Diode laser endoscopic cyclophotocoagulation in the normal equine eye

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Abstract

Objective To determine the clinical and histologic effects of diode endoscopic cyclophotocoagulation (ECP) in the phakic equine eye.

Animals studied Phase I: 10 equine cadaver eyes. Phase II: four normal adult horses.

Procedures Phase I: ECP probe angle of reach (AR) was determined. Multiple ECP energy levels: 0.75, 0.90, 1.05, 1.20, 1.35, 1.50 J, and the resulting visible and histologic ciliary process changes were evaluated. Phase II: Ocular quadrants were treated with ECP at 0.90, 1.14, 1.38 J, and a control. The contralateral eye underwent a sham operation. Tissue changes (clinical and histologic) were evaluated.

Results Phase I: Mean combined AR was 162 ± 29 degrees. Mean visible tissue scores: 2.60 ± 0.58 (0.75 J) to 5.04 ± 0.30 (1.50 J) from possible total of 6. Tissue ‘popping’ was observed at 1.50 J. Histologic ciliary tissue damage was present at all settings.

Phase II: Mean visible tissue scores: 2.90 ± 0.48 (0.90 J), 3.61 ± 0.57 (1.14 J), and 4.52 ± 0.56 (1.38 J). Tissue ‘popping’ was observed at 1.38 J. Histologic ciliary tissue damage was present at all settings. Clinical effects included acute inflammation, intraocular pressure reduction, cataract formation, corneal edema, corneal ulceration, and postoperative ocular hypertension.

Conclusions Diode ECP between 0.90 and 1.14 J is a potential treatment option for glaucoma in horses based on visible tissue effects and target ciliary epithelium damage. Iatrogenic cataract development may limit the use of an anterior chamber approach in phakic horses. Supported in part by an ACVO VAF grant.

Key Words: diode laser, endoscopic cyclophotocoagulation, equine, glaucoma, histopathology

INTRODUCTION

Glaucoma is a disease that results from an increase in intraocular pressure (IOP) which is greater than that compatible with normal function of the eye.¹ In the horse, the most commonly recognized form of equine glaucoma is secondary to intraocular inflammation, while primary and congenital glaucomas are less common.²⁻⁵ The pathogenesis of glaucoma is multifactorial, but vision loss is associated with retinal and optic nerve ischemia and ganglion cell loss.¹⁻³,⁶ Increased IOP can also cause discomfort that may ultimately result in enucleation or even euthanasia. Therefore, early and effective treatment is necessary to prevent or delay these sequelae.

The focus of equine glaucoma therapy is on the management of the underlying cause and maintaining normal IOP.²⁻⁴ Medical management includes nonspecific topical and/or systemic anti-inflammatory therapy and specific topical glaucoma therapy and includes drugs that decrease aqueous humor (AH) formation as well as increase AH outflow. However, the response to medical therapy and long-term prognosis for maintaining vision in equine glaucoma are usually poor.⁴⁻⁶ Surgical management of equine glaucoma includes procedures that increase AH outflow, such as anterior chamber (AC) shunt placement and filtering procedures, or those that decrease AH production, such as cyclophotocoagulation (CPC) and cyclocryosurgery.²⁻⁵,⁶ Shunt placement and filtering procedures are not commonly
performed in horses and are potentially less likely to be successful as a result of the significant equine inflammatory response following intraocular surgery.\textsuperscript{2,3,7} Cyclocryosurgery is a surgical procedure that involves external application of a probe on the sclera to freeze the ciliary epithelium (CE); however, this procedure has lost favor because of numerous side effects.\textsuperscript{2,4,8} Cyclophotocoagulation refers to the induction of coagulation necrosis of the ciliary body (CB) with light energy and is often performed with a semiconductor diode laser that emits light in the near infrared spectrum (810 nm). Diode laser energy is strongly absorbed by tissues containing melanin, such as pigmented CE. Destruction of this tissue along with damage to the ciliary vasculature, postoperative uveitis with prostaglandin release, and architectural change leading to increased uveoscleral outflow are possible mechanisms responsible for IOP reduction. It is likely that a combination of these factors is responsible for IOP reduction.\textsuperscript{9–11}

