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LASER-INDUCED PATHOLOGY OF THE RABBIT RETINA: COMPARISON OF THREE RADIATION WAVELENGTHS

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SUMMARY PAGE

THE PROBLEM

To establish the manner in which the severity of damage to the eye in the form of burns from laser light varies with the energy density and the wavelength of the laser light.

FINDINGS

Three different wavelengths of laser light were used at similar energy density levels to induce damage in the eyes of rabbits, and the amount and type of damage was evaluated by microscopic techniques. The most serious damage apparently resulted from the light of shortest wavelength and decreased for longer wavelengths.

APPLICATIONS

These findings and similar information of a more complete nature will provide a basis for predicting the result of exposure to a given type and intensity of laser light. These predictions are especially useful in medical applications to remedy certain defects of the eye. This information will also be used in prescribing protective measures and devices to guard against accidental exposure to laser light and for determining proper treatment when such incidents occur.

ADMINISTRATIVE INFORMATION

This investigation was conducted as a part of Bureau of Medicine and Surgery Research Work Unit MF022.03-08-9001 - Biomedical Aspects of Naval Laser Applications. This is Report No. 1 on this Work Unit; however, two closely related reports were published under a superceded Work Unit - MR005.08-5203-1. These were SMRL Memorandum Reports 65-1 and 66-3. The present report was approved for publication on 15 March 1968 and designated as Memorandum Report No. 68-4. It has been approved for public release and sale; its distribution is unlimited.

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ABSTRACT

Since laser radiation consists of essentially parellel rays, the wavelengths to which the ocular media is relatively transparent are focused by the eye at or near the fundus, greatly concentrating the energy in the process. Because retinal damage can result, the differential effects in terms of wavelength and power levels is of considerable importance. In this study, damage to the rabbit retina was induced by irradiation with neodymium, ruby and frequency doubled neodymium lasers. The neodymium laser (wavelength 10,600 angstroms) produced the smallest lesions and least damage. The ruby (6943 Å) and frequency doubled neodymium (5300 Å) lasers caused lesions similar in size and seven day post-irradiation histo-pathology except for inter-layer adhesion differences.

LASER-INDUCED PATHOLOGY OF THE RABBIT RETINA: COMPARISON OF THREE RADIATION WAVELENGTHS

INTRODUCTION

The effects of high energy visible or near infrared electromagnetic radiation upon the retina has been the subject of extensive laboratory experimentation on animals, and of clinical observation of accidentally or intentionally exposed humans. Humans may be accidentally exposed while observing an eclipse of the sun or an atomic explosion with insufficient ocular protection, and intentionally exposed during treatment of detached retina, and other retinal anomalies, with light coagulators. Before the advent of the laser most of these exposures were to heterochromatic light. Using the laser it is now possible to study wavelength dependent effects on laboratory animals. In addition, ruby laser photocoagulators are now used clinically on human patients and treatment results have been extensively documented (1, 2, 3, 4).

It has been shown that most of the damage to the retina results from a thermal transformation of energy at the level of the pigment epithelium, resulting in coagulation of adjacent proteins (5). Since retinal structures such as the neural layers, melanin granules and blood vessels have wavelength dependent coefficients of absorption, it has been speculated that laser radiation induced retinal effects would be a function of wavelength and there is evidence that this is true (6). Most investigations to date have been concerned with the effects of the ruby laser (6943 Å), with a few reports of ocular effects of longer wavelengths (6, 7). This study compares the retinal effect of ruby laser irradiation with that induced by wavelengths both shorter and longer.

METHOD AND APPARATUS

Two types of laser devices were used, a clinical laser photocoagulator (Optics Technology, Model M-10, Mk II), and a Q-switched (shortened pulse length) frequency doubled neodymium YAG (Korad, Model KY-19). The clinical photocoagulator had interchangeable ophthalmoscopic heads, one equipped with a 7.6 cm. long by 0.6 cm. diameter ruby crystal, and the other a neodymium-in-glass crystal of the same dimensions. The pulse lengths were approximately 200 milliseconds (200×10^{-3} seconds) for the ruby and neodymium lasers and 9 nanoseconds (9×10^{-9} seconds) for the frequency doubled neodymium YAG laser. Beam divergences were in the 3 to 5 milliradian range for the ruby and primary neodymium lasers and 2 to 4 for the frequency doubled neodymium. Average beam diameters, measured from impacts on carbon paper and film, were approximately 5 millimeters for all three wavelengths.

