Short-Pulsed Neodymium-YAG Laser Trabeculotomy

An In Vivo Morphological Study in the Human Eye

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The in vivo response to short-pulsed Nd-YAG laser damage to the trabecular meshwork has not been studied in the human eye. The nature of the response will determine the potential efficacy of this treatment for glaucoma. We have investigated short-pulsed laser trabeculotomy lesions created in the trabecular meshwork of four human eyes within 18 hr prior to enucleation for intraocular melanoma. Scanning electron micrographs showed irregular craters (150–300 μm diameter) in the trabecular meshwork surrounded by trabecular beams which were splayed towards the anterior chamber. The adjacent damage to trabecular and corneal tissues was characterized by denudation of endothelial cells and deposition of debris. Light and transmission electron micrographs of the edge of the trabeculotomy lesions revealed fragmentation of the endothelial cells and splitting of the trabecular beams. Preservation of normal morphology was noted in the deeper tissues within 50 μm of the edge of the crater. Neutrophils were present within 20 min of laser treatment whilst macrophages characterised the inflammatory response at later stages. Perforation of the canal of Schlemm was only obtained with lesions in the middle of the trabecular meshwork but not with lesions placed more anteriorly. Invest Ophthalmol Vis Sci 29:1698-1707, 1988

The main aim in treatment of chronic open-angle glaucoma is the reduction of intraocular pressure. Theoretical considerations indicate that approximately 20 fistulae, each 10 μm in diameter, between the anterior chamber and the canal of Schlemm should restore normal outflow facility in open-angle glaucoma. Such lesions can be produced by means of a short-pulsed laser and reduction in intraocular pressure has been attained by this means in patients with primary open-angle glaucoma, although no one has shown that this has a therapeutic effect in the long term.

Previous clinical studies have been based upon an empirical choice of the placement and number of lesions, of the energy levels employed and of the number of laser applications at each site. Superimposition of lesions has been used as a strategy to allow adequate penetration through the trabecular meshwork into Schlemm’s canal. However, this strategy has been thwarted by the subsequent closure of the lesions, both in animal studies and in man. In contrast, in an animal study in which single pulses were employed, persistent fistula formation was obtained and in analogous clinical studies in man, a persistent reduction in intraocular pressure has been achieved.

The morphological effects on fixed human trabecular meshwork of Q-switched neodymium-YAG laser applications have been investigated in vitro. These and subsequent studies have indicated that, with appropriately designed apparatus, focal perforations could be produced in the trabecular meshwork into the canal of Schlemm with little damage to adjacent structures and minimal disruption of the outer wall of the canal of Schlemm.

We have investigated the morphological changes and the morphometric characteristics of trabeculotomies produced by a short-pulsed Nd-YAG laser in vivo, in order to determine the position and extent of the lesions produced, and to investigate the early healing responses. Four patients, for whom enucleation was indicated for treatment of choroidal melanoma, consented to the gonioscopic application of short-pulsed Nd-YAG laser lesions to the trabecular meshwork prior to surgery.

Materials and Methods

Laser Apparatus

The experimental Q-switched Nd-YAG laser employed was the Hyper-YAG 2000 (JK Lasers, Ltd.,...
Lumonics, Rugby, England) described previously by Venkatesh et al.\textsuperscript{8,9} but now connected to a slit-lamp microscope delivery system. The output beam from the Nd-YAG laser cavity was expanded two-fold, passed through an articulated arm to a second six-fold beam expansion telescope mounted on a Zeiss 30 S-L slit lamp and reflected through 90° by a dielectric mirror to emerge forwards from the slit lamp coaxial with the visual optics of the observer. The standard objective lens of the slit lamp was replaced with an achromatic doublet lens of focal length 90 mm. This served as the objective for the microscope optics and brought the laser beam to a final focus.

The laser cavity was adjusted to give as pure as possible a TEM\textsubscript{00} transverse mode pattern.\textsuperscript{8,9} Beam expansion and the final focussing lens resulted in a cone angle for convergence of the Nd-YAG beam of approximately 14° (full angle between 1/e contours). The working distance was 90 mm from the slit lamp objective. Nd-YAG laser pulse durations were approximately 50 nsec.

Laser pulse energies were recorded by an in-line energy monitor unit fitted to sample the beam at the laser cavity output. The efficiency of transmission of Nd-YAG radiation from the monitor position to the delivery system. Monitor readings were therefore corrected by this factor to yield the actual pulse energy output from the slit-lamp unit.

