td>
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<tr>
<td>Mitochondrial rupture</td>
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<td>15</td>
</tr>
<tr>
<td>Densification of cytoplasm</td>
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<td>30</td>
</tr>
<tr>
<td>Proteinaceous fluid in cytoplasm</td>
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<td>Severe disturbances of disc stacking</td>
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<td>Nuclear changes</td>
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The minimum ischemic periods required to produce specific structural changes are compared. For the majority of features, the addition of light reduced the times of appearance by 50% or more.

*Effects of ischemia in dark-adapted eyes.7, 8

The light intensity employed in this investigation, primarily by diffusion.22 At the low level of illumination employed in this investigation, retinal heating effects in the absence of choroidal blood flow would be minimal, probably less than 0.5° C.

In the course of prolonged periods of ischemia (30 to 60 min) the retina changes in appearance from transparent to translucent. This change in the optical characteristics of the retina will result in less light reaching the outer retina. Therefore, to some degree the outer retina will be protected from illumination. The inner retina, due to its decreased ability to transmit light, may suffer slightly more damage than would be expected because of local heating. However, from theoretical considerations,22 this heating effect should be very slight.

During the ischemic insult, cessation of the choroidal blood flow must affect the ability of the choroid to dissipate heat. However, it has been shown by theoretical calculation that under conditions of constant illumination, heat removal by the choroidal circulation is fairly limited, heat dissipation being
tigation produced little or no damage to the retina or RPE, yet when combined with various periods of ischemia it resulted in much more severe retinal and RPE damage than would be expected from the effects of the ischemia alone. It would appear that the actions of light and ischemia are synergistic in their ability to produce retinal damage.

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REFERENCES