Histopathology of Continuous Wave Neodymium: Yttrium Aluminum Garnet and Diode Laser Contact Transscleral Lesions in Rabbit Ciliary Body

A Comparative Study

Rosario Brancaro, Giovanni Leoni, Giuseppe Trabucchi, and Anna Cappellini

Neodymium: yttrium aluminum garnet (Nd:YAG) laser transscleral cyclophotocoagulation has been shown to be an effective method of lowering intraocular pressure (IOP). Transmission and absorption features of diode laser radiation (810 nm) make these new laser sources suitable for production of transscleral thermal lesions. The transscleral effects on rabbit ciliary body of Nd:YAG and diode laser wavelengths were compared using a CW Nd:YAG laser and a CW Aluminum Gallium Arsenide diode laser. Both lasers were delivered by silica optic fibers, 600 μm in diameter. Eight rabbits were treated by applying the optic fiber 0.5 mm from limbus while increasing energy values from 0.2-2 J. The lesions produced at equal energy values underwent gross and histologic and ultrastructural comparison. Gross examination revealed threshold lesions at 1 J energy for the Nd:YAG laser and 0.8 J for the diode laser. The diode laser produced transscleral thermal lesions of the rabbit ciliary body comparable to those achieved by the Nd:YAG laser. The histologic and ultrastructural study showed that diode laser radiation produced more remarkable damage to the ciliary pigmented structures, causing deep coagulation necrosis of the pigmented epithelium, wide disorganization of the collagen in the stroma, and intravascular coagulation phenomena in the ciliary vessels. Before the introduction of these new laser sources in clinical transscleral procedures, further investigation is needed to determine optimal energy levels. Invest Ophthalmol Vis Sci 32:1586-1592, 1991
Materials and Methods

Laser Systems

For this study, a continuous wave AlGaAs diode laser unit (Laser Science, Milano, Italy) emitting at 811 nm was used in conjunction with a CW Nd:YAG laser (Surgical Laser Technology, Malvern, PA) emitting at 1064 nm. Considering that the maximum output power at the end of the fiber for the diode laser was 800 mW, the output power was set at 800 mW for both lasers. Exposure times were increased progressively from 0.2 sec obtaining discrete energy values from 0.2–2.0 J. The radiation produced by both lasers was delivered by silica optic fibers 0.6 mm in diameter.

Laser Treatment

Eight pigmented rabbits, weighing between 2.5–3.2 kg, were used for this experiment. All the animals were sedated with intramuscular ketamine hydrochlor-
ide (25 mg/kg), and topical proparacaine (0.4%) was instilled in each eye. The end of the fiber, without a focusing tip, was placed perpendicularly on the conjunctiva 0.5 mm from the corneal limbus (positioned on the sclera overlying the iris root).

To study lesions produced in tissues of similar pigmentation, each animal was treated in the right eye with the Nd:YAG laser and in the left eye with the diode laser. Groups of two photocoagulations were done, 1 mm apart over 360°. The energy values were increased at discrete intervals of 0.2 J. After every two photocoagulations, a power meter was used to measure the output laser power at the end of the fiber (Scientech 362; Boulder, CO).

Morphologic Study

Two hours after treatment, the animals underwent biomicroscopic examination of the ocular surface, and 24 hr after laser treatment they were killed with an overdose of intravenous sodium pentobarbital. After the enucleation, the eyes were immersed in a fixative solution of 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). The eyes were then divided into two segments by posteriorly cutting from the laser...
spots. After removing the lens the ciliary body was examined with a stereo microscope (Zeiss SR, Oberkochen, West Germany) and further dissected. The impact areas underwent preparatory procedures for light and ultrastructural study. The preparatory procedure for transmission electron microscopy has previously been described.

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

**Results**

**Biomicroscopic Examination**

Biomicroscopic examination revealed very slight, superficial, whitish marks on the conjunctiva, corresponding to the areas where the end of the optical fiber was applied. After the irradiation a slight flare was present in those eyes that received higher energy levels.

**Gross Examination**

Gross examination of the ciliary body showed that there were no lesions when energy values less than 0.4 J were used. Threshold lesions (1–2 mm wide whitening and consisting of 2–3 ciliary processes) appeared at an energy level of 0.8 J when the diode laser was used (Fig. 1A). The same lesions were obtained using the Nd:YAG laser at an energy level of 1 J (Fig. 2A). With energy levels above 1.6 J, both wavelengths produced a wider cyclodestructive effect, with extensive loss of the normal integrity of the ciliary body (Fig. 1B, Fig. 2B). Iris and lens capsule damage was detected when energy levels above 1.6 J were used.

**Microscopic Study**

For both wavelengths, a mild coagulative necrosis of unpigmented and pigmented epithelial layers was evident using energy levels lower than 0.8 J (Fig. 3A, Fig. 4A). Increasing the energy from 0.8–1.4 J, the diode and Nd:YAG lasers produced coagulative necrosis of the stroma and the epithelial layers of the ciliary processes. At the periphery of the lesions, the epithelial layers thinned while the center of the lesions dissolved in cellular debris (Fig. 3B, Fig. 4B). The stroma showed large areas of heat-induced degeneration. Moreover, in the ciliary body stroma, pseudo-

**Fig. 4. Nd:Yag laser treatment. (A) Light microscopy of threshold lesion: widespread alteration of the structure of the ciliary processes (methylene blue, original magnification ×400). (B) Light microscopy: necrotic alterations involving epithelial cells and ciliary stroma (methylene blue, original magnification ×600).**
cyst formation was evident, especially in the Nd:YAG laser above-threshold lesions. In both the diode and Nd:YAG laser lesions, marked vascular congestion and thrombosis phenomena were observed in the lumen of ciliary vessels. When using both radiations with energy values higher than 1.6 J, slight hemorrhaging phenomena were present in the stroma of the ciliary processes. At these higher energy values some ciliary processes were broken, with projection of cellular debris toward the vitreous cavity. When energy values above 1.6 J were used, the microscopic study of the lesions produced by the diode and the Nd:YAG lasers revealed a slight thickening of the inner scleral lamellae.

