Neodymium:YAG Laser Iridotomy in the Cynomolgus Monkey

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The histologic effects of various Neodymium:YAG laser energy levels were evaluated after iridotomy formation in cynomolgus monkey eyes. Scanning electron microscopy of the corneal endothelium above the treated areas revealed no significant cell loss or pleomorphism when compared to the adjacent untreated areas. Light and phase contrast microscopy demonstrated closure of the iridotomies in most cases by a bridge of iris pigment epithelium. In several specimens, stromal tissue and pigment laden cells were present over the attenuated iris pigment epithelium. There were four lens opacities with rupture of the anterior lens capsule and anterior epithelial cell hyperplasia in one. No damage was apparent in the trabecular meshwork or retina with light microscopy or fluorescein angiography.

Q-switched ruby and neodymium:YAG (Nd:YAG) lasers can safely produce iridotomies in monkeys and man with the use of nanosecond bursts and little total energy. 1-6 Thick dark brown and pale blue irides are often difficult to penetrate with the argon laser, but they can easily be treated with the Nd:YAG laser. Furthermore, there appears to be less lens opacification, iridotomy closure, and corneal endothelial cell loss in Nd:YAG laser treated eyes. 3 However, complications include bleeding from the margins of the iridotomy and marked dispersion of pigment and stromal debris. In addition, the short- and long-term effect of the Q-switched laser on lens clarity is unknown.

No subhuman primate studies, to our knowledge, have yet evaluated the histologic effects upon the ocular structures of Nd:YAG laser iridotomies. This study, therefore, describes the effects on ocular structures following the Nd:YAG laser iridotomies in cynomolgus monkeys.

Materials and Methods

We used both the Coherent JK prototype (Coherent; Palo Alto, CA) and the American Medical Optical (AMO) (American Medical Optical; Irvine, CA) YAG-100 Nd:YAG lasers to create iridotomies. The Coherent laser has a maximum energy output of 6 millijoules (mJ) and was capable of delivering up to 9 pulses with 20-millisecond (msec) intervals in the burst mode. The AMO laser had a maximum energy output of 20 mJ in single pulses. The spot sizes were 100 and 25 μm in air for the Coherent and AMO lasers, respectively. The duration of each pulse was between 12 and 20 nanoseconds (nsec) for each laser.

Four cynomolgus monkeys (Macaca fascicularis) of similar weight and of either sex were tranquilized with intramuscular ketamine and intravenous pentobarbital. The investigation utilizing the monkeys in this study conformed to the ARVO Resolution on the Use of Animals in Research. Preoperative evaluations included slit-lamp biomicroscopy, gonioscopy, and dilated fundus examinations. All intraocular pressure examinations were done with the Alcon...
pneumotonograph. Baseline photographs were taken of the anterior segment and fundus. Prior to laser therapy, pilocarpine hydrochloride 2% was instilled into each eye. An Abraham YAG laser contact lens (Ocular Instruments; Bellevue, WA) was used for all iridotomies. Right and left eyes were treated similarly but at different time intervals.

Each eye was treated with three iridotomies at the 10, 2, and 6 o'clock midperipheral iris positions. Two eyes were treated with the Coherent laser utilizing energy levels of 4 and 6 mJ. In each eye, a single burst was followed by a second iridotomy produced with a pulse train of five and a third with a pulse train of nine. The other two eyes were treated with the AMO laser, utilizing single bursts ranging from 10 to 20 mJ at 2-mJ step intervals. The patency of each iridotomy was determined postoperatively by direct visualization of the anterior lens capsule. The right eyes of each monkey were treated on day 1 and killed on day 30; the left eyes were treated on day 21 and killed on day 30.

Intraocular pressures were recorded immediately following and at 90 min after treatment. Slit-lamp photomicrographs also were taken at this time, demonstrating the iridotomy size and patency. Prior to enucleation, the following tests were performed: slit-lamp examination, intraocular pressures, dilated lenticular and fundus examinations, and fluorescein angiography.