A single continuous He-Ne laser beam was directed coaxially with the Nd-YAG beam and was par-focal with the focal plane of the visual optics of the slit lamp.

To protect the operator from any back-scattered Nd-YAG radiation, a safety filter of 3 mm thick Schott KG-3 glass was fitted in the slit lamp. An additional opaque metal moving shutter was fitted because of concern about the intensities of visible light emitted by the laser-induced plasma under some circumstances.\textsuperscript{12}

### Table 1. Clinical details of trabeculotomy lesions

<table>
<thead>
<tr>
<th>Specimen no.</th>
<th>Age</th>
<th>Energy</th>
<th>Interval to enucleation</th>
<th>Calotte</th>
<th>No. of shots with plasma</th>
<th>No. of shots with blood reflux</th>
<th>Intraocular pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1†</td>
<td>65</td>
<td>30-42 mJ</td>
<td>20 min</td>
<td>Temporal</td>
<td>3 out of 13</td>
<td>0*</td>
<td>13</td>
</tr>
<tr>
<td>2†</td>
<td>50</td>
<td>30 mJ</td>
<td>30 min</td>
<td>Superior</td>
<td>3 out of 10</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>3‡</td>
<td>62</td>
<td>30-38 mJ</td>
<td>5 hr</td>
<td>Inferotemporal</td>
<td>3 out of 19</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>4‡</td>
<td>51</td>
<td>37 mJ</td>
<td>18 hr</td>
<td>Inferior</td>
<td>3 out of 16</td>
<td>0</td>
<td>14</td>
</tr>
</tbody>
</table>

* The first shot with plasma formation was followed by immediate emptying of the blood-filled canal of Schlemm and a visible shallowing of the anterior chamber.

† Processed for SEM and then reprocessed for light microscopy and TEM.
‡ Processed for TEM only.

### Patients and Clinical Methods

Four patients gave their informed consent for the study. In three, a uveal melanoma was present at the posterior pole of the eye. One patient had a recurrence of a ciliary body melanoma which had been previously treated by local excision. Each patient was fully informed, approval was obtained from the Hospital Ethical Committee, and the guidelines issued were observed.

Table 1 lists the clinical details of the laser applications. Each patient was seated at the laser apparatus and a gonioscope contact lens (Medical Workshop, Groningen, Holland) suitable for pulsed Nd-YAG laser use was applied. The laser was aimed at the trabecular meshwork most remote from the tumor. To obtain plasma formation, the laser was initially aimed at a single point and the energy was sequentially increased for each shot until plasma formation occurred. Further shots at the energy required to produce plasma bubbles were then fired in the region of the presumptive calotte (see below). The eyes were enucleated at the times indicated in Table 1.

### Preparation of Tissue

Enucleated eyes were immediately fixed by immersion in cacodylate buffered glutaraldehyde (3%). Calottes were orientated to include the treated trabecular meshwork and the anterior segment was excised. The anterior segment tissue was dissected and the iris excised at the root in the majority of specimens; non-lasered segments were used as controls for dissection artefacts. After post-fixation in 1% osmium tetroxide, tissue from three eyes was prepared by conventional techniques for critical point drying prior to scanning electron microscopy (SEM). Triangular sectors from the anterior segment were mounted on aluminum stubs so that the corneal endothelium and the trabecular meshwork were parallel to the stub surface. The tissue was coated with gold-palladium (40 nm thick) and was examined in a JEOL T200 scanning electron microscope.
Table 2. Details of the numbers of lesions examined and the methods of examination employed

<table>
<thead>
<tr>
<th>Specimen no.</th>
<th>Interval</th>
<th>No. of lesions seen by SEM</th>
<th>No. of lesions studied by serial semithin section to determine hole dimensions</th>
<th>No. of lesions studied by TEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20 min</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>30 min</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>5 hr</td>
<td>0</td>
<td>0</td>
<td>2*</td>
</tr>
<tr>
<td>4</td>
<td>18 hr</td>
<td>2*</td>
<td>1</td>
<td>1</td>
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</tbody>
</table>

* Although three lesions were observed clinically, the manner in which calottes are prepared may result in not all lesions being available for pathological assessment.

microscope (SEM). Scanning electron micrographs were taken of the trabecular meshwork in the same orientation as the gonioscopic view. The trabecular meshwork was viewed obliquely so that the anterior meshwork and corneal endothelium are seen at the bottom of the illustrations. The anteroposterior diameter of a trabeculotomy is thus the distance between the lower and upper margins of the hole and the transverse diameter is the distance between the left and right margins.

