Histologic evaluation of ophthalmoscopically subvisible retinal laser exposures

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Four exposures of rhesus monkey macula to helium-neon (He-Ne) laser irradiation of 6.4 mw. at 100 msec. produced no ophthalmoscopically visible lesions; however, microscopic lesions were detected. Of the four exposures, one produced a small burn, another had no detectable effect, and the two remaining exposures produced identical lesions consisting only of vesicular cytoplasmic swelling and depigmentation of two contiguous retinal pigment epithelial cells. The variation in effect of the irradiation is attributed to inhomogeneity of pigment granule distribution in the pigment epithelium. The exposures in this experiment were approximately 40 per cent below the median effective dose for minimal ophthalmoscopically visible change.

Key words: Helium neon laser, macula lutea, radiation effects, histopathology, retinal burn, experimental production, monkeys.

The assessment of the retinal hazard from laser irradiation requires a determination of the energy or power necessary to cause a minimal effect. To properly evaluate the results, however, a "minimal effect" must be carefully defined. In a previous paper, ocular threshold damage dose levels for the helium-neon (He-Ne) laser were reported for the rhesus monkey eye, based on the observation of the minimal ophthalmoscopically visible change. The question must be raised whether retinal effects can be detected at levels below the minimum ophthalmoscopically visible threshold. In order to investigate this, subvisible exposures were placed in the macula of a rhesus monkey fundus and the exposure sites subsequently examined histologically.

Method

After preparing the monkey in a manner previously described, two reference marker burns were provided with an He-Ne laser by placing two small lesions in a vertical plane in the macula about 2 degrees apart. The marker burns, which were made with 11.9 mw. at 100 msec., were just barely visible through the fundus camera. Four exposures of 6.4 mw. at 100 msec. were then carefully placed in the area between the two marker burns. This exposure level was well below the threshold damage dose for the macula at 100 msec. as predicted by the ED_{50} (median effective dose) line determined in the previous study. No effect from these exposures was noted after examining periodically for 24 hours.

Approximately 24 hours after exposure, the monkey was killed by retrograde aortic perfusion with histologic fixative. The fixative, a mixture of gluteraldehyde and paraformaldehyde, was perfused under pressure for 20 to 30 minutes. A strip of posterior hemisphere of the globe containing the macula was excised, postfixed in

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osmium, and embedded in epon. Serial sections, 1 to 2 μ thick, were made through the area of interest and stained with Paragon (Paragon C & C Co., Inc., New York, N. Y.). Sufficient serial sections (about 500) were taken through both marker burns and all the intervening retina to insure that the areas of subthreshold exposure would be examined. Details of the perfusion technique and method of tissue preparation have been previously reported.  

Results

The two marker burns were identical in size and appearance. Of the 4 subthreshold exposures, 3 produced histologic lesions. No histologic change was present in the fourth area of subthreshold exposure. The section of the marker burn shown in Fig. 1 is taken through its largest diameter. The most striking changes illustrated are pyknosis in the outer nuclear layer, bulbous swelling of the tips of the inner segments, swelling of the outer segments with interstitial edema, coagulation of the pigment epithelium, and occlusion of the choriocapillaris. The area of damage was greatest at the level of the pigment epithelium, suggesting that it was the most sensitive structure of those irradiated. Pyknotic photoreceptor nuclei were occasionally seen extruding through the outer limiting membrane into the area of the inner segments. Two pigment epithelial cells in the center of the burn were coagulated and slightly shrunken, and the pigment epithelial cell at the right margin was swollen by hydropic vesicles. Occlusion of the choriocapillaris resulted from swelling of capillary endothelium.

Fig. 2 illustrates a small retinal burn
produced by one of the subthreshold exposures. This was the largest lesion resulting from the subthreshold exposures, with effects basically resembling those noted in the marker burns but less extensive. Pyknosis can be seen in the outer nuclear layer. These appear to be mostly rod nuclei because of their location in the inner portion of this layer. The cone nuclei in the outer portion of this layer are mostly unchanged; however, a pyknotic cone nucleus is demonstrated in the center of the lesion adjacent to the outer limiting membrane and attached to its swollen, hyperchromatic inner segment. There is considerable interstitial edema in the area of the outer segments. Fibrin was demonstrated histologically in this edema fluid. The pigment epithelial cells had vesicular hydropic cytoplasm and those in the center of the lesion had ruptured. There was some slight swelling of the endothelium of the choriocapillaris beneath the lesion but no occlusion.

