Evaluation of diode endoscopic cyclophotocoagulation in bovine cadaver eyes

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Objective—To evaluate the anterior chamber approach and energy levels for endoscopic cyclophotocoagulation (ECPC) and assess ECPC-induced tissue damage in phakic eyes of bovine cadavers.

Sample—12 bovine cadaver eyes.

Procedures—Angle of reach was measured in 6 eyes following placement of a curved endoscopic probe through multiple corneal incisions. In another 6 eyes, each ocular quadrant underwent ECPC at 1 of 3 energy levels (0.75, 0.90, and 1.05 J) or remained untreated. Visible effects on tissues (whitening and contraction of ciliary processes) were scored (scale of 0 [no effects] to 6 [severe effects]), and severity and extent of histologic damage to the pigmented and nonpigmented ciliary epithelium and fibromuscular stroma were each scored (scale of 0 [no effect] to 3 [severe effect]) and summed for each quadrant. Overall mean scores for 6 quadrants/treatment were calculated.

Results—Mean ± SD combined angle of reach was 148 ± 24° (range, 123 ± 23° [ventromedial] to 174 ± 11° [dorsolateral]). At the 0.75-, 0.90-, and 1.05-J levels, mean visible tissue effect scores were 3.12 ± 0.47, 3.86 ± 0.35, and 4.68 ± 0.58, respectively; mean histologic damage scores were 4.79 ± 1.38 (mild damage), 6.82 ± 1.47 (moderate damage), and 9.37 ± 1.42 (severe damage), respectively. Occasional popping noises (venting of vaporized interstitial water) were heard at the 1.05-J level.

Conclusions and Clinical Relevance—Multiple incisions were necessary to facilitate 360° ECPC treatment in bovine eyes. For ECPC in vivo, the 0.75- and 0.90-J energy levels had the potential to effectively treat the ciliary epithelium. (*Am J Vet Res* 2012;73:1445–1452)

Glaucoma is a disease that results from an increase in IOP that is greater than that compatible with normal function of the eyes.[1–3] The pathogenesis of glaucoma is multifactorial, but vision loss is associated with retinal and optic nerve ischemia and ganglion cell loss.[1–4] High IOP can also cause discomfort that may ultimately result in an irreversibly blind, painful eye that requires enucleation or another end-stage procedure. Therefore, early and effective treatment is recommended to preserve vision and maintain comfort.

The focus of glaucoma treatment is the management of the underlying cause and maintaining normal IOP.[1–3] Medical management includes nonspecific topical or systemic anti-inflammatory treatment and specific topical glaucoma treatment, including drugs that decrease AH formation as well as increase AH outflow. Surgical management of glaucoma includes procedures that increase AH outflow (AC shunt placement and filtering procedures) or those that decrease AH production (CPC and cyclocryosurgery).[1–3]

Cyclophotocoagulation refers to the induction of coagulation necrosis of the CB with light energy.[5] Cyclophotocoagulation is often performed with a semiconductor diode laser that emits light in the near-infrared spectrum (810 nm), although other laser systems (neodymium-yttrium aluminum garnet, argon, and krypton) have been described.[6–8] Diode laser energy is strongly absorbed by tissues containing melanin, such

Abbreviations

AC	Anterior chamber
AH	Aqueous humor
CB	Ciliary body
CCI	Clear corneal incision
CE	Ciliary epithelium
CP	Ciliary process
CPC	Cyclophotocoagulation
ECPC	Endoscopic cyclophotocoagulation
IOP	Intraocular pressure
TSCPC	Transscleral cyclophotocoagulation

Received June 4, 2011.
Accepted September 1, 2011.
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Presented in abstract form at the 41st Annual Meeting of the American College of Veterinary Ophthalmologists, San Diego, October 2010.
The authors thank Paula Ender from Endo Optiks Inc for providing the integrated laser and endoscope and Nathan Whitehurst and Sandra Horton for technical assistance.
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as pigmented CE. Possible mechanisms for IOP reduction include destruction of the CE, damage to the ciliary vasculature, postoperative uveitis and prostaglandin release, and architectural change leading to increased uveoscleral outflow. It is likely that a combination of these factors is responsible for IOP reduction.[5,9,10] The goal of all cyclodestructive procedures is coagulation of the CE and stroma of the pars plicata where AH is produced.[5]

Two cyclodestructive procedures performed most commonly in veterinary patients are TSCPC and ECPC. Transscleral CPC is a technique in which laser energy is delivered to the pars plicata through the sclera without a surgical incision. Transscleral CPC has been routinely used in veterinary medicine, including the treatment of glaucoma in horses, with variable results.[6–8,11–20] The advantages of TSCPC are the ease of use, potential for IOP control, and avoidance of intraocular surgery. The main disadvantage of TSCPC is the lack of direct inspection of the CB during the procedure, which may lead to an increased risk of collateral damage to nontarget tissues and prohibits an ophthalmologist from safely applying the laser to all quadrants of the eye.[9,10]