Trans-scleral cyclophotocoagulation (TSCP) has been routinely used in veterinary medicine, including the treatment of equine glaucoma, with variable results.\textsuperscript{12–24} The main disadvantage of TSCP is the lack of direct visualization of the CB during the procedure that may lead to an increased risk of collateral damage to nontarget tissues.\textsuperscript{10,11} Endoscopic cyclophotocoagulation (ECP) involves the application of laser energy to the CB under direct endoscopic visualization. Real-time visualization of the ECP probe and acute tissue effects may allow a precise application of the laser energy, thus decreasing the risk of overtreatment or collateral tissue damage. However, this procedure requires surgical entry into the eye, which may be associated with other complications.\textsuperscript{10,11,25}

Use of ECP in veterinary ophthalmology as the primary treatment of glaucoma and in combination with phacoemulsification surgery has been reported\textsuperscript{26–29}; but to date, no peer-reviewed studies on the use of ECP have been published in the veterinary literature. A recent study in large animal cadaver eyes evaluated an AC approach and correlated the visible and histologic effects of ECP at various energy settings.\textsuperscript{30} This report established a reproducible surgical approach and ECP treatment parameters in eyes \textit{in vitro}.

The purposes of this study were to evaluate the AC approach through multiple clear corneal incisions (CCI) for performing ECP, to compare the \textit{in vitro} and \textit{in vivo} visual tissue effects of ECP on the CB, and to determine the clinical and histologic effects of diode laser ECP via an AC approach on the normal equine eye.

**MATERIALS AND METHODS**

\textbf{Phase I: \textit{in vitro} evaluation}

Ten fresh equine cadaver eyes, free from ocular abnormalities, were obtained within 2 h of humane euthanasia for reasons unrelated to this study. The eyes were stored at 4 °C for <24 h before use. Thirty minutes prior to the procedure, the eyes were warmed to room temperature, mounted on Styrofoam, and re-inflated to normal IOP with saline injections through a limbal injection.

\textit{In vitro surgical approach}

Eight trilaminar CCIs were made 45° apart, starting at the 12 o’clock position, in four fresh cadaver eyes using a #64 beaver microsurgical blade (Medical Sterile Products, Rincon, Puerto Rico, USA) and 2.8-mm beveled keratome (Unique Technologies, Inc., Mohnton, PA, USA).\textsuperscript{31,32} Methylcellulose-based viscoelastic substance (First Priority, Inc., Elgin, IL, USA) was used to fill the AC using a cannula (Randolph Cyclodialysis Cannula, Storz E0506; Bausch & Lomb, Inc., Tampa, FL, USA) (Fig. 1). Viscoelastic was then inserted into the posterior chamber (PC) to anteriorly displace the iris and expand the ciliary sulcus, facilitating visualization of the ciliary processes (CPs).

An integrated laser and endoscope (Endo Optiks Inc., Little Silver, NJ, USA) was used with a large animal endolaser probe (Endo Optiks Inc.). The system incorporates an 810-nm pulsed continuous-wave diode laser (Iris Medical Di-Vet Laser System, Iridex, Inc., Mountain View, CA, USA), 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-g, extended fiber, curved tip (Fig. 1). The probe was introduced into each CCI and advanced into the opposite ciliary sulcus under direct visualization on the video monitor. Five CPs were kept in view through the endoscope to maintain a constant distance (approximately 2 mm) between the probe and CB (Fig. 2).\textsuperscript{10,13} The probe was advanced horizontally in both directions within the sulcus, and the angle of reach (AR) was determined.