Figs. 3 and 4 illustrate the two remaining subthreshold exposure lesions, which are possibly the most interesting of all. They had essentially the same histology so that they will be described together. Each lesion consisted of two contiguous pigment epithelial cells which had vesicular, hydropic change of their cytoplasms and loss of the fusiform pigment granules normally located in the microvilli. There was no demonstrable change in the contiguous retina or choriocapillaris. In neighboring sections the nuclei of the pigment epithelial cells demonstrated in Fig. 3 were...
Fig. 3. The first small lesion 24 hours after exposure to He-Ne laser, 6.4 mw. at 100 msec., approximately 40 per cent of the ED\textsubscript{50} for a minimal ophthalmoscopically visible lesion. Note the two contiguous pigment epithelial cells which have swollen, vesicular, hydropic cytoplasm and have lost their fusiform pigment granules. No definite change is seen in the adjacent receptors or choriocapillaris. (Paragon stain; approximately $\times 450$.)

Fig. 4 illustrates the cytoplasmic and nuclear changes described above.

**Discussion**

Of the 4 subthreshold exposures for ophthalmoscopically visible lesions, one resulted in a small but frank burn, one caused no effect, and two produced only changes in two contiguous epithelial cells when examined histologically. The histologic changes produced by these subthreshold exposures differ only in severity from those demonstrated in ophthalmoscopically visible burns and no evidence suggests a new or different mechanism of injury. The pigment epithelium appears to be the retinal cell most sensitive to injury by He-Ne laser irradiation. Klein and associates\textsuperscript{3} have shown evidence of regeneration of pigment in damaged epidermal epithelial cells after exposure to pulsed lasers. This suggests the possibility that complete recovery might have occurred from the two exposures illustrated in Figs. 3 and 4. Thus damage in the pigment epithelium may not be the limiting factor in assessing the long-term effects of retinal laser irradiation.

The variation in the magnitude of the effects produced by subthreshold exposures of minimum spot size may be related to the inhomogeneity of the retinal pigment epithelium. Although the subthreshold exposures were placed in the macula with
Fig. 4. The second small lesion 24 hours after exposure to He-Ne laser, 6.4 mw. at 100 msec., approximately 40 per cent below the EDₙ for a minimal ophthalmoscopically visible lesion. Note the two contiguous pigment epithelial cells which have lost their fusiform pigment granules and have undergone vesicular, hydropic cytoplasmic change which distorts the nucleus of the cell on the left. No definite change is seen in the adjacent receptors or choriocapillaris. (Paragon stain; approximately ×900.)

Fig. 5. Flat preparation of retinal pigment epithelium of the rhesus. Note the variation in the distribution of the pigment. (Unstained; approximately ×100.)
identical power and shutter settings, about 5 per cent variation was measured in the energy output of the four exposures. It is doubtful, however, that this variation in energy can account for the differences in effect seen in the four cases. A more likely explanation is a difference in energy absorption in different macular areas. Inhomogeneity in melanin granule distribution in the pigment epithelium of the dog has been described by Hayes and Wolbarsht. Fig. 5 illustrates an unstained flat preparation of the pigment epithelium of a rhesus monkey (prepared by George Bresnick, Major, USA, MC). Pigment granule inhomogeneity, similar to that described in the dog retina, is clearly demonstrated. Since each hexagonal pigment epithelial cell measures about 15 μ across, and the exposures were made with a minimal spot, estimated at 20 to 25 μ, it appears likely that photon absorption would vary somewhat in different areas. Thus any determination of a threshold of damage in the retina using a minimal spot size must be made on a statistical basis with multiple exposures. Nevertheless, an approximation of the ratio of ophthalmoscopic and microscopic minimum visible damage levels can be made in the present case because of the fortuitous choice of exposure parameters. The results of the four exposures, i.e., one small burn, one with no effect and two with minimal and possibly reversible effects, indicate that the exposure level of 6.4 mw. at 100 msec. represents a close approximation to the microscopic “damage threshold” in the eye under study. Relating this value to the threshold damage dose line for He-Ne lasers as determined in the previous study, the level for minimal microscopic change for this eye is approximately 40 per cent below the 50 per cent probability level for minimal ophthalmoscopically visible change.

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REFERENCES