The SEM magnification scales were calibrated using a set of two stub-mounted calibration grating replicas (BioRad, Polaron Division, Watford, England).

Specimens which showed laser lesions (see Figs. 1, 2) were photographed in detail by SEM. High power photographs were "grid-referenced" with respect to a low power reference photograph. The tissue was reprocessed for resin embedding by transfer through amyl acetate, ethanol and a 50/50 mix of propylene oxide and resin and finally undiluted Araldite. Semithin (1 μm) serial sections were cut and every tenth section was mounted and stained with toluidine blue for light microscopy. For selected lesions measurement of the maximum anteroposterior diameter of each hole was ascertained from the Vernier scale on the microscope stage and from calibrated photomicrographs. Ultrathin serial sections (60–80 nm) were cut from representative trabeculotomy sites and were stained with uranyl acetate and lead citrate, and studied by transmission electron microscopy (Phillips EM301, Eindhoven, Holland).

The morphologic information obtained from the serial sections was cross-related to the grid-referenced SEM pictures in order to allow approximate correlation between the SEM and the TEM features.

Tissue from one eye (specimen 3, Tables 1, 2) was post-fixed in osmium and processed directly to araldite for study by serial section light microscopy and transmission electron microscopy; this obviated the artefact of double processing.

Table 2 gives details of the number of lesions observed and the manner in which they were investigated.

**Results**

**Scanning Electron Microscopy**

Eight trabeculotomy lesions were identified in different parts (anterior, central and posterior) of the trabecular meshwork. Five holes from three specimens showed no evidence of blood reflux from Schlemm's canal. When viewed from the internal surface these were irregular with ragged or stellate margins (Fig. 1a–d). The impression was gained that adjacent trabecular tissues were splayed away from the crater. In none of these five examples had blood reflux into the anterior chamber been seen at the time of laser application. The trabecular tissues at the edges of the holes were elevated and fragments of cells and fractured trabecular beams were present across the adjacent inner trabecular surface. Higher magnification revealed denudation of the surface of the trabeculae (Fig. 2a,b). In one eye with three trabeculotomy lesions, which was enucleated 30 min after laser application, blood reflux had been observed in vivo and the trabeculotomies were filled with red cells and fibrin (Fig. 3a,b). In the example illustrated, a fortuitous trimming cut transected the lesion and demonstrated an intact outer wall in the canal of Schlemm (Fig. 3a).

When the lesions were located in the anterior trabecular meshwork there was cellular debris on the corneal endothelium: the monolayer here appeared flattened and contained prominent nuclear bulges (Fig. 4). In two cases some corneal endothelial cells were disrupted and cytoplasmic debris lined the inner surface of Descemet's membrane (Fig. 4b,d). Elongated spindle cells with prominent cytoplasmic processes were present on the surface of the damaged endothelium. These cells were accompanied by more spherical macrophages (Fig. 4a,c).

**Morphometric Studies**

The anteroposterior and transverse diameters of the holes were measured from the scanning electron micrographs, as was the extent of surrounding corneal endothelial damage. The calculated internal diameters of the holes ranged between 150 and 300 μm and the radius of the extent of the surrounding corneal endothelial disturbance, when present, (as measured from the edge of the hole) ranged between 500 and 900 μm.
Fig. 1. Examples of laser trabeculotomies as seen at low power by SEM and LM. In (a) and (b) the anterior trabeculae are splayed away (arrows) from the irregular crater (c) and debris is scattered on the corneal endothelium (e). In (c) and (d) the defects (c) are located in the center of the meshwork. A light micrograph (e) shows an anterior crater (c): note the splaying effect (arrows). (a) 20 min, X300, (b) 20 min, X400, (c) 20 min, X200, (d) 20 min, X200, (e) 20 min, X250.
Fig. 2. Denudation of trabeculae at edge of crater, in areas analogous to those arrowed in Figure 1a. In (a) the collagenous core persists and the interspaces contain red blood cells (arrow) and macrophages (open arrow). In (b) some trabecular denudation is shown (arrows) adjacent to separating endothelial cells (e). Macrophages (m) and red blood cells are present in the trabecular interspaces. (a) 20 min, x3600, (b) 18 hr, x3600.

Light Microscopy

Three trabeculotomy lesions were studied by serial section (Table 2). The section which contained the hole with the widest diameter was assumed to be the central section in the series (Fig. 1e). The anteroposterior diameters of the holes at their deepest and widest extent varied between 120 ± 10 μm and 200 ± 10 μm. From the serial sections the calculated transverse diameters in microns were approximately the same as those determined from the SEM photographs; thus shrinkage artefact following processing from scanning to light microscopy was minimal.

