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BIOLOGIC TESTING OF LASER PROTECTIVE MATERIALS:

A PRELIMINARY REPORT

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With the increasing use of systems incorporating lasers in scientific research and industry, the potential for accidental injury is increased. Ocular injury that can occur on such accidental exposure may frequently result in permanent loss of visual function. Such injury almost invariably leaves a demonstrable tissue alteration.

Attempts have been made to determine various thresholds of injury for some of the ocular tissues (e.g., retina, cornea) for several laser wavelengths (e.g., 694.3 nm, 1060 nm), and devices or materials have been developed to protect the eye. The required characteristics of such protective materials, in particular the optical density (o.d.) at the laser wavelengths, have been based primarily on calculations and physical measurements (including direct irradiation of the material).

It seemed reasonable to use the eye as a sensitive biologic indicator for evaluating the efficiency with which these materials attenuate the laser radiation at relatively high levels of power density, since it is not known whether physical measurements alone can accurately predict the degree of safety offered by a specified material at a given optical density. At higher power levels unexpected injury might occur in spite of the use of a device calculated to afford adequate protection. The eye *in vivo* would also assist in determining whether injury to tissues other than the retina could occur when the eye is protected by safety material against laser irradiation.

The short-term studies presented here were carried out to determine the degree of protection actually afforded by a number of such materials interposed between ruby laser radiation and rabbit eyes in vivo. An attempt was then made to correlate the optical density of the protective material at the ruby wavelength with the severity of ocular tissue alterations.

Materials and Methods

A Maser Optics normal-mode (~1 msec) and a Korad K-1500 Q-switched (~100 nsec) ruby laser were used as the radiation sources to irradiate rabbit eyes with and without interposition of the sample protective materials. These protective plastic shields were secured one-half to one inch in front of the eye. The samples evaluated were selected plastic materials used in the Glendale Optical Company Laser-Gard spectacles, thin plastic films manufactured by the American Cyanamid Company, and glass used in American Optical Company spectacles protecting against ruby lasers. The optical density of all the samples at 694.3 nm was supplied by the manufacturer.

For irradiation of both the protected and unprotected rabbit eyes, energy levels up to 4 joules were utilized in the Q-switched mode and up to 20 joules in the normal mode.

Because of the diameter of the beam, 25 to 50% of the energy entered the pupil of the unprotected eye. In general, five exposures of the protected eye were made at intervals of several minutes without moving either the animal or the filter. This was done to increase the probability of producing a lesion in a specific location and to test the filter material against repeated laser exposure. An exception was made for the thinner plastic material, which burned on irradiation. In this instance, a new, unexposed region of the plastic was used for each exposure.

The rabbits used in these experiments* weighed about 4 to 6 lb. and had darkly pigmented eyes. Prior to laser irradiation they were anesthetized with Innovar-vet (fentanyl 0.4 mg/ml and droperidol 20 mg/ml [McNeill])and atropine. The pupils were dilated with 2% atropine sulfate eye drops to a diameter of approximately 7 mm. The exposed eye was held open with a lid speculum, and the cornea was kept moist and clear with intermittent saline irrigation. The radiations were directed to the retina in line with, and

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*In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences - National Research Council.

inferior to, the optic disc. Choroidal markings were utilized to locate the smallest lesions.

Ophthalmoscopic examinations were performed before, and immediately after, irradiation, and at intervals during the following week.

Some of the animals were sacrificed two weeks after irradiation, and these eyes were fixed in 2% buffered glutaraldehyde solution[1] for the histopathologic examinations reported here.

The opened eyes were examined grossly under 15 to 20 X magnification of a dissecting microscope. Small blocks of tissue were removed under direct observation from any region in the irradiated area that appeared to be abnormal, fixed secondarily in Dalton's chrome-osmium fixative, and subsequently embedded in an epoxy resin for light and electron microscopic study as described elsewhere.[2] Sections were cut serially at 1 micron for light microscopic examination.

Results

Normal-Mode (~1 msec) Laser Irradiation

Following 10-joule irradiation of the unprotected eye there was some irregularity of the cornea--enough to prevent visualization of the retina. Approximately three hours after irradiation the retina could be visualized ophthalmoscopically, and a "punched-out" lesion with a ragged, heavily pigmented border was observed. Lesions of this type were regarded as mild to moderate.