Endoscopic CPC is a newer surgical treatment that involves the application of laser energy to the CP within the eye under direct endoscopic observation. Real-time observation of the ECPC probe and acute tissue effects may allow precise application of the laser energy, thereby decreasing the risk of overtreatment or collateral tissue damage. Endoscopic CPC may be more effective than TSCPC because the laser can be safely applied to all quadrants. Endoscopic CPC has the potential to be an effective treatment of glaucoma in veterinary patients, including horses. Use of ECPC in small animal ophthalmology, as the primary treatment of glaucoma and in combination with phacoemulsification surgery, has been reported,[a–c] but to date, no peer-reviewed studies on the use of ECPC in large animals have been reported in the veterinary medical literature, to the authors' knowledge. Establishing a reproducible surgical approach and correlating the visible tissue effects to the histologic effects induced by use of ECPC at specific energy settings are necessary to determine the method and protocol for use of ECPC in veterinary patients. Without evaluation of the histologic changes in the CB, defining the relationships between the clinical effects and the structural pathological changes induced by ECPC is impossible. The purposes of the study reported here were to evaluate an AC approach for ECPC in phakic bovine cadaver eyes, including the number of CCIs necessary to effectively treat the complete circumference (360°) of the CPs, and correlate the acute visible and histologic effects to determine appropriate ECPC energy settings to affect the target CB.

Materials and Methods

Sample—Twelve fresh bovine cadaver eyes from 12 adult cattle of undisclosed breed and sex were obtained within 24 hours after slaughter, and no obvious abnormalities were noted on gross examination. All eyes had brown irides and were stored in a container with saline (0.9% NaCl) solution at 4°C for < 48 hours before the procedure. Thirty minutes prior to the procedure, each eye was warmed to room temperature (approx 20°C). Each eye was mounted on a closed-cell extruded polystyrene foam base; fixed in a stable position with 1.5-inch, 18-gauge needles; and reinflated via limbal injection of saline solution by use of a 25-gauge, 1-inch needle attached to a 3-mL syringe.

Surgical approach—Six fresh cadaver eyes were used for this portion of the study. Multiple trilaminar CCIs, as described for cataract surgery in horses,[21,22] were made with a No. 64 microsurgical blade[d] and 2.8-mm beveled keratome.[e] Methylcellulose-based viscoelastic substance[f] was introduced through the CCI to fill the AC. The tip of the cannula was then directed through the pupil and positioned within the posterior chamber. Viscoelastic material was inserted into the posterior chamber, thereby anteriorly displacing the iris away from the crystalline lens, expanding the ciliary sulcus, and facilitating observation of the CPs. Inflation of the ciliary sulcus was repeated as necessary throughout the procedure to allow an adequate view of the CPs.

An integrated laser and endoscope[g] with a large animal endolaser probe[g] was used. The system incorporates an 810-nm pulsed continuous-wave diode laser,[h] helium-neon aiming beam, 175-W xenon light source, fiber-optic video camera, recorder, and monitor. The prototype probe consisted of a 50-mm, 18-gauge extended fiber tip (**Figure 1**). Trilaminar CCIs at 8 sites (45° apart and starting at the 12 o'clock position) were made around the circumference of the cornea. The sites were measured first with calipers and marked with simple interrupted nylon sutures[i] placed at the limbus. The probe was introduced into each incision, advanced through the pupil, and directed into the opposite ciliary sulcus through direct observation via the video monitor. Five CPs were kept in view through the endoscope to maintain a constant distance (approx 2 mm) between the probe and CB (**Figure 2**). The probe was advanced horizontally in both directions within the sulcus, with an attempt to visualize the largest angle that the single incision would allow. With the aid of a marker and protractor, the angle in degrees of access to the ciliary sulcus was recorded at each site.

Acute tissue effects—Another 6 fresh bovine cadaver eyes, obtained and processed as described, were used for this portion of the study. Trilaminar CCIs were created, and the anterior and posterior chambers were inflated as described and repeated as necessary throughout the procedure to allow adequate observation of the CPs. The globe was divided into 4 quadrants, each 90° apart starting at the 12 o'clock position, and marked at the limbus with nylon suture material.[i] The large animal endolaser probe was introduced into the CCI opposite the treatment quadrant. The probe tip was positioned opposite the central CP, constantly maintaining 5 CPs in view (Figure 2). This technique allowed a distance of approximately 2 mm to be maintained between the tip of the ECPC probe and target CP. Fifteen CPs within each quadrant were treated in the same manner with the diode laser, controlled by the surgeon using a foot pedal. Exposure time for each CP was held constant at 3,000 milliseconds, which allowed for 1 complete vertical passage across the in-

dividual CP during treatment. Power settings of 250 (low), 300 (medium), and 350 mW (high) were applied to each CP within the first, second, and third quadrants, respectively. These power and duration settings correlated with total energy values of 0.75, 0.90, and 1.05 J applied to each CP in the first, second, and third quadrants, respectively. The remaining quadrant served as an untreated control. These energy levels were selected on the basis of previous studies[9,10,23–32,a–c] and preliminary evaluation of desired visible tissue responses (data not shown). Energy levels < 0.75 J resulted in minimal to no visible tissue effect, and energy levels > 1.05 J were associated with frequent popping noises emanating from the tissues, which were indicative of possible overtreatment. The sudden formation of vapor from interstitial water results in rapid volume expansion in an enclosed space and, when the vapor pressure exceeds the internal tissue pressure, an explosion (popping noise) as the vapor is vented.

