Effects of cyclophotocoagulation with a neodymium:yttrium-aluminum-garnet laser on corneal sensitivity, intraocular pressure, aqueous tear production, and corneal nerve morphology in eyes of dogs

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Objective—To determine effects of cyclophotocoagulation via administration of 100 J with a neodymium:yttrium-aluminum-garnet (Nd:YAG) laser on corneal touch threshold (CTT), intraocular pressure (IOP), aqueous tear production, and corneal nerve morphology in eyes of dogs.

Animals—15 dogs.

Procedure—Noncontact Nd:YAG laser was transsclerally applied (10 applications; 25 W for 0.1 seconds for each application to each of 4 quadrants) to the ciliary body of the left eye of 15 dogs; the right eye was the control eye. Corneal integrity, CTT, tear production as measured by the Schirmer tear test (STT), and IOP were evaluated for 14 days following laser treatment. On day 14, dogs were euthanatized, eyes harvested, and corneas stained with gold chloride. Major nerve bundles were analyzed by use of a drawing tube attached to a light microscope, and maximum diameters were measured by use of image analysis software.

Results—All laser-treated eyes had significantly higher CTT values, compared with control eyes. Six of 15 laser-treated eyes developed ulcerative keratitis. On most days, IOP was significantly lower in laser-treated eyes in both morning and evening. Laser-treated eyes had a significant decrease of approximately 1 nerve bundle/corneal quadrant. Values for STT or nerve bundle diameters did not differ significantly.

Conclusions and Clinical Relevance—Administration of 100 J with a Nd:YAG laser effectively reduced IOP while increasing CTT and caused a significant decrease in number, but not diameter, of major corneal nerve bundles. Nerve damage and corneal hypoesthesia are etiologic factors in ulcerative keratitis following Nd:YAG cyclophotocoagulation.

Glucoma is a progressive disease in dogs that includes a number of pathophysiologic processes attributable to an increase in intraocular pressure (IOP). This increase in IOP is partly responsible for pathologic changes in the optic disk and corresponding deficits in the field of vision. Normal IOP is dictated by a balance between production of aqueous humor by the ciliary body and aqueous outflow, which is primarily through the iridocorneal angle.

One surgical treatment for glaucoma is cyclophotocoagulation accomplished by use of a neodymium:yttrium aluminum garnet (Nd:YAG) laser. Cyclophotocoagulation is used to selectively damage the ciliary body to decrease production of aqueous humor. The laser beam is directed perpendicular to the sclera at a point 5 to 7 mm caudal to the limbus, and it passes through the sclera and is absorbed by melanin in pigmented tissue. Mechanisms for decreasing IOP by use of cyclophotocoagulation include direct destruction of the pigmented ciliary epithelium, indirect destruction of ciliary epithelial cells by ischemia and inflammation, increased uveoscleral outflow, and creation of alternate drainage routes such as transscleral flow. Optimal clinical effects are achieved when energy (approx 100 to 200 J) is delivered in short bursts, although fewer adverse effects are reported with the application of an increased amount of energy at a slower delivery rate to fewer sites. Cyclodestructive procedures are associated with many clinical complications, including anterior uveitis, rubeosis irides, dyscoria, lenticular opacities, corneal neovascularization and opacifications, corneal ulcers, transient postoperative ocular hypertension, choroidal effusions, serous retinal detachment, hypHEMA, and phthisis bulbi.

At our veterinary medical teaching hospital, the Nd:YAG laser has been used to successfully perform this procedure on several dogs and cause a decrease in IOP. Affected dogs received between 79 and 250 J of energy (10 to 35 W/application site). The number of application sites and watts delivered was extremely variable, because we were attempting to develop an effective protocol. Between 1995 and 1998, 9 of 15 dogs treated in accordance with this protocol developed corneal erosions or ulcers that required 1 week to 3 months to heal. Affected dogs also were observed to have a subjective decrease in corneal sensitivity and blink rate. Such ulcers have been documented in other...
reports. It was hypothesized that these dogs sustained nerve damage related to the Nd:YAG laser procedure and that the damage had resulted in neurotrophic keratitis. Corneal edema, ulcers, and hyposthesia have been reported in dogs and humans following laser cycloablation.