Two of the lesions sectioned had been seen by SEM to be located in the anterior trabecular meshwork; in neither case was a fistula into Schlemm's canal created. The more posterior lesions, however, entered

Fig. 3. Large craters which reached Schlemm's canal as seen by SEM (a) and LM (b) were filled with blood clot (arrowheads); the outer wall of the canal (arrows) is not significantly damaged. (a) X600, (b) X250.
the canal of Schlemm and the intertrabecular spaces contained red cells (Fig. 3b).

Transmission Electron Microscopy

The tissue available for this part of the study allowed examination of lesions of varying severity at 20–30 min and 5–18 hr post-damage and assessment of the cellular responses in different parts of the trabecular meshwork. In general, double processing provided morphology of a poorer quality than single processing but nevertheless the tissue appearances were comparable (Fig. 5).

The smallest lesions were observed in the central meshwork in specimen 3, which provided the best morphology (Fig. 6). These were studied by serial section after single processing. At the edge of the defect cell remnants were seen as loose bundles of cytoplasm containing vesicles of varying size. Nuclear damage of varying degree with chromatin condensation and loss of the nuclear membrane were prominent features in the affected cells (Fig. 6a,b).

The trabeculae showed loss of basement membrane and dispersion of their collagenous cores with extrusion of clumps of wide-banded collagen (Fig. 6c). There was some evidence to suggest that the central collagenous components of the beams were separating and in some cases splitting (Fig. 6d). In these areas the surviving endothelial cells with intact cytoplasmic membranes were separating from the trabecular beams (Fig. 6c). Within approximately 50 μm of obvious damage it was possible to identify cells in the

**Fig. 4.** Corneal endothelial damage adjacent to craters in the anterior trabecular meshwork. In (a) and (c) spindle cells (s) and macrophage-like spherical cells are present on the surviving endothelium. In (b) and (d) there are defects in the endothelium which expose granular debris. (c) and (d) correspond to boxed areas in (a) and (b). (a) 18 hr, ×400, (b) 18 hr, ×400, (c) 18 hr, ×3500, (d) 18 hr, ×3500.
Fig. 5. The anterior and posterior edges of a trabeculotomy crater as seen by TEM after processing for SEM (Fig. 1d). The inset shows preservation of trabecular endothelial cells. Note the apparent splitting of the scleral spur (arrow). X600, inset X2500.

adjacent tissues which were very close to normal architecture (Fig. 5).

The onset of an inflammatory cell response to the laser application was rapid and polymorphonuclear leucocytes were present in the trabecular interspaces within 20 min (Fig. 7a). After 5 hr, mononuclear macrophages were identified (Fig. 7b,d) and the surviving trabecular endothelial cells were enlarged, with lysosomal bodies present in the cytoplasm (Fig. 7c). In the 18 hr specimen, mononuclear macrophages contained secondary lysosomal bodies and were found in the region of damaged trabeculae and fragmented endothelial cells (Fig. 7d).

In none of the anterior segment tissues taken as controls was there any detectable pathology suggestive of indirect damage due to the laser treatment or artefactual damage resembling the changes seen in the experimental tissues.

Discussion

Three morphological techniques were used for this study for the following reasons. Scanning electron microscopy showed exactly where the laser perforations occurred in relation to Schwalbe's line and thus gave an indication of the accuracy of the aiming system. Correlation of the results obtained by SEM with serial sections studied by light microscopy highlighted the importance of the location of the puncture wound in the human trabecular meshwork and allowed demonstration and measurement of the details of the deeper aspects of the lesions. Transmission electron microscopy allowed the study of endothelial cell and trabecular damage and characteristics of the inflammatory cell response.

The human outflow system differs from that of other primates in that an operculum is absent and, with age, there are two important changes. The first is that the anterior part of the meshwork elongates, the second is that the scleral spur increases in size. In terms of the location of therapeutic laser trabeculopunctures, the anterior and posterior thirds of the meshwork are the least likely to be effective in creating a channel which would give access for aqueous to reach Schlemm's canal or the juxtacanalicular layer adjacent to it (see Figs. 1e, 3b). While scanning electron microscopy has the advantage of providing quick results and providing two-dimensional (or with stereoscopy, three-dimensional) data of the size of the hole, it is often difficult to know, from this modality alone, how close the lesion is to Schlemm's canal. This is particularly so when the subhuman primate eye is used for a laser study. An important disadvantage in the use of subhuman primate tissue is that the response of the (possibly) more pliable monkey trabecular tissue differs from that of the (probably) more rigid elderly human tissue. We had been surprised in our previous in vitro studies that the pulsed Nd-YAG laser explosion seemed to produce a stellate irregular hole with radiating cracks. This was suspected to be due to fixation rigidity of the tissue, so it was of interest to note that the same response to the explosion was obtained in vivo. Thus there is sufficient morphological evidence to believe that the laser
produced an internal explosion which caused the trabecular tissues at the epicenter to erupt and splay towards the anterior chamber. The persistence of this distortion after 18 hr was of interest, because it has been suggested that the adjacent unsupported trabecular tissues collapses down into the crater.14

