Ultrastructural Changes of Human Trabecular Meshwork After Photocoagulation With a Diode Laser

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A diode laser, which emitted infrared radiation at a wavelength of 810 nm, was used to perform trabecular photocoagulation in a human eye due for enucleation for malignant melanoma. For comparison, burns were applied with an argon blue-green laser (488–514.5 nm). With each laser, the treatment spot size was 100 μm and the pulse duration was 0.20 sec. Visible lesions were produced with a power of between 750 mW and 1.2 W with the diode laser, and 500–900 mW with the argon laser. The pattern of damage produced by both modalities was similar and essentially consisted of contraction or expansion of trabecular beams, with trabecular destruction occurring only in relation to high power exposures. These findings confirm that trabecular photocoagulation is not a process that depends upon the wavelength of the incident energy at the two spectral extremes of 488 nm and 810 nm. Invest Ophthalmol Vis Sci 33:2664–2671, 1992

Laser trabeculoplasty (LTP) for the treatment of uncontrolled glaucoma was first described by Wise and Witter in 1979.1 It has since become an established treatment modality, and several studies have demonstrated its effectiveness.2,3

LTP usually is performed with an argon laser, which emits blue-green radiation (wavelength 488–514.5 nm), although a similar ocular hypotensive action has been demonstrated with krypton red (647 nm)4 and cw neodymium YAG (1064 nm) laser radiation.5

The precise mechanism for the pressure-lowering effect of LTP is unknown. One theory suggests that photocoagulation results in contraction of trabecular fibers, which opens Schlemm’s canal by drawing the juxtacanalicular trabecular fibers inward.6 Another concept is that laser irradiation causes degenerative decay of the trabecular fibers, with a secondary widening of the intertrabecular spaces.7 Each of these proposed mechanisms could contribute to an improvement in aqueous outflow facility and, thus, an ocular hypotensive reaction. A recently promulgated mechanism of action depends upon a more complex and multifactorial biologic response to irradiation that may influence the outflow facility.8–10

In the present study, a diode laser emitting at 810 nm (infrared) and an argon blue-green laser were used to irradiate human trabecular meshwork. By comparing the histologic findings, it was hoped that an indication of the potential efficacy of diode lasers in the treatment of glaucoma could be obtained.

Materials and Methods

Laser Equipment

Diode laser: A Spectra Physics SDL2430 laser diode (Spectra Diode Technology, San Jose, CA) was used throughout this study with a spectral emission at 810 nm and with a maximum output power of 1.4 W. The laser diode was driven by a custom designed unit that provided control of the pulse duration and power. Power and energy levels at the eye were calibrated with a UDT S390 photometer (United Diode Technology, Orlando, FL) and were referenced to an EG & G (Salem, MA) radiometer model 810. The laser operated in a continuous mode but was a power-on-demand system.

The diode and its delivery optics were attached to the tonometer stand of a standard Haag-Streit (Bern, Switzerland) 900 slit-lamp microscope. An aiming beam was provided by a second laser diode that was red-emitting (680 nm) and low powered (less than 1 mW). Viewing of the trabecular meshwork was accomplished with the optics and illumination source of the slit-lamp microscope in conjunction with a Goldmann (Haag-Streit) three mirror contact lens.

The wavelength of emission and dielectric coatings on the optics within the laser delivery head obviated the need for a mechanical safety shutter mechanism during exposures. This afforded an unimpeded view throughout the treatment session. The power source...
of the laser was from a standard single phase 13 amp mains supply. Ancillary cooling facilities were not necessary because collateral heat production was extremely low. When not in use, the instrument could be stored in a carrying case that measured $46 \times 33 \times 15$ cm. Its light weight made it easily transportable.

**Argon laser** A Lasertek (Dallas, TX) argon laser system was used in the study. This laser has an integral power monitor, and all figures quoted are those registered on the manufacturer's instruments. No study was undertaken regarding the relative energy distribution within the laser beams. Exposures were delivered via the integral slit-lamp system in conjunction with a Goldmann fundus contact lens.

**Human Exposures**

A submission regarding the study was made to the local ethical committee, which gave its approval. A full explanation of the nature of the trial was given to the patient, who gave consent for the procedure. The subject was a 65-year-old female with a right ciliary malignant melanoma. Gonioscopy prior to treatment and subsequent microscopic examination confirmed that the tumor did not involve the trabecular meshwork.

Topical anesthesia was instilled and a Goldmann contact lens was applied to the eye. Three quadrants of the trabecular meshwork were irradiated with the diode laser. Seventy burns were applied. The target area was the pigmented portion of the trabecular meshwork, and the goal was to produce a blanching reaction. The spot size was $100 \mu m$, the minimum available on the system, and the exposure duration was 200 msec. The required output power was varied between 750 and 1200 mW to produce lesions that varied in appearance from an altered light reflex at the site of impact to a visible blanching of the trabecular meshwork.