The anterior segment of the eye is innervated by neural processes from 3 ganglia: trigeminal (sensory), superior cervical (sympathetic), and ciliary (parasympathetic). The cornea is innervated mainly by the ophthalmic branch of the trigeminal nerve. The ophthalmic branch divides within the orbital fissure to give rise to the nasociliary nerve, which subdivides into the long and short ciliary nerves that innervate the anterior segment, including the cornea. The long ciliary nerves travel rostrally through the suprachoroidal space at the 3- and 9-o’clock positions medially and laterally. Prior to reaching the limbus, the sensory nerves are joined by variable numbers of sympathetic and parasympathetic fibers. Nerve axon bundles are radially arranged and enter the cornea at the limbus through the middle third of the stroma. These axons are closely associated with the blood vessels and vascular nerves. The cornea is innervated mainly by the superior cervical (sympathetic), and ciliary (parasympathetic) nerves. The cornea is richly innervated by thin, unmyelinated, axons, some of which form epithelial leashes comprising 2 to 6 axons attached to a single subepithelial fiber. There are species differences as to the absolute numbers of corneal nerve trunks.

In dogs, nerve fibers enter the peripheral cornea at the corneoscleral limbus in a series of 11 to 18 prominent, radially directed, superficial stromal nerve bundles. Each large bundle contains approximately 30 to 40 axons visible by use of light microscopy, with smaller nerve fascicles located between and superficial to those bundles. After entering the cornea, the main stromal nerve bundle branches repeatedly to form an elaborate axonal network throughout the rostral 0.4 to 0.5 mm of the corneal stroma. Corneal epithelium is richly innervated by thin, unmyelinated, axons, some of which form epithelial leashes comprising 2 to 6 axons attached to a single subepithelial fiber. The leashes give off thin, prominently beaded, ascending branches that divide extensively into a bulbous terminal expansion throughout the basal, wing, and squamous corneal epithelial layers.

The Cochet-Bonnet esthesiometer has a 0.12-mm-diameter monofilament nylon thread attached to a weight. When applied to the cornea, it produces pressures from 11 to 200 mg/0.0113 mm². There are specific A-β corneal nerve fibers that respond to mechanical forces exclusively and are the predominant nerve type stimulated by the esthesiometer. On the basis of neutral density counts, the Cochet-Bonnet esthesiometer filament can stimulate as many as 100 terminal endings with a single application, because single intraepithelial terminals never innervate more than a few hundred square micrometers of tissue. However, a single axon can travel a great distance across the cornea and may innervate as much as 20 to 50% of the corneal surface. It has been theorized that corneal nerve endings can transmit impulses that enable the brain to discern the difference between pain and touch by the amount of pressure applied. Corneal sensitivity is essential for the maintenance of normal corneal physiologic processes. The blink reflex and normal tear secretion are essential to the well-being of the cornea and are adversely affected by corneal hyposthesia.

The purpose of the study reported here was to study the effects of cyclophotocoagulation by use of a Nd:YAG laser on corneal touch threshold (CTT) in dogs as well as the laser’s effects on the number and diameter of major nerve bundles in the cornea of dogs. In addition, aqueous tear production and IOP were measured after cyclophotocoagulation.

Materials and Methods

Animals—Fifteen conditioned adult mixed-breed dogs (1 female, 14 males) of various ages that weighed between 19.7 and 27.9 kg were used in the study. Physical examinations, including ophthalmic and neurologic examinations, were performed prior to the cyclophotocoagulation procedure, and results were within reference ranges. Results of a CBC and serum biochemical analyses were within reference ranges for all dogs. Baseline measurements of CTT, IOP, and aqueous tear production were recorded. Dogs were acquired from the research population at the Virginia-Maryland Regional College of Veterinary Medicine. The protocol for this study was approved by our institution’s animal care and use committee.

Cyclophotocoagulation with a Nd:YAG laser—Dogs were medicated with acepromazine maleate (0.01 mg/kg, SC) and butorphanol tartrate (0.1 mg/kg, SC). Anesthesia was induced by IV administration of a bolus of propofol (1 mg/kg). Dogs were intubated, and anesthesia was maintained with a constant-rate infusion of propofol at a dose necessary to provide the desired effect. The left eye of each dog was prepared by rinsing it with a dilute (1:50) povidone-iodine solution, and the eyelids were immobilized with an eyelid speculum. A continuous-wave Nd:YAG laser with a handheld noncontact optical fiber was used. The fiber tip was positioned perpendicular to and 1 to 2 mm offset from the conjunctival surface. The beam was directed at a point 3 mm caudal to the limbus over the approximate location of the pars plicata of the ciliary body. A total of 100 J of laser energy was delivered to the left eye, as was performed in another study in which investigators tested various energy settings and their effects. Ten laser applications (25 W for 0.1 seconds for each application) were delivered to each quadrant, avoiding the 3- and 9-o’clock positions; thus, 2.5 J (25 W X 0.1 seconds) was used in each application, for a total of 100 J/eye. The right eye in each dog served as an untreated control eye. Five dogs were treated each day; treatments were administered on 3 separate days. For consistency, the same investigator (AKW) performed all treatments and measurements.