The observation that the laser produced an explosion within the trabecular tissues is supported by the demonstration of trabecular and cellular debris on the inner surface of the adjacent cornea and the inner layer of the meshwork. It is surprising that this debris was not washed quickly through the trabeculotomy by aqueous flow, but presumably the mucopolysaccharides which are present within the tissues promoted some form of adhesion.15

Even in the short periods studied (within 18 hr) there was evidence that facultative macrophages had appeared within the tissue and were phagocytosing the fragmented debris released by the explosion. These cells were not present in large numbers and their presence probably reflects a nonspecific response in the population of mononuclear cells which normally pass from iris to aqueous and through the meshwork.15 The pattern of YAG laser damage and cellular response contrasts with that demonstrated after argon laser trabeculoplasty in which the tissue has the appearance of heat fixation and preservation, and disruption and distortion are not so prominent.16

One of the most worrisome theoretical considerations of pulsed Nd-YAG laser damage to the trabecu-
Fig. 7. Ultrastructural features of cellular responses in meshwork adjacent to crater. (a), At 20 min, polymorphonuclear leucocytes (arrows) are lying between fractured trabeculae. (b) and (c), At 5 hr, mononuclear macrophages (arrows) are present and the cytoplasm of the enlarged surviving endothelial cells (e) contains electron-dense bodies. (d), At 18 hr, macrophages (m) containing secondary lysosomal bodies are present in addition to residual cell debris (arrow). (a) 20 min, ×300, (b) 5 hr, ×1000, (c) 5 hr, ×2300, (d) 18 hr, ×4000.

ular meshwork which we have observed is the possibility that a shock wave would cause extensive damage to the trabecular (and corneal) endothelial cells around the explosion. If this side effect were to occur, the denuded trabecular beams would swell and fuse and form an ideal platform for the spread of corneal endothelial cells over the defect. This has been shown to occur after Q-switched laser trabeculotomy in the young adult monkey. Transmission electron microscopy demonstrated that trabecular endothelial cells on the surface exhibited normal morphology at a distance of 200–300 μm from the edge of the eruption and that damage to the outer wall of Schlemm’s canal was limited. Moreover, viable trabecular endothelial cells were present within 50 μm in the deeper tissues. The ultrastructure of the cells close to the edge of the rupture was one of fragmentation of cytoplasmic membranes and disruption of the cytoplasm, the organelles and the nuclear chromatin. The use of higher magnification revealed linear tears across the cells in some instances; this was considered to be artefactual and it must be conceded that preparation artefact with double processing for SEM and light microscopy promotes the risk of confusing tissue damage with...
artefact. Hence it was reassuring to find that measurement of laser crater diameter by two techniques provided values which were within an acceptably close range. The unknown effect of tissue fixation on laser trabeculotomies will be elucidated only with difficulty. It was useful to study lesions in tissue (case 3) in which there was not prior preparation for critical point drying. In this specimen the morphological features of the endothelial cells in the traumatized and nontraumatized regions were similar to those observed in cases 1, 2 and 4 and, in the nontraumatized regions, the endothelial and trabecular morphology was entirely within normal limits.

There is every reason to believe that treatment protocols could be devised which will reduce resistance to aqueous outflow and will lower intraocular pressure in the short term. So far some clinical reports have been encouraging. This study indicates that the clinician must aim the laser just anterior to the scleral spur in order to perforate Schlemm's canal. The problem which may prove difficult to solve by means of morphological methods will be the clinically observed failure to maintain the pressure lowering effect. Trabeculectomy procedures would provide tissue from a previously lasered area in cases in which the procedure has failed, but the surgical procedure almost always causes damage to the delicate uveal tissue and hence interpretation is difficult. As a long-term experiment in a patient suffering from a uveal melanoma, the protocol would be ethically unacceptable.

Key words: glaucoma, morphology, neodymium-YAG laser, human trabecular meshwork, trabeculotomy

References