The acute visible changes in each CP (whitening and contraction) were recorded with a computer software program[j] at the time of CP. The primary investigator (JTH) reviewed all recordings and assigned visible tissue effect scores to each CP on the basis of a scale from 0 (no effect) to 6 (audible popping noises during the procedure; **Appendix**). The maximum score assigned to each of the 15 CPs/quadrant was 6. For each quadrant (different power settings or control treatment), a mean total visible tissue effect score was calculated from the 15 CP scores; a mean total visible tissue effect score for each power setting or control treatment was calculated from 6 mean quadrant scores (90 CPs).

Histologic evaluation—Following ECPC treatment, as previously described, the globes were injected with and fixed in a combination formaldehyde-glutaraldehyde (4:1) solution and sectioned on the basis of treatment quadrant. The anterior segment was cut into 4 wedges on the basis of assigned quadrants, and serial 100-µm sections (perpendicular to the ciliary ridge and parallel to the CPs) were cut. Fifteen sections from each quadrant were evaluated. The paraffin tissue sections were stained with H&E stain and evaluated by the primary investigator (JTH) and a board-certified veterinary pathologist (JMC) using light microscopy. General histologic observations were recorded for all sections examined, with specific areas of interest including damage to the pigmented and nonpigmented CE, separation of the epithelial bilayers, pigment clumping and dispersion, and damage to the fibromuscular stroma. Histologic changes in the adjacent cornea, iris, pars plana, and sclera were also noted. In addition, damage to the pigmented CE, nonpigmented CE, and fibromuscular stroma was each scored on a semiquantitative 4-point scale (0 = absent, 1 = mild, 2 = moderate, and 3 = severe). The percentage of CB affected was also scored on a 4-point scale (0 = none, 1 = < 10%, 2 = 10% to 50%, and 3 = > 50%). For each section, the 4 scores were added together to provide a total histologic score (maximum cumulative score for each section, 12). For each quadrant (different power settings or control treatment), a mean total histologic score was calculated from the 15 section scores; a mean total histologic score for each power setting or control treatment was calculated from 6 mean quadrant scores (90 CPs).

Statistical analysis—Normally distributed data were expressed as mean ± SD. A Kruskal-Wallis test was used to compare mean total visible tissue effect scores and mean total histologic scores following treatments at the 3 power settings and the control treatment. A 1-way ANOVA with the Tukey-Kramer test was used to evaluate the difference between CCI locations and mean angle of reach in the ciliary sulcus. Correspondence analysis was used to determine relationships between mean total visible tissue effect and histologic scores. For all analyses, a value of $P < 0.05$ was considered significant.

Results

Surgical approach—The entire 360° ciliary sulcus was accessible through an AC approach by use of mul-

