Histopathology of Diode and Argon Laser Lesions in Rabbit Retina

A Comparative Study

Rosario Brancato,* Riccardo Pratesi,† Giovanni Leoni,* Giuseppe Trabucchi,* and Umberto Vanni†

Argon lasers are the most frequently used laser source for retinal photocoagulation. These are expensive, bulky and low-efficiency devices. In contrast, diode semiconductor lasers have a number of characteristics that make them attractive for medical application: compact size, high electrical-to-optical efficiency, and long operating lifetime. Comparable irradiance lesions (about 120 W/cm²), ophthalmoscopically similar to those obtained therapeutically in humans, were obtained by a diode and an argon laser. Twenty-four hours after the treatment, a study of these lesions was made by light and electron microscopy. Argon irradiations resulted in damage to both the inner and the outer retinal layers, while the diode laser radiation produced damage to the outer retina and choroid. A sufficient diode laser light passed into the choroid to induce small vessel occlusions and/or edema. The histological characteristics of the lesions produced by the two lasers suggest the use of the diode lasers, as well as argon lasers, in the treatment of retinal and subretinal pathologies. Invest Ophthalmol Vis Sci 30:1504–1510, 1989

Materials and Methods

Diode Semiconductor Laser

A Spectra Diode Laboratories, Inc. (Palo Alto, CA) AlGaAs diode laser model SDL 2420 HI, emitting 200 mW continuous wave at 811 nm, was used. The laser head (Fig. 1), consisting of a box containing the laser source, the thermoelectrical cooler and the optics, was placed above the microscope of a slit-lamp system (CSO, Florence, Italy).

The output beam (with longitudinal and transversal angular widths of 15° and 35°, respectively) was collimated by a large numerical aperture lens system with antireflection coating. The laser beam was deflected 90° along the microscope viewing axis by a prism positioned in front of the exit cornea. The cross-section of the beam at the position of the rabbit's eye was 400 μm horizontally and 1 mm vertically, with a convergence angle of 2° 30'. A Peyman 25 mm focal length lens (Ocular Instruments, Bellevue, WA) was used to image the beam on the retina to a 200 × 500 μm rectangular spot. The input laser power at the cornea, measured using a power meter (Scientech 362, Boulder, CO), was 120 mW.

Argon Laser

The model used was the 920 argon manufactured by Coherent Medical (Palo Alto, CA). Its continuous emission was 70% in the region of 488 nm and 30% in the 514 nm spectra. A Peyman lens with a focal

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From the *Department of Ophthalmology, S. Raffaele Hospital, University of Milan, Milan, Italy, and the †Quantum Electronic Institute, CNR, Florence, Italy.

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Reprint requests: Prof. R. Brancato, Clinica Oculistica dell'Università di Milano, Hospital S. Raffaele, Via Olgettina 60, 20123 Milano, Italy.

1504
length of 25 mm was used to obtain a circular retinal spot of about 350 μm diameter. Thus the area of the irradiated surface was comparable to that irradiated with the diode laser. The selected power was 120 mW.

**Laser Photocoagulation**

For this experiment, six chinchilla rabbits with weights between 2.5–3.2 kg were used. The animals were anaesthetized by the intravenous injection of pentobarbital (Nembutal, 1–2 cc).

In order to study lesions produced in ocular fundi of similar pigmentation, each animal was photocoagulated in the right eye with the argon laser and in the left eye with the diode laser. All the photocoagulations were performed in the inferior parapapillary zone. The lesions selected for histological and ultrastructural examinations were those that appeared ophthalmoscopically similar to those obtained therapeutically in human eyes. The power used in the experiment was maintained at 120 mW for both lasers, while the exposure time was varied by intervals of 0.1 to 1.0 sec. Immediately after the photocoagulation, serial photographs of the fundus lesions were taken with a retinograph Kowa ReXr (Kowa Co., Ltd., Tokyo, Japan).