Monitoring intraocular changes and corneal integrity—Testing conducted after the laser procedure was performed in a specified order. All dogs were examined daily by use of a transilluminator, slit lamp biomicroscopy, and indirect ophthalmoscopy. Clinical signs such as conjunctival hyperemia, blepharospasm, aqueous flare and cells, and dyscoria were recorded daily. Conjunctival hyperemia, aqueous flare, and aqueous cells were graded on a subjective scale of 0 to 4, with 0 being normal and 4 being the most severe. Both eyes of each dog were stained with fluorescein impregnated paper strip, and any area that had evidence of stain retention indicative of a defect in the corneal surface was recorded qualitatively and photographed. Chloramphenicol ophthalmic solution was administered to both eyes of all dogs twice daily throughout the study.

Aqueous tear production—Aqueous tear production was monitored by use of the Schirmer tear test (STT). Schirmer I tear tests were conducted in each eye by placing the strip in
the middle to lateral third of the lower conjunctival fornix. The strip was removed 60 seconds after insertion, and the amount of strip that was wet was recorded as number of millimeters per minute. The STT was performed in each eye 1 week before and 2, 4, 6, 8, 10, 12, and 14 days after laser treatment.

**Measurement of IOP**—Intraocular pressure was measured by use of an applanation tonometer. The IOP was measured and recorded for both eyes of each dog 1 week before laser treatment and twice daily (between 7 and 10 AM and again between 3 and 6 PM) for 14 days after laser treatment. Only pressure readings with an error of < 5% were considered acceptable for use.

**Measurement of CTT**—A Cochet-Bonnet hand-held esthesiometer was used to stimulate the corneal surface until a corneal blink reflex was elicited. The cornea was divided into 5 testing regions that comprised a 5-mm-diameter oval central region and 4 oval areas (4 × 2 mm) 3 mm axially to the limbus in the dorsal, ventral, nasal, and temporal corneal regions, respectively (Fig 1). Initial stimulation was performed with the nylon monofilament of the esthesiometer at a length of 3.0 cm.

To obtain a measurement, the observer viewed the cornea from the side at close range while the filament was applied at a steady speed perpendicular to the cornea and contact was established. Once the filament tip touched the cornea, the rate of movement continued until the filament had a slight bend or deflection of approximately 5%. When that point had been reached without a blink response, the monofilament was shortened by 0.3 cm, and the process was repeated until a positive blink was detected for 3 consecutive attempts at the same monofilament length. Filament length was converted to a value of milligrams of force, which was recorded as the CTT. Dogs generally were cooperative throughout the procedure; however, when a dog was not cooperative, testing was suspended for a short period and then resumed. Measurement of CTT was performed on both eyes the week before and 1, 3, 5, 7, 9, 11, and 13 days after laser treatment. For consistency, the same investigator (AKW) performed all measurements at the same time each day (ie, between 7 and 10 AM).

**Collection and processing of tissue samples**—On day 15 following laser treatment, the dogs were euthanatized by administration of an overdose of pentobarbitone sodium (150 mg/kg, IV). Eyes from 10 dogs were harvested within 30 minutes after the dogs were euthanatized, and the dorsal and temporal or lateral aspects of the globes were marked with 5-0 silk sutures placed at the limbus. Whole eyes were immersion-fixed in 4% paraformaldehyde-0.2% picric acid in 0.1 M PBS solution, pH 7.4, for 2 to 3 hours. Each cornea was then removed, along with 1 to 2 mm of continuous corneoscleral limbus and stored in fresh fixative solution at 4 C. Each cornea was placed in 30% sucrose in 0.1 M PBS solution for 12 to 18 hours and was then sectioned with a razor blade into 4 wedge-shaped segments extending from the corneal limbus to the center. The dorsolateral and ventrolateral quadrants from each dog were placed in optimal cutting temperature compound for 5 minutes and then sectioned with a cryostat in the rostral-caudal direction. Sections were cut tangential to the corneal surface, and serial, 40-µm-thick sections were collected into tissue wells filled with chilled PBS solution.

**Staining with gold chloride**—Dorsolateral and ventrolateral corneal sections from 9 pairs of corneas were rinsed twice in 0.1 M PBS solution, placed in 0.1 M sodium citrate, pH 5.4, for 5 minutes, and then placed in 100% pure, unfiltered lemon juice for 15 minutes in ambient room light. Corneal sections then were placed in 1% aqueous gold chloride solution for 25 minutes. Corneal sections were transferred to acidulated water (6 drops of glacial acetic acid/100 ml of distilled water) for 4 to 5 hours (ie, until nerves were visible by use of a dissecting microscope and before excessive background staining was evident). The sections were rinsed in 0.1 M PBS solution for 10 minutes, placed in a rapid fixer solution with hardener (to prevent progressive darkening of tissue) for 10 minutes, and then rinsed in 0.1 M PBS solution for 10 minutes. Stained sections were dehydrated in a graded series of alcohol solutions (70, 95, and 100%) and cleared in xylene. They were mounted in serial order on slides coated with chrome alum-gelatin, and a coverslip was applied with a commercial adhesive. The optimal incubation time in gold chloride and acidified water used in this study was determined on the basis of results of preliminary experiments in a manner that would enable us to maximize staining quality and minimize the amount of background staining.