Following 20-joule normal-mode irradiation of the unprotected eye, slightly hemorrhagic lesions were observed immediately. These lesions were classified as moderate to severe. Therefore, this level of irradiation was used to test the effectiveness of the filters as protective devices. The irradiated eye was examined ophthalmoscopically after each exposure. If a lesion was observed, further irradiation of this type was not made.

Interposition of plastic filter material of 2.8 o.d. resulted only in decrease of the size and severity of the lesion. Interposition of filters with 3.5 to 4.0 o.d. resulted in one mild lesion with a pale center and pigmented edge at o.d. 3.5 and a single questionable lesion at o.d. 4.0 (total of 17 irradiations).

Q-Switched Mode (~100 nanosec) Laser Irradiation

Following 1-joule irradiation of the unprotected eye, there was marked disruption of the retina and vitreous. At 2.7 joules, bubbles were present in the anterior chamber, together with scattered iris pigment. The posterior portion of the eye could not be visualized.

On irradiation at 2.7 joules, with the 2.8-o.d. plastic filter interposed, several retinal lesions were produced without detectable injuries to the anterior segment. In one instance a sharply localized hemorrhagic lesion was confined to the retina. In a second instance, the retinal lesion was associated with hemorrhage into the vitreous.. The blood in the vitreous slowly cleared over the next several days.

On Q-switched irradiation at 2.7 joules (~50 megawatts), the lesions produced on interposing plastic filters of 3.5 and 3.9 o.d. appeared as moderate to mild. On interposition of 4.0.-o.d. filters, the lesions produced were mild to questionable. There was no evident lesion on interposition of 6.3-o.d. filter after five shots to the eye.

Histologically, tissue alterations were demonstrable in every case in which a lesion could be detected or was even suspected by either ophthalmoscopy or by gross examination. The tissue responses varied from well-defined retinal adhesions (figs. 1, 2) through a milder response of focal destruction of the pigment epithelium associated with changes in the overlying photoreceptor cells (figs. 3, 4) to the most subtle alteration, that of the presence of a few pigment-filled macrophages within an otherwise normally adherent rod and cone layer (fig. 5).

Discussion

The energy density required to produce a threshold retinal lesion with Q-switched irradiation has been shown by Geeraets et al[3] to be about one-tenth (retinal energy density of 0.1 joule/cm^2)that required with normal-mode irradiation (retinal energy density of 1.0 joule/cm^2).

If this 10:1 ratio were continued into the suprathreshold levels of irradiation, one might then expect to find lesions of similar relative severity for Q-switched and normal-mode irradiation.

From our studies here on suprathreshold Q-switched irradiation of the unprotected eye at approximately one-tenth the levels of normal-mode irradiation (2.7 joules Q-switched vs. 20 joules normal-mode), the Q-switched lesions were much more severe than anticipated. Even with 1-joule Q-switched irradiation of the unprotected eye, the lesion was more severe than after 20-joule normal-mode irradiation. Interposition of a filter of 2.8 o.d. between the 2.7-joule Q-switched beam and the rabbit eye was necessary to produce a lesion that could be considered roughly equivalent to one produced on 20-joule normal-mode irradiation of the unprotected eye. Consequently, at suprathreshold levels, Q-switched irradiation can be considered significantly more hazardous than expected from the 10:1 ratio obtained on the basis of threshold studies. This increase in hazard may be due to generation of pressure waves within the eye, which can be considered as a closed, fluid-filled cavity.[4-6]

In our studies, the calculated energy entering the eye required to produce a retinal threshold lesion of much greater than minimal spot size (~10 microns) was of the order of 10^{-3} joules on normal-mode irradiation and 0.1×10^{-3} joules on Q-switched irradiation. Rough calculations indicate that the energy density required at the retina for production of threshold lesions in the rabbit eye is in general agreement with the levels obtained by Geeraets et al; that is, about 1 joule/cm^2 after normal-mode irradiation, 0.1 joule/cm^2 with Q-switched irradiation.[3]

In general, there was no detectable injury to tissues of the eye other than the retina following interposition of the filters. The filter material therefore appeared to protect as anticipated. The degree of protection offered by the plastic materials increased as the optical density increased, both for the normal and for the Q-switched modes of irradiation.

In some instances, what appeared to be alterations in the corneal epithelium after normal-mode irradiation cleared within 24 hours. These surface irregularities would occasionally limit our examination of the retina immediately following irradiation. With clearing, the fundus could be examined within 24 hours.