Chincilla grey rabbits weighting from four to seven pounds were used as subjects. The rabbit's pupils were maximally dilated by instillation of three drops of 2 per cent homatropine hydrobromide prior to the experiments. A retinoscopic estimate of the refractive error of each eye was made after instillation of the drug. The ophthalmoscopic head of the laser photocoagulator afforded direct observation of the retina through the drug-dilated pupil, making possible the selection of lesion sites which, in all cases, were located in a horizontal series from one-quarter to one disc diameter below the optic nerve disc.

A different procedure was necessary with the frequency doubled neodymium laser. After anesthesia with sodium pentobarbital, the rabbit was placed with one eye in apposition to an adjustable iris diaphragm. The laser beam was then directed normally to the plane of the diaphragm aperture and through the dilated pupil by interposing a 90 degree deflecting prism in the beam path. This procedure was usually applied to both eyes. Only those lesions occurring in the same areas below the optic disc as were produced by the photocoagulator were used for comparison.

Using a Korad, model KJ-2, calorimeter and Keithley strip-chart recorder, the energy of three laser firings was measured and averaged immediately before and after each of the primary neodymium and ruby laser experiments. These averages, 25 millijoules for the ruby (6943 Å) and 65 millijoules for the neodymium (10, 600 Å), were then assumed to be close approximations of the energy per pulse incident upon the rabbit's cornea during the intervening experiments. A pulse energy of 0.3 millijoule was used in the case of the frequency doubled neodymium laser (5300 Å), determined by averaging the energy of ten pulses, measured with an E.G.&G. model 585 radiometer just prior to the experiments. Energy per pulse variations ranging to ± 10 per cent were found for all three laser wavelengths. Retinal photographs were made immediately after irradiation and at intervals for approximately one week, at which time the animal was sacrificed and the eyes enucleated.

The retinal lesions resulting from 6943 Å radiation presented as small, round, well delineated greyish areas immediately after impact. There was some variation in appearance over a series of thirty lesions with size being the principal variant. A small hemorrhage was produced at the lesion site in one instance. It should be pointed out that these were moderately severe lesions since the energy densities used were almost an order of magnitude greater than Campbell et al (8) found necessary to induce threshold lesions in the grey chinchilla rabbit retina.

Radiation at 10,600 Å produced lesions that were similar in appearance but smaller than those induced by the ruby laser. No hemorrhages were seen.

It was not possible to observe the retina while firing the Q-switched laser (5300 Å) but an ophthalmoscopic inspection was made immediately after impact. Without exception the impact sites were round and blood red in color. There was some shot to shot variation in apparent lesion size. Observation of the lesion sites over a week-long period revealed diminishing evidence of hemorrhage, with apparently, almost complete absorption by the end of seven days.

All of the enucleated eyes were fixed in formalin and imbedded in paraffin. Several sections were cut at five microns and stained with hemaloxylin and eosin.

RESULTS

There is significant variation in the histological evidence of retinal damage produced by each laser. The more severe lesions produced by the ruby laser (Figure 1) show disruption of all the nuclear and neural layers of the retina out to the pigment epithelium. Many of the pigment cells are destroyed and their pigment is scattered inward through the damaged retina where much of it is engulfed by macrophages. Remaining in the pigment layer are large cells containing pigment and spaces where pigment is lost. There is formation of fibrous adhesions between the damaged pigment epithelium and the destroyed inner retina. The burn is 300 microns in diameter but is surrounded by an area of detachment of the pigment epithelium from the layer of rods measuring 800 microns in diameter. Although frequently this separation occurs as a histological artifact, it is consistently present in this site and possibly represents a lesion produced by vaporized fluid at the time of the burn and subsequent edema. Less severe ruby induced lesions reveal preservation of the layer of nerve fibres and the inner nuclear layer and destruction and adhesions in the remainder of the retina similar to the more severe lesions.