**Evaluation of corneal nerves**—Corneal sections were critically examined by use of a light microscope. Location and distribution patterns of all major corneal stromal nerve bundles were documented by making a series of composite line drawings by use of a drawing tube attached to the microscope (Fig 2). Camera lucida sections were examined, and the number of stromal nerve bundles entering the peripheral aspect of the cornea at the corneoscleral limbus in each corneal quadrant was quantified independently by 2 investigators (AKW, CFM) who were not aware of the source of each tissue section.

Corneal sections were also photographed at 1.5X magnification by use of a high-speed color film. These photomicro-

![Figure 1](image)

Figure 1—Schematic of the canine cornea, depicting areas that were stimulated by use of an esthesiometer. Five distinct test regions are outlined, and the central ovals within those regions indicate the test areas for the corneal touch threshold.
graphs were scanned into an imaging program at a resolution of 1,000 dots/in. Using image analysis software, the diameter of each nerve bundle was measured at its widest point as it exited the limbus (Fig 3). The investigator (AKW) who measured diameters was not informed of whether it was the dog's right or left eye. Two quadrants of each cornea were examined in 7 dogs. Because of ineffective staining, only 1 quadrant was examined in the right eye of 2 dogs, and only 1 quadrant was examined in the left eye of 2 other dogs. Nerve diameters were measured in arbitrary units and then converted to the number of micrometers.

Statistical analysis—Differences in values for IOP, STT, and CTT between the laser-treated and control eyes were calculated, and means of this difference were analyzed by use of a repeated-measures ANOVA. In addition, baseline values for IOP, STT, and CTT for the right and left eye of each dog were compared by use of a paired Student t-test.

The P values for IOP and STT were compared with the
Bonferroni-corrected $\alpha$ value to hold the experiment-wise error rate to 0.05. For response variables in which 14 comparisons were made (ie, IOP), the $\alpha$ value for each test was decreased to 0.003938 to bring the overall $\alpha$ value back to 0.05. For response variables in which 7 comparisons were made (ie, STT), the $\alpha$ value was decreased for each test to 0.0073008 to bring the overall $\alpha$ value back to 0.05. The $P$ values for CTT were compared with an $\alpha$ value of 0.05.

Number of nerve bundles and mean diameter of nerves in laser-treated and control eyes were compared by use of a paired t-test. Because the number of nerves counted and measured varied among dogs as a result of the inequality of the number of quadrants examined, weighted and nonweighted analyses were performed.

Results

Clinical signs and corneal integrity—All laser-treated eyes had varying degrees of conjunctival hyperemia, dyscoria, or aqueous flare (Fig 4). Most of these clinical signs had resolved by 8 days after laser treatment, with only 2 dogs retaining signs of conjunctival hyperemia on days 8 to 12. Aqueous flare had resolved in all but 2 dogs by 8 days after laser treatment. Eight dogs had a few aqueous cells on day 5 of the study, 7 still had a few cells on day 9, and 1 had aqueous cells evident throughout the entire 14 days after laser treatment. Ten of 15 dogs remained mildly to moderately dyscoric throughout the study. Three dogs had a fibrin clot in the anterior chamber the day after the laser procedure; the clot resolved by day 4 in all 3 dogs. One dog developed a cystic mass in the ventral bulbar conjunctiva on day 10. The cyst grew in size for 2 days and ruptured on day 12 with an extrusion of purulent material. The cyst became flat and was almost totally resolved by day 14 of the study. Two dogs had evidence of iris hemorrhage that resolved in 1 dog by day 3 and in the other dog by day 10.

Six of 15 dogs developed corneal epithelial defects. One day after laser treatment, 4 of 15 dogs had evidence of roughened corneal epithelium in the laser-treated eye. This corresponded to multifocal, punctate areas of the cornea that retained fluorescein stain. Two of these dogs also had such roughened epithelium on days 2 and 8, respectively, which resolved in each within 48 hours. Another 1 of the 4 dogs developed a rough corneal epithelial surface in the laser-treated eye on day 5 that resolved by day 8, and the fourth dog had a rough corneal epithelial surface in the laser-treated eye throughout the 14-day period after laser treatment, with new punctate areas arising and previous areas healing during that time. These epithelial defects were always in the same location for each dog.