\textit{In vitro acute tissue effects}

An AC surgical approach was created, as described previously, in six fresh equine cadaver eyes. The endolaser probe tip was positioned opposite the central CP while constantly maintaining five CPs in view (Fig. 2). Fifteen CPs within each quadrant were treated and power settings of 250, 300,
and 350 mW were applied to each CP within the first, second, and third quadrants, respectively, of three globes. Exposure time was held constant at 3000 ms, allowing one complete vertical passage across the individual CP. These settings correlated with total energy values of 0.75, 0.90, and 1.05 J, respectively. Power settings of 400, 450, and 500 mW were applied to the first, second, and third quadrants, respectively, of three additional globes. These settings correlated with total energy values of 1.20, 1.35, and 1.50 J, respectively. The remaining quadrant in each globe served as an untreated control. These energy levels were selected based on previous studies.\(^\text{10,11,25–30,34–42}\)

Visible changes in each CP (whitening and contraction) were recorded using a computer software program (DSCALER 4.1.15 TV viewing application for Microsoft Windows, DScaler Project Team 2009) at the time of CPC and scored according to the scale in Table 1.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Effects</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No effect</td>
<td>No noticeable whitening or contraction</td>
</tr>
<tr>
<td>1</td>
<td>Faint</td>
<td>Mild tissue whitening without contraction</td>
</tr>
<tr>
<td>2</td>
<td>Minimal</td>
<td>Moderate tissue whitening without contraction</td>
</tr>
<tr>
<td>3</td>
<td>Mild</td>
<td>Tissue whitening with mild contraction</td>
</tr>
<tr>
<td>4</td>
<td>Moderate</td>
<td>Tissue whitening with moderate contraction; occasional bubble formation</td>
</tr>
<tr>
<td>5</td>
<td>Severe</td>
<td>Tissue whitening with rapid contraction; occasional bubble formation</td>
</tr>
<tr>
<td>6</td>
<td>Excessive</td>
<td>Tissue ‘popping’ or explosion</td>
</tr>
</tbody>
</table>

In vitro histologic evaluation

Following ECP, globes were fixed in a combination of formaldehyde/glutaraldehyde (4:1) solution and sectioned based on treatment quadrant. Serial, 100-\(\mu\)m sections were cut perpendicular to the ciliary ridge (parallel to the CP), and paraffin tissue sections were stained with hematoxylin and eosin. Fifteen sections were evaluated per quadrant using light microscopy. General histologic observations were recorded, 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. Changes in the pigmented CE, nonpigmented CE, and fibromuscular stroma were scored on a previously described, semiquantitative scale.\(^\text{50}\)

In summary, the histologic changes (0 = absent, 1 = mild, 2 = moderate, 3 = severe) and percentage of CB affected (0 = none, 1 = <10%, 2 = 10–50%, 3 = >50%) were scored and added together for a total histologic score of a possible 12.

Phase II: in vivo evaluation

Preoperative evaluation

Four adult horses (three Thoroughbreds, one Quarterhorse) weighing (mean ± SD) 532.5 ± 23.6 kg were used. The experimental protocol was approved by the NCSU Institutional Animal Use and Care Committee and adhered to the Association in Vision and Ophthalmology guidelines for the use of animals in research. All horses were healthy, as judged by complete physical examination, complete blood count, and serum chemistry panel, and found free of ocular disease by a complete ophthalmic examination, including slit-lamp biomicroscopy (Kowa SL-14; Kowa Company, Ltd., Tokyo, Japan), indirect ophthalmoscopy, and applanation tonometry (Tono-Pen XL; Mentor, Norwell, MA, USA). Baseline IOPs were measured between 7:00 and 8:00 AM for two consecutive days.