The more severe of the frequency doubled neodymium induced lesions (Figure 2) reveal disruption of the retinal layers down to the pigment epithelium and damage to this layer similar to that in the severe ruby lesions. In none of the lesions in this group is there formation of adhesions between the destroyed neural layers and the damaged pigment epithelium. The diameter of the destroyed retina and of the surrounding detachment is similar to the ruby lesions. The less intense lesions in this group reveal preservation of the inner retinal layers and destruction of the outer layers similar to the less intense ruby lesions.

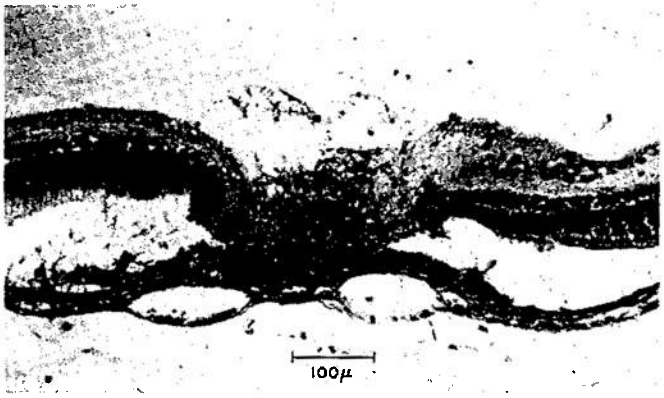

Figure 1 - Cross section of rabbit retina one week after burn by ruby laser (wavelength 6943 Å). There is destruction of the neural retina with formation of adhesion to the damaged pigment epithelium. The retinal detachment ceases just beyond the bounds of the photomicrograph.

Figure 2 - One week after burn by frequency doubled neodymium laser (5300 Å). There is similar damage to that in Figure 1 except that no adhesion has formed.

The neodymium laser, at the energy densities used, produced lesions (Figure 3) consistently sparing the nerve fibre and inner nuclear layers. The outer layer and layer of rods are destroyed. Here also there is scattering of pigment through the lesion and alteration of the pigment epithelium. In this group, there are no preserved pigment containing cells in the pigment epithelium immediately under the destroyed neural retina. There is reactive tissue over the depigmented area but no adhesions bind the neural retina to this tissue. The destroyed neural retina is approximately 100 microns in diameter and the area of detachment 300 microns in diameter.