Three dogs had corneal erosions that developed after laser treatment. A fluorescein-positive corneal erosion developed in 1 dog in the laser-treated eye on day 8. This erosion remained static for 3 days before improving on day 12, but it was worse again on day 13. Both of the other dogs developed a corneal erosion in the laser-treated eye on day 4 that healed by day 5.

Table 1—Mean corneal touch threshold values for control and laser-treated eyes of 15 dogs

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Nasal</th>
<th>Temporal</th>
<th>Dorsal</th>
<th>Ventral</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monofilament length (cm)</td>
<td>1.85</td>
<td>1.31</td>
<td>1.57</td>
<td>1.40</td>
<td>1.40</td>
</tr>
<tr>
<td>Force (g/mm$^2$)</td>
<td>1.34</td>
<td>0.83</td>
<td>0.97</td>
<td>0.88</td>
<td>0.93</td>
</tr>
<tr>
<td>Control eye</td>
<td>7.31</td>
<td>10.66</td>
<td>8.30</td>
<td>10.07</td>
<td>10.19</td>
</tr>
<tr>
<td>Laser-treated eye</td>
<td>10.22</td>
<td>14.40</td>
<td>13.15</td>
<td>12.66</td>
<td>13.64</td>
</tr>
<tr>
<td>Difference (g/mm$^2$)</td>
<td>2.91</td>
<td>3.74</td>
<td>5.25</td>
<td>2.59</td>
<td>3.45</td>
</tr>
<tr>
<td>Decrease in corneal sensitivity (%)</td>
<td>28.5</td>
<td>26.0</td>
<td>36.9</td>
<td>20.5</td>
<td>25.3</td>
</tr>
</tbody>
</table>

Values for the laser-treated eyes were significantly ($P < 0.05$) less, compared with the control eye, in all regions of the cornea tested.

Figure 5—Mean ± SD results of a Schirmer tear test (STT) in control (circle) and laser-treated (diamond) eyes of 15 dogs. Results were obtained for 14 days after laser treatment. *Values did not differ significantly ($P < 0.05$) between groups.

a = Baseline measurement obtained before laser treatment.
Corneal sensitivity—Mean regional CTT differences were calculated (Table 1). Before treatment, CTT values did not differ significantly between the treatment and control eyes for any of the corneal regions measured. Following laser treatment, a significant decrease in corneal sensitivity was identified in the laser-treated eyes, compared with values for the control eyes, in all regions of the cornea tested. This difference was already evident on the first day after laser treatment and remained evident throughout the study. The difference in CTT values between laser-treated and control eyes in the central cornea ranged from 0.43 to 0.57 mm in monofilament length with a mean difference in CTT of 2.91 g/mm², which corresponded to a 28.5% decrease in corneal sensitivity. The differences in CTT values for the dorsal, ventral, temporal, and nasal regions ranged from 0.43 to 0.75, 0.37 to 0.77, 0.47 to 0.83, and 0.53 to 0.77 cm of monofilament, respectively, which corresponded to a decrease in corneal sensitivity of 20.3, 25.3, 36.9, and 26%, respectively. The mean overall difference for all corneal regions in all dogs ranged from 0.55 to 0.64 mm in monofilament length, for an overall mean difference in force of 3.5 g/mm², which corresponded to an overall decrease of 27.4% between the laser-treated and control eyes.

Results of STT—Baseline values for STT did not differ significantly between laser-treated and control eyes. Over the entire observation period after laser treatment, the mean difference in STT values between laser-treated and control eyes ranged from 0.5 to 2.5 mm/min (SD, 0.95 mm/min; Fig 5). We did not detect significant differences between laser-treated and control eyes (P values ranged between 0.57 and 0.0112; Bonferroni corrected, P = 0.007).

Results of IOP—Baseline values for IOP did not differ significantly between laser-treated and control
eyes. However, after laser treatment, IOP in the laser-treated eyes was significantly lower than in the control eyes at most measurement points. Laser-treated eyes had a significantly (P = 0.004) lower IOP, compared with IOP for the control eyes, in the morning on days 2 through 14 (Fig 6). Mean IOP in the control eyes ranged from 15 to 18.2 mm Hg for the 14 days, compared with a range of 12.9 to 14.9 mm Hg in the laser-treated eyes. Overall mean decrease of IOP in the morning in laser-treated eyes was 3.1 mm Hg (an overall decrease of 20.3%). Laser-treated eyes also had a significantly (P = 0.004) lower IOP compared with values for control eyes, in the evening on days 2 through 11. Mean IOP of control eyes in the evening ranged from 16.1 to 21.1 mm Hg for the 14 days, compared with a range of 12.5 to 15.5 mm Hg for laser-treated eyes. Overall mean decrease of IOP in the evening for laser-treated eyes was 3.7 mm Hg (an overall decrease of 22.1%).