In vivo surgical approach

Prior to surgery, horses were treated with flunixin meglumine (1.0 mg/kg, IV, Banamine®; Schering-Plough Animal Health Corp., Omaha, NE, USA), gentamicin (6.6 mg/kg, IV; Sparhawk Laboratories, Inc., Lenexa, KS, USA), and penicillin G potassium (22 000 IU/kg, IV, Pfizerpen®; Roerig, Division of Pfizer, Inc., New York, NY, USA). Anesthesia was induced with xylazine hydrochloride (1.1 mg/kg, IV), ketamine (2.2 mg/kg, IV), and midazolam (0.1 mg/kg, IV), and a surgical plane of anesthesia was maintained, after orotracheal intubation, with isoflurane vaporized in 100% oxygen. Horses were monitored by a board-certified veterinary anesthesiologist and received routine administration of intravenous fluids. After the placement in dorsal recumbency, one eye was randomly selected and routinely prepped for intraocular surgery. A retrobulbar block was performed using a 22G, 3.5-inch needle and 10 mL of 0.1% lidocaine hydrochloride (Vedco Inc., St. Joseph, MO, USA) to provide akinesia and analgesia to the globe. Trilaminar CCIs were made, as described in Phase I, using a 2.8-mm angled sapphire crescent blade and 2.8-mm angled sapphire slit/phaco blade.
Diode laser ECP was performed, as described earlier, using a modified, equine-specific endo-laser probe (Endo Optiks Inc.), designed with a 50-mm, 18-g extended fiber tip and S-shaped curve (Fig. 1). Power and duration settings were selected based on Phase I results and included 300 mW × 3000 ms (0.90 J), 380 mW × 3000 ms (1.14 J), and 460 mW × 3000 ms (1.38 J). A total of 25 CPs were treated in the first (0.90 J), second (1.14 J), and third (1.38 J) quadrants, respectively. One quadrant served as a control and received no ECP treatment. Visible changes in each CP (whitening and contraction) were recorded at the time of CPC and scored according to the scale in Table 1. Following ECP, manual irrigation and aspiration of the viscoelastic were performed using a balanced salt solution. The CCIs were closed using 8-0 polyglactin 910 (Polyglactin 910; Ethicon, Inc., Somerville, NJ, USA) in a double-saw-tooth continuous pattern. A sham operation, as described earlier, using a modified, equine-specific endolaser probe, was performed in the contralateral eye under the same anesthetic episode.

In vivo acute tissue effects Diode laser ECP was performed, as described earlier, using a modified, equine-specific endolaser probe (Endo Optiks Inc.), designed with a 50-mm, 18-g extended fiber tip and S-shaped curve (Fig. 1). Power and duration settings were selected based on Phase I results and included 300 mW × 3000 ms (0.90 J), 380 mW × 3000 ms (1.14 J), and 460 mW × 3000 ms (1.38 J). A total of 25 CPs were treated in the first (0.90 J), second (1.14 J), and third (1.38 J) quadrants, respectively. One quadrant served as a control and received no ECP treatment. Visible changes in each CP (whitening and contraction) were recorded at the time of CPC and scored according to the scale in Table 1. Following ECP, manual irrigation and aspiration of the viscoelastic were performed using a balanced salt solution. The CCIs were closed using 8-0 polyglactin 910 (Polyglactin 910; Ethicon, Inc., Somerville, NJ, USA) in a double-saw-tooth continuous pattern. A sham operation, as described for the previous eye without the administration of laser energy, was performed in the contralateral eye under the same anesthetic episode.

In vivo histologic evaluation At postoperative days 3, 7, 14, and 28, one horse was randomly selected and humanely euthanized. The globes were immediately enucleated, fixed, sectioned, and stained, as described in Phase I. Individual treatment quadrants were subdivided into three sections, and sixteen serial 100-μm sections were cut in each subdivision for a total of 48 sections per quadrant. Using light microscopy, histologic observations were recorded and scored using a semiquantitative scale, as described in Phase I, for a total histologic score of a possible 12.