Eyes were enucleated and placed in 4% glutaraldehyde solution buffered with 0.1 N sodium cacodylate. The corneas were trephined from the globes and prepared for scanning electron microscopy. These corneal buttons were rinsed in 10% sucrose/0.05 N sodium cacodylate buffer for 10 min. Post fixation was in 1% osmium tetroxide in 0.05 N sodium cacodylate for 2 hr, followed by dehydration in graduated alcohols, and critical point drying. The Technics Hummer II sputter coating machine (Technics; San Jose, CA) was used to coat each specimen with gold/palladium 300 angstroms (Å) thick. The corneas were then examined with the AMR Model 1000 scanning electron microscope (Amray; Bedford, MA). Endothelial cell counts were then made in the peripheral cornea, through which the laser beam passed, and in the adjacent untreated areas.

The remainder of the anterior segment was washed in water and then transferred to 60% alcohol. Each lesion was identified grossly, excised with the contiguous structures, processed in paraffin, and sectioned. Each specimen was monitored during sectioning by examination of unstained and sometimes quick stained sections at 0.1-mm levels until the lesion was identified. Serial sections were then prepared through the entire lesion. All sections were stained and studied, but the representative sections were considered to be those which were at the midpoint in the serial sections. With this technique, over 2,400 serial sections of 19 lesions (16 iris, 3 lens) were generated in the study. Sections of the optic nerve, optic nerve head, and macula were also prepared.

Eight iris specimens were prepared for transmission electron microscopy (TEM). Each was placed in Karnovsky’s fixative overnight, following which each specimen was rinsed with 10% sucrose/0.05 N sodium cacodylate buffer. The specimens were refixed with 1% osmium oxide in 0.05 N sodium cacodylate/10% sucrose solution overnight. 10% sucrose/0.05 N sodium cacodylate was used to wash the specimens, following which each was dehydrated with graduated strengths of alcohol, and embedded in epoxy resin. They were examined by phase contrast microscopy and with a JEOL 100B transmission electron microscope (JEOL Ltd; Japan, Tokyo).

After examination for lens opacities beneath the dissecting microscope, one lens was submitted for phase contrast microscopy and the others were submitted for light microscopy.

### Results

#### Clinical Results

Twenty of 24 iridotomies were patent at the end of the treatment session postoperatively (Fig. 1). The four nonpatent iridotomies followed treatment with the single 6-mJ burst in the right and left eyes and with the 18- and 20-mJ single bursts in the left eye. The failed 18- and 20-mJ bursts followed a successful 16-mJ burst, resulting in much pigment dispersion that may have decreased the efficiency of the laser with subsequent bursts. In the right eye, the 18- and 20-mJ bursts were successful but produced smaller iridotomies than the initial 16 mJ burst.

The diameter of the iris holes varied from 50 to 75 μm (Table 1). By 9 days postoperatively, every iridotomy had closed. A grayish stromal discoloration surrounded each treatment site (Fig. 2).

Postoperative slit-lamp examinations showed marked pigment dispersion and fibrinous anterior chamber reactions in nearly every case. No postoperative topical steroids were given.

Minimal bleeding was noted at each iridotomy edge following treatment. In two cases, bleeding lasted for 10 sec and resulted in a barely detectable hyphema (Fig. 3).

No significant intraocular pressure rise was detected in any animal at 90 min, 1 wk, or 1 mo postoperatively.

Prior to enucleation, no posterior segment abnormality was found by indirect ophthalmoscopy or by fluorescein angiography.
Fig. 1. Patent Nd:YAG iridotomy created with a 10 mJ single pulse (arrows). Spotty hemorrhages appear on the iris surface surrounding the iridotomy.

Fig. 2. Iridotomy site 1 mo after treatment that has completely closed and shows stromal discoloration surrounding the area of maximal damage.

Histopathology

Cornea: Scanning electron microscopy of the corneal endothelium revealed no areas of cell dropout, pleomorphism, or cellular alteration. There were no differences between the endothelial cell counts in the treated cornea, through which the laser beam passed, compared with the untreated corneal zones.

Lens: The dissecting microscope was used to examine the lens beneath all 24 iridotomies. Only four focal anterior subcapsular opacities were found. These were directly beneath the 4-mJ single burst (Fig. 4), the 6-mJ five-pulse train burst, and beneath both 14-mJ single burst iridotomies. The 6-mJ five-pulse train burst caused anterior lens capsule disruption with epithelial cell hyperplasia extending through the capsular break and onto the surface of the lens (Fig. 5). None of the lens specimens developed diffuse opacification in the 1-mo postoperative period.