In those irradiated eyes that were examined histologically, it was found that changes could be demonstrated even in lesions that appeared clinically to be extremely mild or questionable. It seems that if a retinal lesion cannot be found by ophthalmoscopy combined with careful fixation and serial sectioning of the suspected site of impact, the exposure must be near threshold or subthreshold. Such a minimal area of tissue alteration is illustrated in Figure 5. By ophthalmoscopy this lesion was so extremely minute that it was considered questionable clinically and doubtfully present by gross microscopic examination, but minimal pathologic changes could be demonstrated histologically.

These studies are considered to be exploratory in nature. Eyes in vivo were used as sensitive biologic indicators to study the effectiveness of filter materials. More detailed studies will be necessary to evaluate the exact capabilities of representative protective materials. The use of eyes in vivo may be of considerable significance if these materials exhibit nonlinear protection, particularly at very high power densities, such as those occurring with picosecond pulses. Indeed, as expected, the thin plastic materials tested did undergo change during irradiation at the levels of power and energy density used, leaving a permanent record of the site of impact. Since such thin plastic was not considered suitable or indeed had not been designed as material for safety spectacles by the manufacturer, repeated irradiation of these plastics in the same location was not done as part of this study.

These experiments were acute studies in order to determine whether unpredictable injuries may occur at suprathreshold irradiation of eyes protected by filters. Longer term follow-up studies are required. Delayed effects cannot be evaluated unless a biologic system is used as a detector.

Summary

A series of filters having considerable attenuation at the ruby laser wavelength were evaluated as protective devices for the eye in short-term studies by using the sensitivity of in vivo ocular tissue response. The ability of the filter to protect the eye increased with increasing optical density at the laser wavelength, as expected. Long-term studies are needed to evaluate possible delayed effects.

Compared with normal-mode irradiation, the relative hazard of Q-switched irradiation at suprathreshold levels is much greater than expected from threshold studies.

The use of multiple irradiations rather than single exposures to an eye with and without interposition of filters might provide a better criterion for establishing thresholds for injury as well as determining the degree of protection offered by laser protective glasses.

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Legends

Fig. 1 (Fine, Berkow, MacKeen, and Fine). Q-switched ruby, 2.7 joules, incident on filter (2.8 o.d.). A chorioretinal adhesion is present that involves all of the retinal layers in the center of the lesion two weeks after a single test exposure into this eye. A grossly disrupted layer of cells occupies the plane of the pigment epithelium in the zone between the two free arrows. The choriocapillaris appears occluded in this zone, but there is no scarring between retina and choroid (paraphenylenediamine, X145, AFIP Neg. 69-2831).

Fig. 2 (Fine, Berkow, MacKeen, and Fine). Q-switched ruby, 2.9 joules, incident on filter (3.5 o.d.). Small, apparently adherent retinal adhesions involve the pigment epithelium and overlying photoreceptor cells. The injury has extended to the bipolar cell layer, but the ganglion cell layer appears normal. The underlying choriocapillaris and choroid appear normal. Pigment (melanin) granules are present within the retinal layers that were injured. From an eye that received five exposures (paraphenylenediamine, X300, AFIP Neg. 69-2834).

Fig. 3 (Fine, Berkow, MacKeen, and Fine). Q-switched ruby, 2.7 joules, incident on filter (4.0 o.d.). Dense accumulations (D) (mitochondrial changes) are present within the overlying photoreceptor inner segments. The retinal layers anterior to the photoreceptors appear morphologically normal, as do the underlying choriocapillaris and choroid. Eye with single exposure, (paraphenylenediamine, X265, AFIP Neg. 69-2830).

Fig. 4 (Fine, Berkow, MacKeen, and Fine). The densities observed in the acutely injured photoreceptor inner segments in Figure 3 are due to the characteristic[7] accumulation of spicules of dense material resembling a crystalline deposit within the mitochondria of the inner segments. The adjacent photoreceptors (inner-segment mitochondria [M] as well as outer segments) are normal because of the obliquity of the section and are therefore interpreted to be uninvolved in this lesion (paraphenylenediamine, X16,500, AFIP Neg. 70-5365).

Fig. 5 (Fine, Berkow, MacKeen, and Fine). Q-switched ruby, 2.7 joules, incident on filter (3.5 o.d.). Two small foci of tissue alteration are present (free arrows). The retinal layers on each side appear normal, as do the choriocapillaris and choroid. This suggests either original injury in another plane of section, with migration of a few cells (macrophages) into this region, or possibly repair of a minor injury. From a single exposure into the eye (paraphenylenediamine, X265, AFIP Neg. 69-2832).