Delayed Retinal Effects of the Frequency-Doubled YAG Laser (532 nm)

Marjorie A. Mosier,* Jean Champion,† Lih-Huei L. Liaw,‡ and Michael W. Berns‡

In order to compare the retinal effects of the frequency-doubled YAG laser (532 nm) with those of argon laser, rabbit eyes were exposed to green YAG laser irradiation and processed for light and electron microscopic study at 24 hr, 2 weeks and 4 weeks. Detailed analysis was conducted on tissue exposed to 7.3 and 7.6 millijoules (mj). Response of the photoreceptors and retinal pigmented epithelium to green YAG was very similar to that described for argon laser over the same time period. By 2 weeks post-exposure, there was histologic evidence of partial recovery with absence of damaged, pyknotic photoreceptor nuclei, increased numbers of typical photoreceptor outer segment lamellae and repair of retinal pigmented epithelium. Four weeks after irradiation, normal-appearing photoreceptor nuclei were present although inner photoreceptor segments still showed mitochondrial damage. Outer segments at 4 weeks showed regular lamellar structures. We conclude that the frequency-doubled YAG laser is equivalent to the argon laser with respect to the production of thermal lesions in the retina. Its additional advantages include increased efficiency, portability, reliability and lack of absorption by macular xanthophyll pigment. Invest Ophthalmol Vis Sci 28:1298-1305, 1987

Although the argon laser (488 and 514 nm) has been widely used for the treatment of retinal disease, it has certain disadvantages. These include its inefficiency and resultant power loss, its considerable maintenance requirement, non-portability and the absorption of energy by macular xanthophyll pigment. These drawbacks, except for xanthophyll absorption, also apply to krypton green laser. An ideal replacement for these lasers would be an efficient, low-maintenance, portable laser using a wavelength absorbed only by pigment in the retinal epithelium and by hemoglobin. These features are present in the green YAG laser.

The frequency-doubled (532 nm) green YAG laser was first suggested as a possible replacement for the argon laser in 1971 by L’Esperance, who showed that the morphologic effects of the two lasers were similar. In that study, an experimental prototype continuous wave laser was used to treat seven human eyes, later examined histologically. Puliafito reported preliminary experiments on rabbit and monkey eyes using the pulsed “green” YAG laser of Laserscope, Inc. Simultaneously, we reported the short-term effects on rabbit eyes. In both our studies and those of Puliafito, the gross morphology and light microscopic histology showed that the YAG and argon wavelengths produced similar lesions when a high repetition pulse rate and low peak powers were used.

Our early experiments used a Quantronix research laser (Quantronix Corp., Smithtown, NY) operating at 8–12 kHz with peak powers of 25–50 W. Rabbit eyes were fixed for histologic analysis within 2 hr of laser exposure. Only subtle and probably insignificant differences were noted between the lesions produced by argon and green YAG lasers.

Support for the possibility of true equivalency of green YAG and argon laser effects requires studies of the late histopathologic response of irradiated tissue. The present study examines retinal lesions up to 1 month following exposure to green YAG laser and compares the changes to those seen following argon laser irradiation.

Materials and Methods

Animals

A total of 63 sites were irradiated in both eyes of five Dutch Cross rabbits. The animals were maintained pre- and postoperatively under standard conditions in the Animal Resource Facility, University of California, Irvine, and were anesthetized and sacrificed as described previously and in accord with the ARVO Resolution on the Use of Animals in Research. Enucleation was performed at 24 hr, 2 weeks and 4 weeks postoperatively.
Laser Exposure

The source of irradiation was the 532 nm beam from the Humphrey Instruments frequency-doubled YAG laser (Humphrey Instruments, San Leandro, CA). Measurements taken on the day preceding exposure indicated that the laser was operating at 12.8 kHz, with individual pulse durations of 400 nanoseconds. A 25 mm focal length lens was used to image the beam on the retina to a spot diameter of 200 μm. The total energy in each spot for this study ranged from 1.27 to 7.61 millijoules (mj). Detailed histology was conducted on exposures of 7.3 and 7.6 mj.

Histopathology

The eyes were enucleated, fixed and sectioned for both light and electron microscopic analysis as described previously.4 Tissues containing 35 of the 63 lesions were embedded in plastic and prepared for sectioning and microscopic examination. Initial histopathologic analysis was performed on lesions produced with energies of 7.3 and 7.6 mj. This was chosen because previous experiments indicated that 7–12 mj produced clinically visible, equivalent lesions with both argon and the green YAG lasers.4 The resulting lesions at 24 hr, 2 weeks and 4 weeks appeared similar to standard argon lesions by slit lamp examination. At least two lesions at each time point were examined by light microscopy, and one of each was further examined by electron microscopy.

Results

Threshold Lesion

Initial experiments were designed to determine the energy required to produce a visible blanching of the retina 50% of the time, ie, the threshold energy. This was determined to be 1.37 mj in a 200 μm-diameter spot.

Histopathology

Photographs of histopathological results are grouped into three sets: the 24-hr lesion is shown in Figures 1–3, the 2-week lesion in Figures 4–7 and the 4-week lesion in Figures 8–12.