Staining with gold chloride—The number of major corneal nerve bundles was quantified in 9 dogs on the basis of composite drawings of the camera lucida. Laser-treated eyes had a significantly lower number of nerve bundles per quadrant, compared with control eyes (mean, 1.01 nerve bundles/quadrant fewer than the control eyes). There was a greater overall number of nerves in the control eyes, compared with laser-treated eyes (77 vs 57). In the 9 pairs of eyes that were examined, there were between 3 and 7 nerve bundles/corneal quadrant in the control eyes and between 3 and 4 nerve bundles/corneal quadrant in the laser-treated eyes.

Mean diameter of corneal nerves did not differ significantly (P = 0.859) between control and laser-treated eyes. We obtained measurements of 84 nerves from control eyes, and values ranged from 21.24 to 322.02 µm, (mean, 95.77 µm). We obtained measurements of 44 nerves from laser-treated eyes, and values ranged from 37.29 to 307.74 µm (mean, 100.47 µm). Statistical analysis was performed by use of weighted numbers of observations.

Discussion

Analysis of the findings reported here supported the hypothesis that cyclophotocoagulation with an Nd:YAG laser results in corneal nerve damage and corneal hypesthesia. It is likely that the ulcerative keratitis seen following the use of the Nd:YAG laser to accomplish cyclophotocoagulation in dogs with glaucoma is related to laser-induced ocular nerve damage.

Corneal sensitivity has been studied in several species of domestic animals, including dogs, cats, and horses. Trainable threshold sensitivities differed significantly among breeds of dogs, depending on skull type of the dogs, with brachycephalic breeds having corneas that are the least sensitive. Similar to other species, there is regional variation in sensitivity in the cornea of dogs, with the greatest sensitivity in the central cornea. The CCT values determined before treatment in the study reported here were similar to those reported for dogs. Following cyclophotocoagulation, corneal sensitivity was significantly decreased in laser-treated eyes of all dogs for the entire 14-day monitoring period. Sensitivity was diminished throughout all test regions of the cornea. This finding supports the hypothesis that cyclophotocoagulation with a Nd:YAG laser damages corneal sensory nerve fibers at a location caudal to the limbus. Avoiding the 3- and 9-o’clock positions on the globe during cyclophotocoagulation may reduce but does not negate this complication.

Diminished corneal sensitivity has been documented in human patients with glaucoma. Sensation was especially decreased in older patients and in those with changes in the optic nerve head. Probable mechanisms include direct mechanical damage to corneal nerves by increased IOP and decreased sensitivity caused by corneal edema. Damage to corneal nerves caused by glaucoma could result in partial ophthalmic neuropathy. Responses of sensory nerves to ocular hypertension and subsequent decreased ocular blood flow result in an increased firing rate at the initial onset of ocular hypertension, which contributes to pain. It is feasible that continued mechanical stimulation causing nerve deformation and ischemia could lead to nerve damage. By adding further insult to an already compromised corneal innervation, the effects reported here on corneal sensitivity for cyclophotocoagulation with an Nd:YAG laser may be magnified in glaucomatous dogs.

Corneal sensitivity is affected by several systemic and ocular disease processes. Corneal sensitivity is decreased in human patients with diabetes, leprosy (Hansen’s disease), and myasthenia gravis. Ocular disease processes that affect corneal sensitivity include keratoconus, scleritis and episcleritis, lattice corneal dystrophy, and glaucoma. Parasympathetic denervation (Adie’s syndrome) attributable to lesions of the ciliary ganglion or short ciliary nerves causes decreased corneal sensitivity. Decreased corneal sensitivity is common in people with herpes simplex keratitis. Corneal edema decreases corneal sensitivity by up to 55%. Hyposecretion of tears may lead to pathologic changes in the corneal epithelium, which in turn may lead to a decrease in corneal sensitivity.

Surgical procedures such as cataract surgery and photorefractive keratectomy result in temporary or permanent reduction in corneal sensitivity. Various other keratorefractive procedures including radial keratotomy, epikeratophakia, and laser in situ keratomileusis cause damage to corneal sensory innervation and result in increased sensory thresholds. Photocoagulation of the ocular fundus also decreases corneal sensitivity.