For comparison, an argon blue-green laser was used to apply an additional 25 burns to the remaining quadrant of the same eye. As with the diode exposures, the end point was a visible blanching of the pigmented region of the trabecular meshwork. To allow comparability with the diode exposures, the spot size was set at $100 \mu m$ and the exposure duration was set at 200 msec. The power needed to produce a visible reaction varied between 500 and 900 mW. Gas bubble formation was not observed after diode or argon irradiation. Enucleation was performed 18 hr after laser exposure.

**Microscopic Studies**

Immediately after enucleation, a 5 mm penetrating incision was made at the pars plana, and the eye was immersed in 100 ml of fixative. This initial solution contained 2.5% glutaraldehyde buffered in 0.1 mol/l sodium cacodylate containing 10 mg/ml calcium chloride with a final pH of 7.4. The globe was hemisected so that the irradiated areas were totally isolated from the portion containing the tumor. This permitted the routine diagnostic procedures to be carried out on the latter.

Meridional sections of the iridocorneal angle were isolated under a dissecting microscope. The samples were trimmed so that the laser employed to irradiate a particular portion of trabecular meshwork and the power that was used could be identified.

Tissue for light and transmission electron microscopy was washed briefly in 0.1 mol/l sodium cacodylate buffer containing 7.5% sucrose and glucose, and was post-fixed for 1 hr in 2% osmium tetroxide buffered in 0.1 mol/l sodium cacodylate. Samples were dehydrated through a graded series of concentrations of ethanol in water and embedded in araldite via epoxypropane.

Samples for light microscopy were cut at 1 $\mu m$ on glass knives mounted in a Huxley Mark 1 ultramicrotome (Cambridge Instruments, Cambridge, UK) and were stained with toluidine blue.

Sections for transmission electron microscopy were cut with diamond knives in a Reichert OMU4 ultramicrotome. They were mounted on 200 mesh copper grids and stained with uranyl acetate and lead citrate before they were examined in an AE1 801 transmission electron microscope (Cambridge Instruments, Cambridge, UK).

Sections for scanning electron microscopy were post-fixed overnight in 2% osmium tetroxide buffered in 0.1 mol/l sodium cacodylate. They were dehydrated through a series of ascending concentrations of acetone before being critical-point dried (Sandri 780; Biorad Microscience, Hertfordshire, UK). Dried samples were coated with a 30 nm layer of gold in a sputter coater (Emscope; Biorad Microscience) before being examined in a Hitachi 520 scanning electron microscope (Hitachi Scientific Instruments, Berkshire, UK).

**Results**

**Clinical Data**

The patient experienced no discomfort during diode and argon irradiation. She did comment that the diode treatment was subjectively preferable because of the lack of bright flashes during exposures and the relative silence of the laser system. These findings were typical of observations that have been noted in association with a clinical trial of diode laser trabeculoplasty in progress at St Thomas' and Moorfields Eye Hospitals.11

**Light Microscopy**

Over the energy ranges used in this study, sites of irradiation were difficult to identify in histologic preparations because they were not delineated by
gross disturbances in morphology or by differential staining.

Sites initially were located by gross observation under the dissecting microscope and by subsequent trimming of sample blocks. Under the light microscope, the most characteristic changes were seen in relation to the overall geometry of the trabecular elements and the intervening spaces.

In some areas of irradiation, the trabeculae seemed to have contracted, and, therefore, the intertrabecular spaces became more apparent. In other specimens, however, the trabeculae appeared swollen and there was a complementary reduction in the intertrabecular spaces (Figs. 1a and b). These changes occurred mainly in the uveal and corneoscleral compartments of the trabecular meshwork.

**Electron Microscopy**

*Scanning electron microscopy:* Sites of irradiation could be clearly determined using low power scanning electron microscopy. Although both argon (Figs. 2a and b) and diode (Figs. 3a and b) exposures produced similar disturbances in the trabecular morphol

![Fig. 1. Light micrographs of the trabecular meshwork 18 hr after irradiation with argon (A) and diode (B) lasers. The presence of red blood cells in Schlemm's canal and the juxtacanalicular trabecular meshwork was not related to the laser treatment and was not present at the time of irradiation. In both cases, the beams of the uveal trabecula were slightly swollen and associated with numbers of white blood cells. In the diode, distended trabecula also were seen in the corneoscleral portion of the trabecular meshwork. (Bar 50 µm.)](image)
Fig. 2. Scanning electron micrographs of sites of argon laser treatment 18 hr after irradiation. In (A), five separate areas of irradiation are arrowed, and the resulting lesions are roughly circular in shape. A higher power view of the fourth lesion in (A) is seen in (B). Note the presence of white blood cells associated with the trabecular meshwork around the peripheral portion of the lesion. (Bars = 100 μm (A) and 50 μm (B).