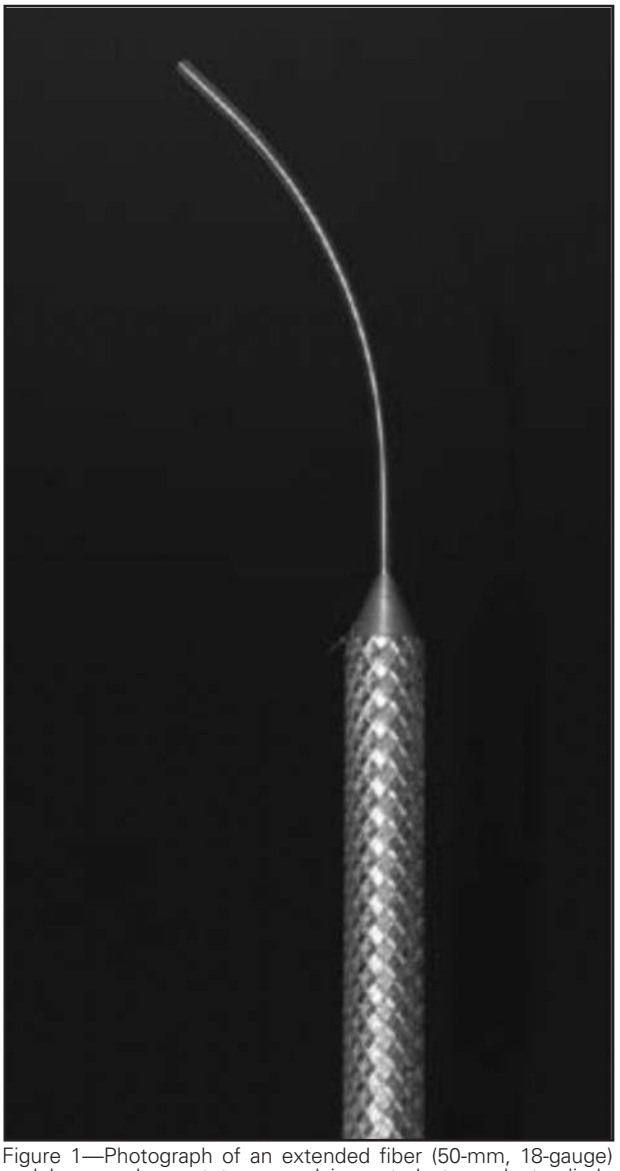

Figure 1—Photograph of an extended fiber (50-mm, 18-gauge) endolaser probe prototype used in a study to evaluate diode ECPC in bovine cadaver eyes.

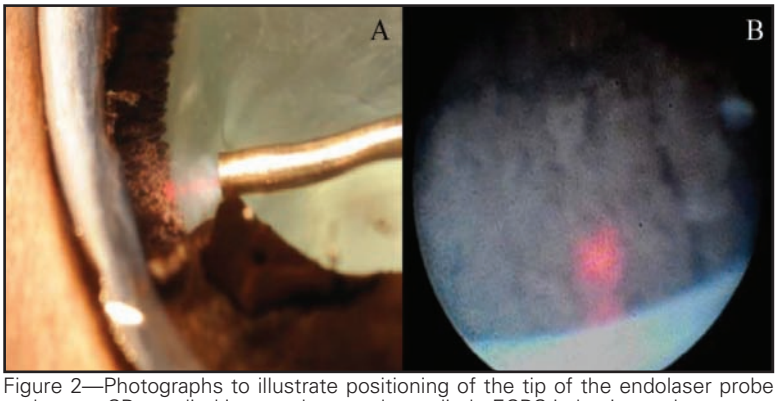

Figure 2—Photographs to illustrate positioning of the tip of the endolaser probe and target CPs applied in a study to evaluate diode ECPC in bovine cadaver eyes. A—Gross view of the ciliary sulcus following sector iridectomy. The distance between the ECPC probe tip and target CPs is approximately 2 mm. B—Endoscopic view through an endolaser probe. Five CPs are in view. The red aiming beam is focused on the central process.

tiple CCIs and the curved large animal ECPC probe. For the dorsal, ventral, lateral, and medial CCI locations, the mean ± SD angle of reach to the ciliary sulcus was 131 ± 11°, 137 ± 11°, 142 ± 25°, and 154 ± 28°, respectively. For the ventromedial, ventrolateral, dorsomedial, and dorsolateral CCI locations, the mean angle of reach to the ciliary sulcus was 123 ± 23°, 157 ± 16°, 162 ± 14°, and 174 ± 11°, respectively. The mean combined angle of reach was 148 ± 24°. The mean angle of reach to the ciliary sulcus for the dorsolateral incision location differed significantly from findings for the ventral ($P = 0.025$), dorsal ($P = 0.005$), and ventromedial ($P < 0.001$) locations. Significant differences in mean angle of reach to the ciliary sulcus were identified between the dorsomedial and ventromedial incision locations ($P = 0.015$) as well as between the ventrolateral and ventromedial incision locations ($P = 0.049$). Comparisons between the other incision locations did not reveal any significant differences in mean angle of reach to the ciliary sulcus. During probe placement, the corpora nigra impeded access to the ciliary sulcus from the dorsal incision and the third eyelid impeded access to the ciliary sulcus from the ventromedial incision. The curved large animal endolaser probe effectively reached the ciliary sulcus through each CCI location.

Acute tissue effects—A pulse duration of 3,000 milliseconds at 250, 300, and 350 mW (0.75, 0.90, and 1.05 J, respectively) resulted in grossly visible lesions in the CB. Mean total visible tissue effect scores were as follows: at the 250 mW × 3,000 milliseconds (0.75-J) ECPC treatment, 3.12 ± 0.47, which corresponded to mild tissue whitening and contraction; at the 300 mW × 3,000 milliseconds (0.90-J) ECPC treatment, 3.86 ± 0.35, which corresponded to moderate tissue whitening and contraction; and at the 350 mW × 3,000 milliseconds (1.05-J) ECPC treatment, 4.68 ± 0.58, which corresponded to moderate to severe tissue whitening and contraction. No abnormalities were detected in the untreated control eyes (total visible tissue effect score, 0). Occasional overtreatment, characterized by audible tissue popping noises, occurred at the 1.05-J energy level. The mean total visible tissue effect score at the 0.75-J energy level was significantly ($P < 0.001$) lower than the mean total visible tissue effect score at the 0.90- or 1.05-J energy level. No significant difference was found between the mean total visible tissue effect scores at the 0.90- and 1.05-J energy levels.