Ultrastructural Study

The transmission electron microscopy study of the threshold lesions produced by both lasers showed that the epithelial layers were swollen with vacuolated endoplasmic reticulum and mitochondria. The diode laser above-threshold lesions were characterized by epithelial layer disruption. Epithelial cells or cell debris were blasted from their basal membrane. In the diode and Nd:YAG laser threshold lesions, and in the above-threshold lesions, the basal membrane of the epithelial cells was spared from heat-induced damage, thus maintaining its characteristic, multilayered, anatomic structure (Figs. 5A–B). The ultrastructure of the pigment granules was undamaged. Both laser sources caused a homogenization of the collagen fibrils of the stroma which dissolved into subfibrillar structures and amorphous masses, losing their periodicity. This finding was more evident in the Nd:YAG laser threshold and above-threshold lesions (Figs. 6A–B). The study of the vessels of the ciliary processes, in the threshold lesions, showed swelling of the endothelial cells with mitochondrial rupture. However, in the above-threshold lesions, endothelial cells lost their normal structure and were mixed in the vessel lumen with a large amount of fibrin and necrotic blood elements.

Discussion

We evaluated the possibility of using the diode laser to produce contact cyclophotocoagulations in rabbit eyes that were comparable to those obtained with the Nd:YAG laser. Emphasis was placed on evaluation of the energetic parameters and comparison of the morphologic aspects. Furthermore, as previous study of rabbit eyes showed, threshold lesions (whitening of

Fig. 5. Diode laser treatment. (A) TEM: cellular debris of pigmented epithelial cells overlying normal basal membrane (×7200). (B) TEM: wide destruction of the ciliary epithelial cells. Basal membrane appear untouched (×7200).
the ciliary body and consisting of two to three ciliary processes) decrease IOP. Therefore, we did not evaluate IOP in this study.

Our results showed that the diode laser produced contact transscleral cyclophotocoagulations in rabbit eyes comparable to those achieved by the Nd:YAG laser. The microscopic and ultrastructural study demonstrated that the degree of thermal damage for both wavelengths was related to the increase in treatment energy levels. However, when the diode laser was used, histologic examination revealed that at corresponding energy values more damage of the epithelial layers was present. When studying the Nd:YAG laser lesions, damage to the collagen of the stroma was more noticeable. These results could be attributed to the transmission and absorption characteristics of 810 nm and 1064 nm wavelengths, respectively. Theoretic and experimental investigations show that the transmission of the sclera increases as the wavelength approaches the near-infrared spectrum (750-1064 nm). Considering the ciliary body as the target tissue, the most important parameter is the absorption curve of the melanin contained in the pigmented epithelium. Melanin absorption at 810-840 nm is about twice the absorption at 1064 nm. Therefore the lower absorption of the scleral tissue for the 1064 nm allows much of the laser energy to pass to the ciliary body stroma. At this site, due to the different optical and morphologic properties of the stroma with respect to the sclera tissue, the scattering of the 1064-nm radiation leads to an increase in its energy absorption. This effect is less evident for shorter wavelengths such as the 800 nm of the diode laser; these can easily reach the epithelial layers and be completely absorbed by the melanin of the pigmented epithelium.

In above-threshold lesions, the basal membrane of the epithelial layers was untouched by the radiation produced by both lasers and showed resistance to thermal laser damage. This is not surprising considering the elastic property of the basal membrane. Some authors report that during the healing period a recovery of the epithelial layers over an intact membrane could result, allowing for restoration of the secretory structure. In our study, the transscleral lesions were obtained using exposure times of 0.4-2.0 sec and power of 0.8 W. Due to the heat dissipation, these energy levels probably do not produce a tissue temperature high enough to damage the basal membrane. By

Fig. 6. Diode laser treatment. (A) TEM: high magnification of ciliary stroma. Note the damage of collagen fibrils, ie, alteration of fibrillar periodicity (x40,000). (B) TEM: high magnification of ciliary stroma. Collagen fibrils near the inner border are dissolved into subfibrillar structures and amorphous mass (x39,000).
delivering high powers with short exposure times, a quasimechanical effect can be added to the thermal effect to produce basal membrane rupture. A longer duration of IOP reduction was produced by higher treatment energies, and a greater degree of damage, to rabbit and human ciliary bodies. However, a wider range of acute and chronic complications were found.

Several variables make it difficult to state the threshold energy values in transscleral procedures, eg, the thickness of the sclera, the pigmentation of the sclera, the inclination of the optical fiber, and the pressure exerted on the sclera by the fiber. Nevertheless, our results show that similar energy levels were required to produce comparable lesions.

These data agree with those in previous studies where diode and Nd:YAG lasers were used in contact transscleral cyclophotocoagulation in rabbit eyes. The wider damage to the ciliary stroma produced by the Nd:YAG laser together with the wide destruction of the epithelial cells by the diode laser suggest that these two wavelengths may complement each other for transscleral treatment and retreatments of the ciliary body.

Technologic characteristics of the diode laser, ie, its compact size, longer operating life, and low maintenance costs, together with our results, suggest that this laser source is suitable for clinical application. However, considering the energy levels used in clinical situations for contact transscleral cyclophotocoagulation with the Nd:YAG laser, a further improvement of the diode laser’s output power emission is needed.

Key words: contact transscleral cyclophotocoagulation, diode laser, CW Nd:YAG laser, rabbits

References