**Histological and Ultrastructural Study**

Twenty-four hours after irradiation the animals were anaesthetized and perfused through the vascular system with Ringer’s solution, to which was added potassium procain 1%, and then with a fixative solution of 2% gluteraldehyde in 0.1 molar phosphate buffer (pH 7.4). The eyes were enucleated after perfusion and fixed in the same fixative for 6 hr after removal of the cornea. Thereafter, the areas of impact were prepared and postfixed with a 1% osmium tetroxide solution in 0.2 molar cacodylate buffer. After the dehydration, the sectioned pieces were embedded in epoxy resin. Thin sections were then cut with an ultratome (Reichert OmU2, Wien, Austria) and stained with methylene blue for light microscopy. Ultrathin sections were stained with uranile acetate for transmission electron microscopy (Philips CM 10, Eindhoven, The Netherlands).

The treatment of experimental animals in this study was in compliance with the ARVO Resolution on the Use of Animals in Research.

**Results**

Suprathreshold lesions similar to those obtained therapeutically on human retinas were produced with an exposure time of 0.8 sec by the diode laser (Fig. 2). Ophthalmoscopically, the photocoagulations appeared as somewhat rounded, small white areas, with
approximate diameters of 350 μm, encircled in the periphery by a whitish gray ring. Lesions, which appeared similarly white, were obtained with the argon laser when an exposure time of 0.5 sec was used (Fig. 3). Ophthalmoscopically, they consisted of round areas, approximately 350 μm in diameter, with a central, whitish-gray zone.

Histological Aspects

Diode laser: The optic microscopic examination of the serial sections of impact areas demonstrated zones of coagulation necrosis in the retinal pigment epithelium layer (Fig. 4A, B). The damage produced at the sensory retina was confined to the photoreceptor cells and nuclei of the internal nuclear layer. Photocoagulations with diode laser produce an intense coagulation of the photoreceptor outer segments, which were in contact with the altered pigment epithelium. The photoreceptor nuclei in the irradiated zone were mostly pyknotic, although some of them appeared normal. The alteration at the choroidal level consisted of an irregular obliteration of the choriocapillaris and a certain degree of disorganization of the choroidal pigment.

Argon laser: In the examined histological sections, even the most internal retinal layers appeared damaged by the radiation (Fig. 5A, B). The orderly alignment of the pigment epithelium was altered and the cells were rarefied in the center of the lesion and swollen towards the borders. The outer segment of photoreceptor cells was severely altered: this was fragmented into a small quantity of subretinal exudate. The nuclear layer was intensely pyknotic and was altered in its linear alignment at the site of the lesion. The plexiform layer was thickened and numerous vacuoles could be seen at its center. There were no significant structural alterations at the level of the choroid.

Transmission Electron Microscopy

Diode laser: In the choriocapillary layer the endothelial cells appeared swollen although the basal
membrane remained intact. Thromboses of the capillary lumens by red blood cells and thrombocytes were found in some specimens. The retinal pigment epithelium cells appeared vastly vacuolized (Fig. 6). The outlines of the pigmented cells were clearly visible, while the cytoplasm remained as residual bands containing ultrastructurally normal melanin granules. The examination of the photoreceptor cells revealed wide disc fragmentation with dispersion of cellular debris. The pyknosis of the photoreceptor nuclei was intense, but irregular, even in the central zone of impact. Numerous intercellular lacunae were present at the level of the ganglion cells layer. A certain grade of edema existed at the plexiform layer, with the loss of the classical patterns in some of the nervous fiber bundles. No alterations were observed at the internal limiting membrane (Fig. 7).

**Argon laser:** The endothelial cells in the choriocapillaris were slightly edematous, while Bruch's membrane appeared spared. The central area of the spots revealed a notable disorganization of the pigmented epithelium and photoreceptors. The pigment epithelial cells appeared highly altered and in some cases they were even destroyed, becoming dislodged from their basal laminae. The disc membranes of the external segment of the photoreceptor cells were disorganized in their normal alignment. Cellular debris, together with agglomerated electron-dense material and fibrin, constituted the visible exudate that was seen with the optical microscope (Fig. 8).

The mitochondria of the internal segment of the photoreceptor cells were dilated with broken cristae. The photoreceptor nuclei showed a notable chromatin accumulation. At the level of the more internal layers numerous intercellular vacuoles were seen with evident damage to the nervous fibers, while ganglion cells were partially spared (Fig. 9). The internal limiting membrane was shown to be thinned while the lengthening of the Müller cells showed electron-dense bodies within the cytoplasm (Fig. 10A, B).