Histologic evaluation—Histologic evidence of tissue damage, specifically coagulation necrosis and tissue shrinkage in the CB, was observed at all energy levels evaluated (0.75, 0.90, and 1.05 J). Thinning of the pigmented and nonpigmented layers of the CB, extracellular dispersion of pigment granules, contraction of the pigmented CE, and loss of the normal lacy appearance on the anterior surface were also observed at all energy levels. Mean total histologic scores (maximum score, 12) were 4.79 ± 1.38, 6.82 ± 1.47, and 9.37 ± 1.42 for the 0.75-, 0.90-, and 1.05-J ECPC treatments, respectively. No abnormalities were detected in the untreated control sections (total histologic score, 0) or in the adjacent cornea, iris, pars plana, or sclera; the untreated control sections had CPs with intact bilayers of normal nonpigmented and pigmented CE surrounding a homogenous stroma (**Figure 3**). At the lowest energy level (0.75 J), histologic changes were mild and characterized by moderate damage to the pigmented and nonpigmented CE, mild separation of the epithelial bilayers, and mild damage to the fibromuscular stroma adjacent to the treated surface. The overall CB structure was preserved relative to the other treated quadrants. At the intermediate energy level (0.90 J), histologic changes were indicative of more widespread CB destruction, characterized by greater damage to the pigmented and nonpigmented CE, separation of the epithelial bilayers, and fibromuscular stromal damage. At the highest energy level (1.05 J), histologic changes included severe ablation of the pigmented and nonpigmented CE, greater pigment dispersion, and moderate fibromuscular stromal damage adjacent to the treated surface. The mean total histologic score at the 1.05-J energy level was significantly ($P < 0.001$) higher than the mean total histologic score at the 0.75- or 0.90-J energy level. No significant difference was found between the mean total histologic scores at the 0.75- and 0.90-J energy levels.

Relationships between mean total visible tissue effect and histologic scores—Results of correspondence analysis suggested multiple relationships between the total visible and histologic scores. A high level of correspondence was found between visible tissue effect score 2 and histologic score 3, between visible tissue effect score 3 and histologic score 4, between visible tissue effect score 4 and histologic scores 6 to 9, and between visible score 5 or 6 and histologic scores 10 to 12. Thus, in bovine cadaver eyes, there was a positive relationship between gross visible tissue changes and histologic changes associated with ECPC.

Discussion

The direct visualization of the CPs through an endoscope during ECPC may allow clinicians to titrate laser energy levels during the treatment of glaucoma,