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Dogs with glaucoma sometimes are treated by topical administration of diclofenac sodium and frequently are treated by topical administration of timolol maleate and dorzolamide. When each of these drugs is used as the sole therapeutic agent in clinically normal humans, there is a decrease in corneal sensitivity.\textsuperscript{42-44} In patients treated with diclofenac, an increase in plasma concentrations of $\beta$-endorphins and a decrease in sensory input from corneal polymodal nociceptive fibers contribute to analgesic activity.\textsuperscript{45} Administration of $\beta$-blocking agents such as timolol maleate has been credited with causing local anesthesia and stabilizing membranes, which contribute to decreased corneal sensitivity.\textsuperscript{42,44,46} We hypothesize that the combination of these drugs in dogs with glaucoma that are undergoing cyclophotocoagulation with a Nd:YAG laser would further decrease corneal sensitivity and contribute to pathologic changes in the cornea observed following cyclophotocoagulation. We used topical administration of a chloramphenicol product in both eyes of all dogs in our study. To our knowledge, this product does not adversely affect corneal sensitivity.

The percentage of dogs that developed corneal erosions and ulcers following cyclophotocoagulation in our study was 20%, compared with 60% in glaucomatous dogs that are patients at our veterinary medical teaching hospital. Fluorescein stain was used to detect these epithelial defects. Rose bengal stain, which detects devitalized corneal epithelium, may have revealed a higher percentage of corneal epithelial lesions in both populations by highlighting subtle irregularities in corneal epithelium. The severity of corneal epithelial defects was much less in the study dogs than in dogs admitted as patients. In dogs treated as clinical cases at our veterinary teaching hospital, however, glaucoma had been previously diagnosed, and an increase in IOP, often with optic nerve head changes, had been documented before the laser procedure. In addition, the clinical cases were commonly being treated with topical medications that may have contributed to pathologic changes in the cornea. The difference in frequency and severity of corneal epithelial defects may have been related to these differences between dogs treated at our clinical facility and the dogs used in the study reported here.

It is possible that the application of the nylon filament of the esthesiometer to the corneal surface can damage the corneal epithelium.\textsuperscript{34,47} It has been hypothesized that the invasive nature of the instrument damages corneal epithelium and can also decrease CTT.\textsuperscript{34,47} It is unlikely that the nylon filament was responsible for the epithelial defects documented in the study reported here, because we did not detect defects in any of the control eyes at any point during the study.

The optimal clinical effect from cyclophotocoagulation with a Nd:YAG laser is associated with application of 100 to 200 J of energy delivered in short bursts,\textsuperscript{3} although there has been a report\textsuperscript{7} of fewer adverse effects associated with an increased amount of energy delivered at a slower rate to only a few sites. Lower amounts of energy (<100 J) have been associated with only transient reduction of IOP.\textsuperscript{44} Because of variations among subjects, amounts of power, exposure times, and degree of scleral and uveal pigmentation, it is difficult to define an optimal treatment protocol.\textsuperscript{34,44} In the study reported here, as expected, there was a significant decrease in IOP in treated eyes at most time points after laser treatment. The significant decrease in corneal sensitivity seen on all days after laser treatment documented that lower total energy delivery (eg, 100 J) is able to compromise corneal sensitivity. However, it is likely that corneal changes associated with this loss of corneal sensitivity would be increased with higher energy delivery.

Aqueous tear production was measured every other day following cyclophotocoagulation and was found to be unchanged within that time period. Decreased corneal sensitivity correlates significantly with insufficient aqueous tear production.\textsuperscript{31,50} Hyposecretion of tears may lead to pathologic changes in corneal epithelium, leading to a decrease in corneal sensitivity.\textsuperscript{48} Conversely, damage to the corneal surface may cause a destructive negative-feedback mechanism on the lacrimal glands.\textsuperscript{31} It is possible that 2 weeks was not a sufficient amount of time to establish such a negative-feedback process on the lacrimal glands to decrease aqueous tear production. Although tear production did not appear to be significantly altered during the study period, there did appear to be a pattern of lower aqueous tear production in the laser-treated eyes. However, aqueous tear production was unlikely to be a contributing factor for the clinical signs of pathologic corneal changes detected during the study.