Iris: Several specimens studied 1 wk postoperatively by phase contrast microscopy showed stromal and iris pigment epithelial discontinuity consistent with a patent iridotomy (Fig. 6). Pigment laden cells were present at the margins above the edges of the iris pigment epithelium. By transmission electron microscopy, cells from the iris margin extending into the iridotomy appeared to originate from the iris pigment epithelium and contained round iris pigment granules and abundant endoplasmic reticulum within long cellular processes. There were also abundant collections of disrupted basement membrane material and pigment containing cells extending into the hole (Fig. 7).

The specimens studied at 1 mo postoperatively demonstrated closure of the iridotomy by an intact but attenuated iris pigment epithelium. Some specimens studied by light microscopy seemed to have thinned stromal tissue present over the attenuated iris pigment epithelium (Fig. 8). By transmission electron microscopy, pigment laden cells, scattered fibrocytes, and disrupted basement membrane material was shown over an attenuated iris pigment epithelial layer that contained fewer round pigment granules (Fig. 9).

Table 1. Size of iridotomy following single pulse or pulse-train with the neodymium:YAG laser

<table>
<thead>
<tr>
<th>Energy* (mJ)</th>
<th>Single pulse (µm)</th>
<th>Train of 5 (µm)</th>
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<tr>
<td>4</td>
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* n = 2 eyes for each energy level.
† Average size of iridotomy.

Fig. 3. Cascade of blood from the edge of an iridotomy immediately after treatment (12 mJ, single pulse).
Fig. 4. Anterior subcapsular lens opacity that lay beneath the 4 mJ single pulse iridotomy (arrow).

Trabecular meshwork: A moderate accumulation of pigment granules were present within the trabecular beams and endothelial cells of the trabecular meshwork. Otherwise, the histologic architecture of the trabecular meshwork was unaltered. In a few specimens, pigment-containing cells were found within the longitudinal muscle of the ciliary body.

Ciliary body: The right and left eyes treated with 4-mJ utilizing pulse trains demonstrated focal inflammatory cell infiltrates within the ciliary body.

Posterior segment: No abnormalities of the posterior pole were demonstrated by light microscopy.

Discussion

The Nd:YAG laser can successfully produce iridotomies in the monkey eye. In most cases, a single pulse between 10 and 14 mJ creates a patent iridotomy whose diameter measures 200–300 μm. Energy levels between 4 and 6 mJ utilizing a train of five pulses can also produce a patent iridotomy. In this experiment, some of the iridotomies that were produced at the higher energy levels were smaller than predicted. Generally these followed initially successful and large iridotomies in an eye, which resulted in much pigment dispersion and clouding of the aqueous. Presumably the energy reaching the iris on subsequent pulses would be reduced, thus accounting for some of the smaller or nonpatent iridotomies. In addition, poor focusing on the iris may be responsible for some of the variation in iridotomy sizes produced. In general, the iridotomies produced with the AMO laser were larger than those produced with the Coherent laser.
Fig. 6. Nd:YAG iridotomy 9 days following treatment with a 20 mJ single pulse. There is thickening of the pigment epithelium at the margins and pigment laden cells in the stroma bordering the iridotomy (phase contrast microscopy, x200).

Fig. 7. TEM at the edge of an iridotomy showing a cell with long processes containing abundant endoplasmic reticulum and few round pigment granules (black arrow). There are numerous pigment laden cells (white arrow) and disrupted basement membrane material (black caret) (x4,200).
Fig. 8. The iridotomy is bridged by an attenuated iris pigment epithelium and thinned stromal tissue. Also there are accumulations of pigment laden cells adjacent to the iridotomy (×490).

Fig. 9. TEM through an iridotomy that closed. There are pigment laden cells (black arrow), scattered fibrocytes (white arrow), and disrupted basement membrane material (black caret) present over an attenuated layer of iris pigment epithelium (×2,500).
This can be predicted by calculating energy densities delivered to the iris surface utilizing these two lasers. Bleeding rarely occurs after argon laser iridotomy. However, bleeding from the cut edges of the iris occurs frequently after a Nd:YAG iridotomy. In the monkey eye, the extent of bleeding is minimal; the blood clears spontaneously and may cause only a small hyphema. No apparent intraocular pressure rise occurs secondary to the bleeding.