24-hr Lesion

At 24 hr, the low power light micrograph (Fig. 1) demonstrates typical thermal photocoagulation histopathology. A zone of damage is clearly demarcated, extending from just below the inner neural layer and extending through photoreceptors and retinal pigmented epithelium (RPE). The dark-staining pycnotic photoreceptor nuclei are prominent, sharply separated from undamaged nuclei (Fig. 1, arrows). Inner and outer photoreceptor segments appear damaged and displaced by serous exudate. Severe intra-cellular disorganization of photoreceptor inner segments is shown by high magnification electron micrography (Fig. 2). Outer photoreceptor segments are highly dis-
organized as well and do not exhibit any of the orderly lamellar membrane stacks (Fig. 3). The RPE is highly disrupted with melanin granules interspersed with necrotic and vacuolar debris (Fig. 3). Loss of RPE integrity, separation from Bruch’s membrane and loss of lamellar organization of photoreceptor outer segments are seen under high magnification electron microscopy (Fig. 3).
Two-Week Lesion

At 2 weeks, low power light microscopy shows considerable change. The zone of damage is still clearly demarcated (Fig. 4, arrow). Pycnotic photoreceptor nuclei are strikingly absent from this area and have been replaced by cellular debris and vacuolation (Fig. 5). The RPE appears somewhat less damaged than previously and is apposed to Bruch's membrane (Fig. 6). Photoreceptor outer segments are greatly disrupted in some areas (Fig. 6, arrow), but approach normal structure in others (Fig. 7). In the mitochondria-rich apex of inner segments, mitochondrial damage is evidenced by loss of many cristae, although outer membranes generally appear intact.

Four-Week Lesion

Considerable recovery in the lesion area has occurred at 4 weeks. The low power light micrograph

Fig. 4. Low power light microscopic appearance of rabbit retina 2 weeks after exposure to 532 nm YAG laser (7.3 mJ). Zone of thermal damage is clearly demarcated (X450).

Fig. 5. Electron micrograph of rabbit photoreceptor nuclear zone 2 weeks after exposure to 532 nm YAG laser (7.3 mJ) showing replacement of pyknotic nuclei with cellular debris and vacuolation (X10,800).
Fig. 6. Electron micrograph of rabbit retina 2 weeks after exposure to 532 nm YAG laser (7.3 mJ). The pigmented epithelium is apposed to Bruch's membrane and shows moderate damage. Photoreceptor outer segments show focal disruption (arrow) (×5000).

demonstrates a buckling in the center of the lesion (Fig. 8, arrow), producing a separation of photoreceptor outer segments and RPE. Normal-appearing photoreceptor nuclei are adjacent to the damaged zone. The RPE appears normal and uniformly apposed to Bruch's membrane. Normal photoreceptor

Fig. 7. Electron micrograph of rabbit photoreceptor outer segments 2 weeks after exposure to 532 nm YAG laser (7.3 mJ), showing significant recovery of normal lamellar structure (×10,800).
outer segments are evident (Figs. 9–11). Photoreceptor nuclei appear normal by electron microscopy (Figs. 10 and 12). Mitochondria in photoreceptor inner segments show some disruption, suggesting that repair is not complete (Figs. 10 and 11).

Discussion

The severe tissue damage observed in the 24-hr samples underwent significant modulation by 2 weeks. At this point, the lesions showed absence of damaged cells from the zone of irradiation, along with repair and regeneration, or the inward movement of undamaged cells from the periphery of the lesion zone. A striking similarity is noted between this appearance and that reported for argon laser lesions. In 1973, Tso et al described 1–4-week argon lesions as follows: "One to four weeks after exposure, most of the pycnotic nuclei in the center of the lesions are absent, with regeneration indicating repair of damaged cells, or movement of undamaged cells inward."

Fig. 8. Low power light micrograph of rabbit retina 4 weeks after exposure to 532 nm YAG laser (7.3 mJ), showing buckling of center of lesion (arrow), with separation of photoreceptor outer segments from flat pigmented epithelium (×450).

Fig. 9. Electron micrograph of rabbit photoreceptor outer segments 4 weeks after exposure to 532 nm YAG laser (7.3 mJ), showing normal lamellar structure (×4400).
were removed . . .” and: “Eight weeks after injury, repair and regeneration were most apparent.\textsuperscript{5}

The present set of experiments shows that the histopathological response of the retina to green YAG laser is very similar to that described for the argon laser, both initially and over a 4-week period post-exposure. During that time, lesion development and repair in both types of laser injury occur in a similar manner. The disappearance of damaged photoreceptor nuclei and the return of relatively normal photoreceptors (as evidenced by normal nuclei and lamellar-patterned outer segments) bears a striking resemblance to the same events described for the argon laser in 1973 by Tso et al.\textsuperscript{5} We conclude, therefore,
that the frequency-doubled YAG laser is equivalent to the argon laser with respect to the production of thermal lesions in the retina.

Key words: laser, retina, histopathology, YAG, green YAG

References