Despite the development of sophisticated and specific methods for examining nerve fibers, such as immunohistologic techniques, methods that use the impregnation of nerve fibers with metallic salts remain useful in the study of neuroanatomic structures.\textsuperscript{15} Quantification of major stromal nerve bundles in the control eyes in the study reported here is comparable to that of other studies.\textsuperscript{13,17} Anatomic differences have not been reported in the number of major nerve bundles in quadrants of the same cornea or between corneas of the same animal. Therefore, it was believed that an extrapolation of total corneal innervation on the basis of observations from representative quadrants could be performed.\textsuperscript{13,17} There was a mean decrease of 1.01 major nerve bundles/corneal quadrant in laser-treated eyes. When this value is extrapolated to the entire cornea, there may be a decrease of as many as 4 bundles/cornea. This value was significantly different between laser-treated and control eyes, and it is likely that this was an effect attributable to cyclophotocoagulation with a Nd:YAG laser. Focal burns and histologic necrosis reported at areas of laser application\textsuperscript{7} would suggest that nerve bundles in those areas could be destroyed, resulting in nerve drop-out. In 1 study,\textsuperscript{52} investigators found a decrease of 1 corneal nerve bundle/eye in diabetics with peripheral neuropathy. They declared this finding to be clinically relevant, even though it was not significantly different. Denervation of the cornea results in impaired healing of epithelial wounds, increased epithelial permeability, decreased metabolic activity of epithelium, and loss of cytoskeletal structures associated with cellular adhesion, which would account for the corneal epithelial
defects seen in the dogs of our study and in clinical populations of dogs with glaucoma.\textsuperscript{55,56} In the study reported here, dogs that developed corneal defects had fewer bundles per corneal quadrant in the laser-treated eye; this was not significantly different from those dogs that did not develop corneal defects.

Diameter of nerve bundles did not differ significantly between laser-treated and control eyes. Although the laser procedure did not produce detectable changes in diameter of nerve fibers, it remains possible that minor trauma to the nerves or transient inflammation may have compromised these nerves functionally (eg, decreased transport and release of trophic factors such as neuropeptides) that contributed to the decrease in corneal sensitivity and keratitic changes. In inflammatory conditions, cytokines are released, and they cause corneal nerve damage.\textsuperscript{30} Cytokines appear to inhibit parasympathetic neural transmission in peripheral nerves, and increased concentrations of cytokines are found in inflammatory conditions associated with decreased corneal sensitivity.\textsuperscript{31,32}

In addition to the observed decrease in corneal nerve bundles in laser-treated eyes, we hypothesize that the prevalence of corneal ulcers in laser-treated eyes was related to diminished corneal neuropeptide expression in the cornea as a result of laser-induced corneal nerve damage. Comparison of neuropeptide expression in eyes that have undergone cyclophotocoagulation with a Nd:YAG laser with that of nontreated eyes is required to validate this hypothesis. Sensory nerves of the cornea may exert effector functions through the release of trophic neuropeptides such as substance P and calcitonin gene-related peptide (CGRP). Nerve fibers that stain positive for substance P are found throughout the corneal stroma, epithelium, and limbus.\textsuperscript{36,39} Calcitonin gene-related peptide is found throughout the cornea and is often colocalized with substance P; the distribution of CGRP is dense and complex.\textsuperscript{38,39} Substance P and CGRP increase the rate of mitosis of corneal epithelial cells in vitro and modulate other aspects of corneal epithelial cells, including adhesion and cellular migration.\textsuperscript{30,36} Clinical effects associated with denervation of the trigeminal nerve include a decrease in healing rate for standardized epithelial abrasions and a high incidence of naturally developing recurrent epithelial erosions.\textsuperscript{30} Corneal sympathetic and parasympathetic fibers have been implicated in the modulation of corneal sensitivity and healing of wounds in the corneal epithelium.\textsuperscript{55,56} Sympathetic receptors have been identified on corneal epithelial cells.\textsuperscript{55,56} Sympathetic corneal fibers exert trophic effects on corneal epithelium by stimulating mitotic activity and promoting wound healing.\textsuperscript{30,36} Loss of corneal sympathetic innervation significantly decreases epithelial proliferation in normal and wounded corneas.\textsuperscript{30}

Tyrosine hydroxylase is a mixed function oxidase found in all cells that synthesize catecholamines and is the rate-limiting enzyme in catecholamine synthesis. In 1 study\textsuperscript{7} tyrosine hydroxylase was found in approximately 30% of the corneal nerves obtained from dogs. Differences among species in the organization and density of tyrosine hydroxylase in immunohistochemically positive nerve fibers may reflect species variation in the amount of sympathetic control over corneal physiologic processes and wound healing.\textsuperscript{32}

Vasointestinal peptide is contained in parasympathetic nerves and may exert regulatory effects on proliferation of corneal epithelial cells.\textsuperscript{56} Quantitative analyses of immunohistochemically labeled corneal nerve fibers in the central corneas of clinically normal dogs reveals that substance P, CGRP, tyrosine hydroxylase, and vasointestinal peptide are expressed within >99, >99, 29.7, and 0%, respectively, of all labeled-protein gene product-9.5-IR corneal nerves.\textsuperscript{17} Immunohistochemical labeling of corneas was attempted on the dogs used in the study reported here. Because of methodologic difficulties associated with inadequate fixation and antigen stabilization, attempts to examine the expression of protein gene product 9.5, substance P, CGRP, and tyrosine hydroxylase in these corneas were largely unsuccessful.

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