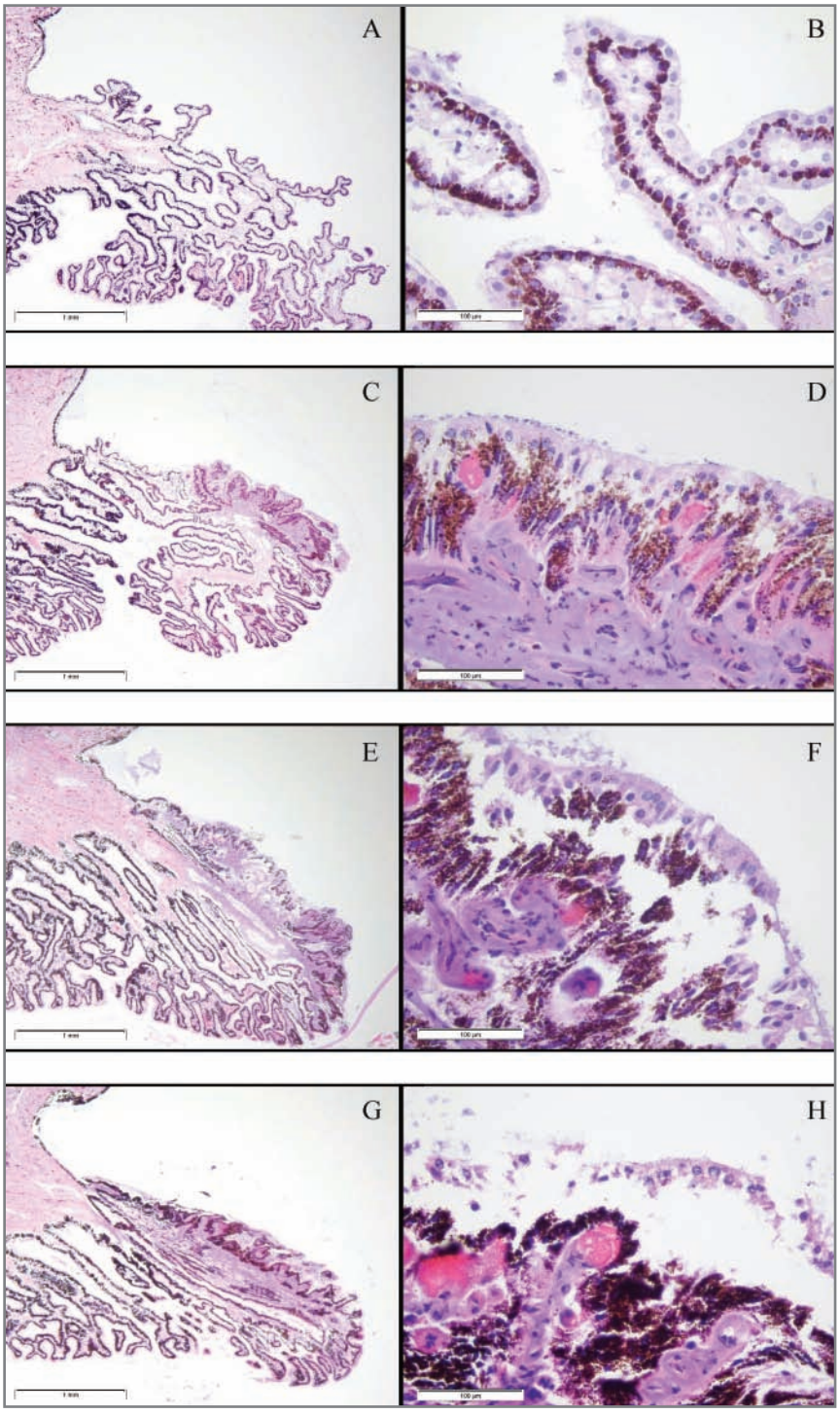

Figure 3—Respresentative light microscopy images of H&E-stained sections of CB tissue in ocular quadrants of bovine cadaver eyes that were or were not treated via ECPC at 1 of 3 energy levels. A—Low-power photomicrograph of a section of untreated CB tissue. Notice the normal lacy contour. Bar = 1 mm. B—Higher-power photomicrograph of the same section of untreated CB tissue in panel A. Notice the normal appearance of the pigmented and nonpigmented CE and fibromuscular stroma. Bar = 100 µm. C—Low-power photomicrograph of a section of CB tissue treated via ECPC at a power setting of 250 mW X 3,000 milliseconds (0.75 J). Notice the loss of the normal lacy appearance on the anterior surface of the CP. Bar = 1 mm. D—Higher-power photomicrograph of the same section of CB tissue in panel C treated via ECPC at a power setting of 250 mW X 3,000 milliseconds (0.75 J). Notice the moderate damage to the pigmented and nonpigmented CE, mild separation of the epithelial bilayers, extracellular dispersion of pigment granules, and mild damage to the fibromuscular stroma adjacent to the treated surface. Bar = 100 µm. E—Low-power photomicrograph of a section of CB tissue treated via ECPC at a power setting of 300 mW X 3,000 milliseconds (0.90 J). Notice the comparatively greater loss of normal lacy appearance and CB damage on the anterior surface of the CP. Bar = 1 mm. F—Higher-power photomicrograph of the same section of CB tissue in panel E treated via ECPC at a power setting of 300 mW X 3,000 milliseconds (0.90 J). Notice the comparatively greater damage to the pigmented and nonpigmented CE, separation of the epithelial bilayers, extracellular pigment dispersion, and fibromuscular stromal damage adjacent to the treated surface. Bar = 100 µm. G—Low-power photomicrograph of a section of CB tissue treated via ECPC at a power setting of 350 mW X 3,000 milliseconds (1.05 J). Notice the marked loss of normal lacy appearance and severe CB damage on the anterior surface of the CP. Bar = 1 mm. H—Higher-power photomicrograph of the same section of CB tissue in panel G treated via ECPC at a power setting of 350 mW X 3,000 milliseconds (1.05 J). Notice the severe ablation of the pigmented and nonpigmented CE, major pigment dispersion, and moderate fibromuscular stromal damage adjacent to the treated surface. Bar = 100 µm.

thereby improving the safety of the procedure, compared with other techniques. However, to date, only anecdotal information on the surgical approach and treatment variables used in ECPC is available within the veterinary medical literature, to our knowledge. Despite the lack of controlled, peer-reviewed studies, there are studies[23–31] reported in human ophthalmology publications of ECPC evaluation in eyes of several species, including humans, nonhuman primates, swine, and rabbits. Shields et al[28] performed ECPC in nonhuman primates and compared structural and histologic effects during an 8-month period following the procedure. Pantcheva et al[24,25] compared structural and histologic changes of TSCPC and ECPC in porcine and human cadaver eyes and concluded that ECPC was a more selective form of CPC than TSCPC, with ECPC resulting in less tissue disruption and also effective destruction of CE. In another study,[26] CB perfusion (assessed via fluorescein angiography) was compared between 20 TSCPC- and 20 ECPC-treated rabbit eyes. At 1 month after treatment, perfusion of the CPs in TSCPC-treated eyes remained poor; the chronic poor perfusion was correlated with