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ogy, those induced by diode irradiation were more obvious and created a more distinctive pattern of disruption. Diode lesions often were ovoid, with a long axis typically 150 μm in diameter and a short axis typically 90 μm in diameter, and they appeared deeper than those induced by argon. Argon burns tended to be circular, and typically were 100 μm in diameter.

No evidence of disruption of trabecular elements was seen in terms of destruction of surface membranes, and severed trabeculae were not identified in any exposure sites. We did not identify fibrin or other surface deposits in any of our specimens, although there was evidence of infiltration of the burn sites by white blood cells.

Transmission electron microscopy: Transmission electron microscopy of low power argon (Figs. 4a and b) and diode (Fig. 4c) lesions showed similar, but limited changes in the morphology of the trabeculae. As was seen in light microscopy specimens, there was some evidence that lesions produced by the infrared radiation of the diode induced changes to a greater depth than those induced by argon. The most significant changes observed were a contraction of the cross-
Fig. 3. Scanning electron micrographs of areas of trabecular meshwork 18 hr after irradiation with the diode laser. Two sites of irradiation are seen in (A) (arrowed). In contrast to argon impacts, those of the diode are more readily apparent and are oval. At higher power (B), the more penetrating nature of the wound is apparent, and again, the site of irradiation shows a large number of white blood cells. (Bars = 100 μm (A) and 50 μm (B).)

sectional aspect of individual trabeculae, coupled with an increase in electron density. No evidence of rupturing or fragmentation of trabeculae was seen, and except for some superficial endothelial cells, most cellular elements appeared relatively normal.

The relatively mild changes induced by argon and diode lasers perhaps could be attributed to the poorly pigmented nature of our target trabecular meshwork. In the higher power exposures emanating from both lasers, there was a more marked response in the internal aspects of the uveal trabecular meshwork, with some zones of endothelial cell sloughing. Displacement of collagen fibrils was rare and usually was associated with aggregations of macrophages or polymorphs (Fig. 4d).

Discussion

Although numerous clinical and laboratory studies have been undertaken to examine the effects of laser irradiation on the trabecular meshwork, the tissue reaction to photocoagulation and the mechanisms underlying this therapeutic regime remain unclear.

The absorption of laser energy in the trabecular
meshwork is a difficult concept to understand because linear optical absorption processes and the resulting thermal damage normally require the presence of a pigmented absorbing system. All previous reports assume that argon laser trabeculoplasty results from thermal transients generated by absorption in melanin within the trabecular meshwork.

Human trabecular meshwork is composed of beams of collagenous tissue, which are lined by endothelial cells and which have an extracellular matrix of glycosaminoglycans, noncollagenous protein, and fibronectin. In reality, despite individual variation, this tissue is a poorly pigmented structure, although pigment density tends to be greatest inferiorly. This is in contrast to the pigmented sheet within the retinal pigment epithelium.

Upon examination of the absorption characteristics of melanin, for a uniform absorbing monolayer, 55% of argon blue, 45% of argon green, and only 7% of diode irradiation at 810 nm will be absorbed. These are maximal figures. Melanin distribution in the trabecular meshwork is discontinuous and, therefore, the percentage of incident energy absorbed will be less. The depth of tissue to which radiation penetrates increases with increasing wavelength in the visible and the near-infrared. Therefore, radiation at 810 nm penetrates to a greater depth than argon.

The initial effect of photocoagulation is one of protein denaturation, and the rate is influenced by the temperature of the tissue. Studies have shown that shrinkage of collagen fibers in cornea and iris occurs at 60–70°C. This would indicate that a similar temperature is needed to induce such changes in the trabecular meshwork. The conversion of light to heat within melanin and the production of thermal transients passing over the trabecular beams thus may result in denaturation of protein if equilibrium temperatures are of sufficient magnitude. In the trabecular meshwork, in contrast to the retinal pigment epithelium, the relatively low pigment density and the diffuse distribution of melanin result in achievement of generally lower temperature gradients after irradiation. The lesions produced therefore tend to be more threshold in quality.

The tissue changes observed in the present study with both laser wavelengths were similar and also
were comparable to those identified in studies of the free running YAG.18,19 All of these studies have identified some thermal damage to trabecular beams and cells.