**Discussion**

We obtained transpupillary chorioretinal photocoagulations that were ophthalmoscopically similar using a diode laser prototype and a commercially available argon laser. Other authors performed retinal endophotocoagulations using a semiconductor prototype laser at 808 nm delivered by a fiber system. They observed retinal lesions that were very similar in their macroscopic and histological aspects to those obtained with argon laser endophotocoagulators. In this study we have shown that retinal lesions that
Fig. 7. Transmission electron microscopy, diode laser: the internal limiting membrane, within the center of the lesion, appears unaffected by the radiation (×4000).

Fig. 8. Transmission electron microscopy, argon laser: wide destruction of the retinal pigmented cells. Note the homogenization of the photoreceptor cells discs (×12,460).

Fig. 9. Transmission electron microscopy, argon laser: intercellular vacuolation at the level of inner nuclear layer (×7280).

were ophthalmoscopically similar could be produced by comparable irradiation levels (about 120 W/cm), although the argon laser exposure time was slightly shorter. This result was rather unexpected since it is well known that melanin absorption decreases as the wavelength of light approaches the infrared spectrum region. On the other hand, some authors have reported that in addition to a radiation range between 800 nm and 900 nm, low energy levels are required in order to produce threshold retinal lesions. Further-
more, diode laser radiation is well transmitted by the ocular optic media, thus reducing the total amount of energy needed to produce a photocoagulative effect.  

Histological and ultrastructural study demonstrated that the chorioretinal lesions produced by the two types of photocoagulators have some structural differences. The lesion produced by the argon blue-green radiation on the rabbit retina involves all the retinal layers, but especially the retinal pigment epithelium. On the contrary, the 811 nm radiation of the diode laser was most evident at the level of the retinal pigment epithelium and the choriocapillaris. Most internal retinal structures were spared. Furthermore, the diode laser irradiation did not damage the internal limiting membrane and produced a coagulative effect without provoking excessive cellular breakage and pigment debris dispersion.

The minimum structural cellular reaction at the
vitreoretinal interface could prevent the post-photo-
coagulative appearance of proliferative vitreoretinopa-
yth.\textsuperscript{9,10} The coagulative necrosis of the retina produced by laser irradiations with exposure times ranging from some tenths of a second to 1.0 sec is a consequence of a thermal effect due to the absorption of the radiation by the cromophores.\textsuperscript{11} Melanin is the principal chro-
mophore for any type of laser radiation in the retina.\textsuperscript{7} This is true also for both blue-green and diode laser radiation. However, some authors\textsuperscript{11,18} have noted that there are other photoactive substances with the sec-
ondary property of absorbing blue light. Among these there are the flavoproteins, glutathione peroxidase, cytochromes and the photoactive enzyme systems.\textsuperscript{11}

We suggest that the alterations of the most internal retinal layers due to the blue-green radiation might be attributed to absorbing chromophores that are widely distributed along the entire retinal thickness. On the contrary, the microscopic retinal pattern after diode laser irradiation (damage confined to the nearby mel-
anized structures) suggests that, with the 811 nm, ra-
diation could provoke less activation of the chromo-
phores in the internal retinal layers than that occurring when blue-green light’s used. These findings are in line with the prediction of mathematical models of laser thermal retinal injury: a maximum temperature increase of about 5°C occurs at 100–150 μm from the pigment epithelium. Thus, no direct thermal damage takes place in the inner retinal layers.\textsuperscript{12,13} Moreover, the reaction of the photoreceptor cells nuclei could be justified by a secondary absorption process.\textsuperscript{12} Therefore, diode lasers emitting in 811 nm spectral range, distanced from the absorption spectrum of xantho-
phyll, could be safely used in the treatment of macu-
lar subretinal pathologies.

Results indicate that these new laser sources, as well as argon lasers, could be useful in the treatment of retinal pathologies.

The avascular anatomical nature of the rabbit ret-
ina did not allow a comparison of the two laser radia-
tion effects on retinal vessels. The emission wave-
length of the diode laser is not within the absorption spectrum of hemoglobin, but is absorbed selectively by some stains such as indiocyain green. This property will allow further studies of the photocoaulation of pathological vascular tissues after intravenous in-
jection of a specific absorption spectrum dye.

**Key words:** diode laser, argon laser, histopathology, retina, rabbits

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