the procedure's efficacy as well as more complications (hypotony and phthisis bulbi), compared with results for ECPC.[26] In the ECPC-treated eyes, some reperfusion was detected 1 week after treatment, with greater late reperfusion.[26] Human clinical trials have been performed to determine the effects of ECPC on IOP in human patients with refractory glaucoma.[27,29] In 68 eyes of 68 patients, 1 or 2 ECPC treatments were successful in IOP control in 61 (90%) eyes at the last follow-up (mean, 12.9 months), with a low rate of severe vision loss (4/68 [6%] eyes) and no development of phthisis or hypotony.[27] Lima et al[29] found ECPC to be equally as effective as the Ahmed valve in reducing and stabilizing IOP at 12 and 24 months after surgery for refractory glaucoma. Despite these studies reported in the human medical literature, important variables, including extent of treatment, exposure power and duration, and laser tissue effects, have not been established for veterinary patients. The goals of the present study were to evaluate an AC surgical approach and establish treatment variables for diode ECPC through demonstration of the acute gross and histologic tissue effects in phakic bovine cadaver eyes.

Bovine eyes were chosen for use in the present study for multiple reasons, including the accessibility to fresh cadaver eyes, globe size comparable with that of equine eyes, and opportunity to evaluate a large animal endolaser probe. Evaluation of ECPC in bovine eyes may serve as a model for the use of ECPC in equine eyes. Furthermore, a clear corneal approach in a phakic eye was chosen to determine the feasibility in performing ECPC without concurrent removal of the lens. Several reports[a,b] of ECPC in veterinary patients describe concurrent removal of the lens, but the necessity of lens removal has not been determined and may not be the optimal treatment in animals that have glaucoma without cataract formation. Previous ECPC studies[23–32] used various surgical approaches, including clear corneal or limbal incisions in phakic, aphakic, or pseudophakic eyes as well as a pars plana approach in aphakic and pseudophakic eyes. Little attention has been given in the human or veterinary medical literature to development of a consistent surgical approach or treatment variables for ECPC. The first part of the present study was aimed at establishing a repeatable protocol for an AC surgical approach for ECPC in bovine cadaver eyes.

On the basis of results of the present study, we concluded that a minimum of 3 CCIs, at a constant distance of approximately 2 mm from the target CPs, is necessary to facilitate 360° ECPC treatment in a bovine eye. Multiple combinations of CCI locations around the cornea will facilitate 360° ECPC treatment. The dorsolateral, dorsomedial, and ventrolateral CCI locations achieved the greatest angle of access to the ciliary sulcus (Figure 3). Therefore, we recommend a dorsolateral incision (approx 1:30 o'clock position in a left eye or 10:30 o'clock position in a right eye), a dorsomedial incision (approx 10:30 o'clock position in a left eye or 1:30 o'clock position in a right eye), and a ventrolateral incision (approx 4:30 o'clock position in a left eye or 7:30 o'clock position in a right eye) to access the entire ciliary sulcus. Observations during probe placement included impedance by the corpora nigra at the dorsal incision location and impedance by the third eyelid at the ventromedial incision location. In a clinical setting, a dorsal CCI should be avoided to limit this damage to and impedance by the corpora nigra, and a ventromedial CCI should also be avoided because of impedance by the third eyelid. Although these results outline a surgical approach to access the entire (360°) ciliary sulcus, further studies are required to determine the number or degree of treated CPs required to achieve a clinically relevant reduction in IOP. It is possible that < 360° of ECPC treatment would result in the desired clinical effect of IOP control; consequently, 1 or 2 CCIs may be sufficient.

Probe position and design are important factors in effective ECPC treatment. Probe position has a major role in the amount of energy delivered to the target tissue and, by extension, overall energy necessary to effectively achieve the desired tissue response. A probe that is positioned too close to the CPs may overtreat the tissue and create popping noises, but a probe positioned too far away from the CPs may not deliver enough energy to treat the tissue and may deliver energy to nontarget tissues. A constant distance of approximately 2 mm from the probe tip to target CPs was maintained in the present study. The distance of 2 mm correlated with maintaining approximately 5 CPs within the field of view through the endoscope. This positioning was chosen on the basis of preliminary evaluation of probe-to-target distances > 2 and < 2 mm and results of a study by Yu et al,[33] which suggested that 2 mm is the optimal distance between the laser probe and tissue under treatment to preserve the intended laser energy setting. Both adequate visualization of tissue effects and application of laser energy were possible with the tip of the probe positioned 2 mm from the CPs.

The probe used in the present study was a 50-mm, 18-gauge curved prototype[g] developed for use in large animal eyes, specifically the eyes of horses (Figure 1). The prototype contains the same components (light source, aiming beam, fiber-optic camera, and diode laser fiber) as the commercially available small animal probe, but the extended fiber allows for access across the larger AC and ciliary sulcus. An extended-fiber, straight-probe prototype[g] has also been developed. In the authors' opinion, the straight probe was difficult to advance horizontally in the ciliary sulcus in a phakic eye while maintaining a consistent distance between the probe tip and target because of the shallow AC and large anterior lens surface.