Currently, three basic theories attempt to explain the beneficial effects of laser trabeculoplasty. Wise and Witter proposed that heat-induced shrinkage of collagen fibers caused a reduction in the circumference of the trabecular ring and an opening of the trabecular spaces.1 Some experimental evidence for this theory has been provided by Weber.6

Van der Zypen and Funkhauer proposed an alternative mechanism to explain the ocular hypotensive effect of photocoagulation. Laser burns applied to the posterior trabecular meshwork in monkeys induced a widening of trabecular spaces adjacent to the burn site, because of primary disruption of collagen fibers. In addition, over 8–12 wk, a secondary degenerative effect extended beyond the primary impact zone and as deep as Schlemm’s canal. The result of this process was further widening of the trabecular spaces.7

Studies by Van Buskirk and others8–10,20 suggest that in addition to the above mechanical mechanisms of action, photocoagulation also may promote beneficial cellular and biochemical processes within the trabecular meshwork.

In Van Buskirk’s studies, it was shown that trabecular cell loss after irradiation was accompanied by trabecular cell hyperplasia. It was hypothesized that these newly formed cells were able to perform the functions that contributed to the reestablishment of a normal outflow resistance.

There is evidence that suggests the proteoglycan components of the extracellular matrix (ECM) represent a significant barrier to aqueous outflow.21 The ECM resides mainly in the deeper portion of the corneoscleral meshwork, within the juxtacanalicular apparatus. Current physiologic and experimental studies indicate that 97% of tissue resistance to aqueous outflow is in the 10 μm of tissue adjacent to Schlemm’s canal.22 Laser irradiation could affect aqueous outflow resistance by stimulating the division of cells, which could, by phagocytosis or synthesis, modify the constituents of the ECM.8,23–25 The deeper penetration of diode laser irradiation into the trabecular meshwork therefore may exert a more immediate effect on the tissue responsible for outflow resistance.

The complexity of the situation is increased by the observation of Fink that laser trabeculoplasty to one eye often was associated with a reduction in pressure in the contralateral eye. This observation indicates the possible influence of neurogenic or biochemical mediators released by cellular elements and initiated by laser irradiation.26

Most current clinical procedures adhere to the parameters that have been defined by Wise and Witter.1,27,28 These parameters are: a spot size of 50 μm, a pulse duration of 50 msec, and a power of approximately 1 W. Clinically, this exposure results in blanching of the trabecular meshwork and frequently in the formation of a gas bubble. These features are assumed to result from the thermal degradation of laser energy and the conversion of cell fluids to the gas phase. The small spot and short pulse duration will result in a very rapid change of energy content of tissue in a relatively confined space. This, in turn, is considered to generate the cellular responses that are a prerequisite to lowering intraocular pressure.

Defining boundary conditions of exposure to optimize the required tissue response is extremely difficult if the mechanisms underlying such a response are unknown. The treatment parameters for the clinical trials of argon laser trabeculoplasty were set on the basis of limited pilot studies and largely hypothetical mechanisms of action.

Given that the optimal tissue reaction underlying argon laser trabeculoplasty is unknown, it is difficult to understand why a gas bubble is a prerequisite of the current protocol. The presence of a gas bubble indicates that a phase change within the tissue has occurred, which identifies a very rapid change of energy content and adiabatic expansion of cellular constituents. This implies a two stage damage mechanism to the target cells, the first resulting from the passage of thermal transients and the second from mechanical displacement due to the expanding gas bubble. In diode exposures, heating takes place as a more gradual process, resulting from absorption and energy conversion over a greater volume of tissue. The more diffuse absorption and less marked rate of change of energy did not result in a gas bubble in the present study. Therefore, such damage involved only a thermal mechanism.

The lower tissue absorption and greater penetration depth of the diode are comparable to those of cw YAG laser irradiation. With both of these laser wavelengths, higher energy levels must be used compared to those of argon. Typically in this study, power levels of greater than 1 W were required with diode exposures, whereas lesions resulted from powers of 900 mW or less with the argon system. The more diffuse zone of tissue reaction also was perhaps responsible for the less defined nature of the diode lesions. Clinically, in some patients, it is difficult to perceive any change in the trabecular meshwork, other than a slight altered light reflex.

Current protocols suggest a spot size of 50 μm for argon LTP 28 whereas the minimum available spot size with the diode laser was 100 μm. This did not
appear to have a detrimental effect and, in practice, a
given spot size with any laser results in a wide range of
burn diameters as a consequence of variations in
focus and power settings.

The results of this study indicate that the diode laser
produces comparable histologic changes to the argon
laser, and early clinical trials have shown it to be an
effective instrument for performing photocoeagulation
of the trabecular meshwork. The similarity to le-
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advantages of diode lasers regarding their portability
and reliability, certainly will stimulate further interest
in their therapeutic potential for the treatment of
glaucoma.

Key words: glaucoma, photocoeagulation, trabecular mesh-
work, diode laser trabeculoplasty, argon laser trabeculo-
plasty

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