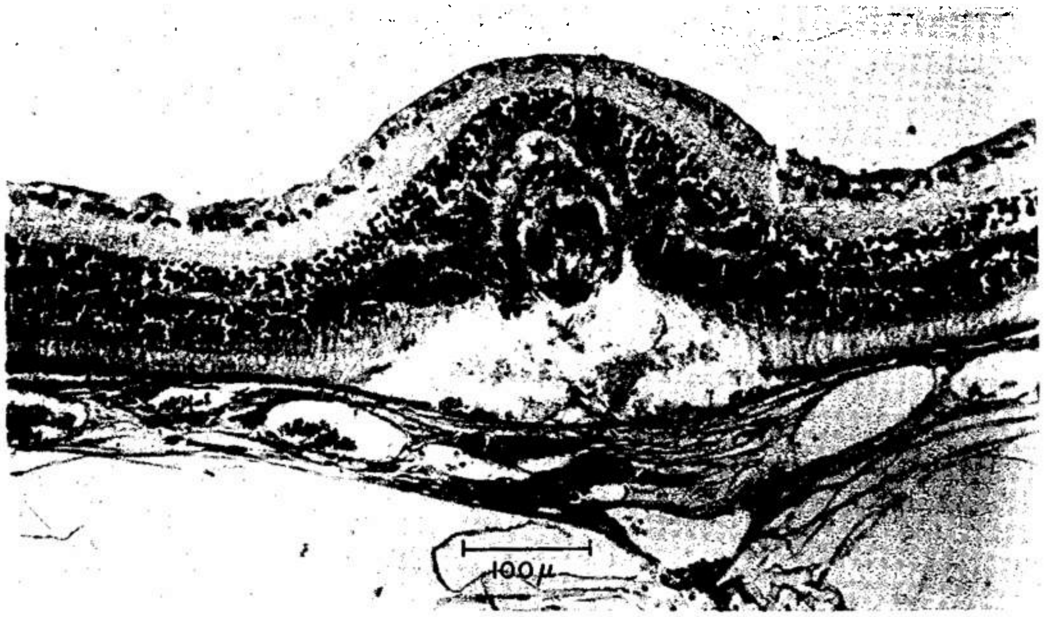

Figure 3: One week after burn by neodymium laser (10, 600 Å). There is preservation of the inner neural retina and destruction of the outer layers without adhesion formation. There is loss of pigment from the pigment epithelium.

DISCUSSION

Since it is well known that thermally induced retinal lesions differ in size in proportion to the density of the inducing energy and its absorptance by pigmented retinal structures, the variations in lesion size noted for each wavelength used in this study can be explained by the small variations in pulse energy and particularly by the differing pigmentary and vascular characteristics of the lesion sites. The lesions produced by 10, 600 Å radiation were consistently smaller than those resulting from 6943 Å radiation, although the energy per pulse, with similar beam diameter and divergence characteristics, was 2-1/2 times greater. Retinoscopic estimates of the refractive state of each rabbit revealed that all were hyperopic ranging from two to four and a half diopters. From purely geometrical optical considerations, therefore, the retinal area irradiated should have been larger in the case of the longer wavelength since for all visible and near infrared wavelengths the focus would have fallen behind the retina with the amount increasing with wavelength in proportion to the dispersion of the rabbit eye. It is likely that the comparatively smaller lesions induced by 10, 600 Å radiation resulted from insufficient energy compensation for the known lower ocular media transmission (9) and particularly the lower retinal pigment epithelium absorptance (9, 10) at this wavelength.

When comparing the effects of 5300 Å radiation to that of 6943 Å and 10, 600 Å, the shorter pulse length must also be considered since it has been shown that laser radiation retinal effects vary with the rate of energy delivery. According to Geeraets et al (11), a given amount of ruby laser radiant energy delivered in 200 to 300 milliseconds can be roughly equated in severity of retinal damage (although histological differences exist) with that induced by 1/10 this amount delivered in 30 nanoseconds. Kohtiao and his group (12) found that 6943 Å radiation with pulse lengths of 500 milliseconds equated with 80 nanosecond Q-switched pulses of 1/40th the energy in producing threshold retinal damage. These rate effects have been attributed to steam production, thermal conduction differences and to largely unexplored non-linear processes (5, 13). If optical media transmission and pigment epithelium absorption are considered as a product and plotted against wavelength, as Geeraets has done (14), 5300 Å radiation lies at or near the peak of a curve showing spectral effectiveness in inducing damage at the pigment epithelium level of the rabbit retina. These are probably the principal factors to be considered in explanation of the histological similarities of the retinal lesions resulting from 0.3 millijoules of Q-switched 5300 Å radiation and 25 millijoules of normally pulsed 6943 Å radiation.

Radiation at 5300 Å is within a narrow band of visible light wavelengths highly absorbed by hemoglobin (15). This probably predisposed to the hemorrhaging noted at the impact sites when this wavelength was used, with separation of the neural retina from the pigment epithelium being one consequence. The resulting interference with formation of a fibrous adhesion by the slowly absorbing hemorrhage would explain the major difference noted in the histopathology induced by this laser (frequency doubled neodymium) and the ruby device.

SUMMARY

Since laser radiation consists of essentially parallel rays, the wavelengths to which the ocular media is relatively transparent are focused by the eye at or near the fundus, greatly concentrating the energy in the process. Because retinal damage can result, the differential effects in terms of wavelength and power levels is of considerable importance. In this study, damage to the rabbit retina was induced by irradiation with neodymium, ruby and frequency doubled neodymium lasers. The neodymium laser (wavelength 10, 600 angstroms) produced the smallest lesions and least damage. The ruby (6943 Å) and frequency doubled neodymium (5400 Å) lasers caused lesions similar in size and seven day post-irradiation histo-pathology except for inter-layer adhesion differences.

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13. ABSTRACT

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