The goals of ECPC are whitening and contraction of the CPs (gross change) and destruction of the CE and stroma (histologic change).[9,10] Correlation of the visible tissue effects with the histologic effects created by following specific ECPC protocols is necessary to determine whether ECPC is an appropriate treatment in veterinary patients. In addition to establishing the appropriate ECPC power settings to obtain a predictable and safe tissue response, defining the relationship between clinical effects and structural pathological changes allows surgeons to titrate the visible tissue effects during surgery on the basis of the desired histologic changes. The present study revealed that diode ECPC can create reproducible and visible lesions isolated to

the pars plicata at energy levels of 0.75, 0.90, and 1.05 J. The highest energy level (1.05 J) resulted in moderate to severe tissue whitening and contraction, with a mean visible tissue effect score of 4.68 ± 0.58 (maximum possible score, 6) and occasional tissue popping noises that were indicative of overtreatment. This power setting may be excessive in vivo given the potential for occasional overtreatment. The visible tissue effects at the low (0.75-J) and medium (0.90-J) energy levels ranged from mild to moderate tissue whitening and contraction, with mean visible tissue effect scores of 3.12 ± 0.47 and 3.86 ± 0.35, respectively. Therefore, these power settings may be more appropriate in vivo, resulting in reproducible visible tissue effects without overtreatment.

Endoscopic CPC energy levels evaluated in the present study also resulted in predictable histologic changes, producing most of its structural changes in the CE and stroma (Figure 4). All energy levels had the potential to treat glaucoma, as evidenced by destruction of the aqueous-producing CE. The histologic changes corresponded positively with the total visible tissue effect scores for the 3 ECPC treatments; in general, total visible tissue effects and histologic scores increased with increasing ECPC energy levels. Mild epithelial and stromal damage to the CB was observed at the lowest energy level (0.75 J), and epithelial and stromal damage were comparatively greater at the medium and high energy levels (0.90 and 1.05 J). The 0.75- and 0.90-J energy levels may be appropriate choices in vivo when taking into account the visible tissue and histologic changes, which resulted in CE damage without visible overtreatment. The 0.75- and 0.90-J energy levels may have the potential to minimize underlying tissue damage in vivo.

An additional goal of ECPC is to minimize loss of anatomic integrity of the CB and damage to adjacent ocular structures. A noteworthy finding of the present study was the localized damage in the CB resulting from the ECPC procedure. No histologic evidence of peripheral laser damage was identified in sections of the CB or the adjacent cornea, iris, pars plana, or sclera from untreated ocular quadrants. Given that the histologic evaluation was limited to the CB and immediately adjacent structures, the influence of ECPC on other ocular structures, such as the retina, is uncertain. Interestingly, only the anterior portion of the CPs was damaged via ECPC treatment, whereas the posterior portion remained more or less unaffected. The posterior portion of the CPs may be more effectively treated through a pars plana approach.

The present study had a few limitations, particularly because of the use of ECPC in bovine cadaver eyes. The tissue effects following ECPC treatment in bovine cadaver eyes may not translate well to other species or in vivo. There may be differences of energy absorption, dissipation, and ultimately tissue effects in living tissue. Horses are the target species for the prototype probe, and tissue changes in this species may also be different at the energy levels evaluated. There are differences in ocular characteristics between bovine and equine eyes, including globe size and CB structure, that have the potential to make extrapolations between these species difficult.

Endoscopic CPC treatment protocols currently being used in veterinary patients have been extrapolated from the human medical literature, anecdotal reports, or personal observations. The present study has provided a starting point for evidence-based evaluation of ECPC as a treatment option for glaucoma in animals other than humans, but species-specific procedures must be developed prior to routine clinical use. The AC approach used in the present study was repeatable, and it is now known that destruction of the CB may be accomplished in cadaver eyes via diode ECPC. This treatment may prove useful in treating patients with glaucoma; however, findings of the present study do not indicate whether ECPC would be effective in treating eyes in vivo or how it compares with other treatments. Further study is needed to determine the number of CPs that must be treated to achieve a particular reduction in IOP as well as the appropriate treatment protocols and tissue effects of ECPC in vivo.

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Appendix

Criteria applied to gross lesions created via diode ECPC and used to determine visible tissue effect scores for ocular quadrants in bovine cadaver eyes.

Grade	Description	Criteria
0	No effect	No noticeable effect
1	Faint	Mild tissue whitening without contraction
2	Minimal	Moderate tissue whitening without contraction
3	Mild	Tissue whitening with mild contraction
4	Moderate	Tissue whitening with moderate contraction
5	Severe	Tissue whitening with rapid contraction; occasional bubble formation
6	Excessive	Audible tissue popping noises (due to vaporization of interstitial water that results in rapid volume expansion in an enclosed space, followed by an explosion [popping noise] when the vapor pressure exceeds the internal tissue pressure and the vapor is vented)