In this study, every iridotomy closed by the ninth postoperative day. Other studies utilizing animals have also demonstrated frequent closure of iridotomies. Fortunately, in the human, this tendency for closure is much less frequent and may be less when utilizing the Nd:YAG laser, as compared with the argon laser. In the past, the mechanism for iridotomy closure was assumed to be iris pigment epithelial migration. In this study, there was migration of cells, most likely to represent the iris pigment epithelium over the iridotomy. Light microscopy in several specimens shows stromal tissue of reduced cellularity and thickness overlying the pigment epithelium. This may represent reformation of stromal connective tissue once the posterior pigmented cells have initially bridged the iridotomy. Hanna and Roy demonstrated limited capability for stromal replication in rabbits after iris injury. Further studies are necessary to clarify the mechanism for iridotomy closure.

The lens can clearly be damaged by the Nd:YAG laser during iridotomy treatment. Precise focusing on the iris or just anterior to the iris might minimize this risk. The chance of lens damage might be further reduced by treating the extreme periphery of the iris beyond the anterior curvature of the lens, where the iris is least likely to be in contact with the lens. The argon laser is commonly used to enlarge a small patent iridotomy. It may not be wise to utilize the Nd:YAG laser to enlarge an iridotomy as one might with the argon. Instead, a new site should be selected, as the potential for lenticular damage has been shown to be several times greater when enlarging an iridotomy with the Nd:YAG laser. Previous studies have shown that focal lens opacities occur following argon laser iridotomies. However, the long-term incidence of rapid cataract formation was not found to be greater when using the argon laser to produce an iridotomy as compared with conventional surgery. Therefore, long-term follow-up studies will be necessary to determine whether generalized cataract formation is greater when using the Nd:YAG laser to produce an iridotomy.

Because of our study design, we are unable to detect whether there is any diffuse loss of corneal endothelial cells as no preoperative cell counts were obtained. However, no corneal abnormalities were found. Previous animal studies utilizing the argon laser also reported no significant corneal endothelial cell loss. However, when utilizing either the Nd:YAG or argon laser in human eyes to treat pupillary block glaucoma, focal opacities are frequently encountered in the corneal zone through which the laser energy passes. Possibly the shallow anterior chamber in the human eye with pupillary block can account for the greater incidence of focal damage to the corneal endothelium as compared with the deep anterior chamber of the monkey eye. Recent study with the Nd:YAG laser in the monkey eye demonstrates that the iris must be within 1.0 mm of the corneal endothelium in order to produce significant damage to the endothelium when producing iridotomies. In the human eye with pupillary block glaucoma, the proximity of the iris to the corneal endothelium is increased. Thus, even greater attention to precise focusing on the iris is justified in order to lessen the risk of corneal endothelial damage when producing iridotomies. However, encouraging clinical trials comparing the argon laser to Nd:YAG laser suggest that corneal endothelial cell loss may be lower when utilizing the Nd:YAG laser.

Histologic examination of the trabecular meshwork demonstrates an accumulation of both free and phagocytized pigment within the endothelial cells and trabecular beams. No apparent trabecular endothelial cell loss or damage to the trabecular architecture is apparent. The findings suggest that most of the pigment is removed by bulk aqueous flow and phagocytosis. This is similar to prior subhuman primate studies with the argon laser. However, the finding of pigment laden cells within the longitudinal muscle of the ciliary body suggest a partial contribution of uveoscleral outflow to the removal of debris following laser iridotomy.

The lack of significant intraocular pressure elevation postoperatively, despite marked pigment dispersion and inflammatory debris, is surprising. Previous studies in monkeys of the intraocular pressure response following posterior capsulotomy also showed no significant pressure elevations. However, in human clinical trials, the intraocular pressure rises, commonly following Nd:YAG posterior capsulotomy, and peaks between 1 and 3 hr. Thus, monkeys may not be an appropriate model to study intraocular pressure increases following anterior segment laser surgery.

Key words: neodymium:YAG laser, iridotomy, glaucoma, cataract, monkeys
Acknowledgments

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References