Data evaluation and statistical analysis Normally distributed data were expressed as mean ± SD. Kruskal–Wallis and paired Student’s t-tests were used to compare mean visible scores and mean histologic scores between ECP energy levels. A one-way ANOVA with Tukey–Kramer test was used to evaluate the difference between CCI locations and mean AR in the ciliary sulcus. As no demonstrable contralateral effect of ECP is expected and an effort was made to minimize the number of animals used in the study, the sham operation was performed in the contralateral eye of each animal rather than in additional animals. Because of a low number of animals, only one animal remained after day 14. Graphs and descriptive terms were used to demonstrate the effects of surgery on IOP. For all analyses, a value of P < 0.05 was considered significant.

RESULTS

Phase I: in vitro evaluation

In vitro surgical approach The entire 360° ciliary sulcus was accessible through an AC approach using multiple CCIs and the large animal ECP probe. The lateral, dorsal, medial, and ventral CCI locations achieved a mean of 124 ± [SD] 13 degrees, 146 ± 11 degrees, 160 ± 14 degrees, and 164 ± 5 degrees in AR to the ciliary sulcus, respectively. The ventrolateral, dorsolateral, dorsomedial, and ventromedial CCI locations achieved a mean of 196 ± 19 degrees, 182 ± 13 degrees, 181 ± 46 degrees, and 149 ± 22 degrees in AR to the ciliary sulcus, respectively. The mean combined AR was 162 ± 29 degrees (Fig. 3). A significant difference was present with the lateral incision location when compared with the dorsolateral, dorsomedial, and ventrolateral locations. No statistically significant differences were measured between the other incision locations. During probe placement, the corpora nigra impeded access to the ciliary sulcus from the dorsal incision and the nictitating membrane impeded access to the ciliary sulcus from the ventromedial incision.

In vitro acute tissue effects Diode laser pulse duration of 3000 ms from 250 to 500 mW (0.75–1.50 J) resulted in grossly visible lesions in the CB. Visible tissue effect scoring resulted in a mean of 2.60 ± [SD] 0.58, 2.96 ± 0.47, 3.84 ± 0.42, 3.93 ± 0.33, 4.31 ± 0.47, and 5.04 ± 0.30 at the
0.75, 0.90, 1.05, 1.20, 1.35, and 1.50 J energy levels, respectively (Fig. 4). Visible tissue effects at 0.90 J resulted in mild tissue whitening, and little to no contraction, while occasional overtreatment, characterized by tissue ‘popping’, was observed at 1.50 J. The mean visible scores at all energy levels evaluated were significantly different \((P < 0.001)\) with respect to one another, with the exception of no significant difference between the mean visible scores at 1.05 and 1.20 J.

**In vitro histologic evaluation**  Histologic evidence of tissue damage, specifically coagulation necrosis and tissue shrinkage in the CB, was observed at all energy levels evaluated. Thinning of the pigmented and nonpigmented layers of 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. Histologic scoring resulted in a mean of 3.93 ± 1.47, 5.27 ± 1.34, 6.69 ± 2.17, 7.58 ± 1.01, 7.82 ± 0.91, and 8.91 ± 0.82 from a possible total of 12 for the 0.75, 0.90, 1.05, 1.20, 1.35, and 1.50 J energy levels, respectively (Fig. 5). The mean histologic scores at all energy levels evaluated were significantly different \((P < 0.001)\) with respect to one another and control, with the exception of no significant difference between the mean histologic scores at 1.20 and 1.35 J.

Changes observed at the lowest energy (0.75 J) were mild 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 (<10% affected) relative to the other treated quadrants (Fig. 6). At the intermediate energy levels of 0.90–1.35 J, there was increasing CB destruction, characterized by greater damage to the pigmented and nonpigmented CE, separation of the epithelial bilayers, and fibromuscular stromal damage, with an increase in power setting (Fig. 6).

The untreated control sections possessed CPs with intact bilayers of normal nonpigmented and pigmented CE surrounding a homogenous stroma (Fig. 6). No histologic changes were identified in the control sections or in the adjacent cornea, iris, pars plana, or sclera.

**Phase II: in vivo evaluation**

**Preoperative evaluation** Baseline physical examination, routine bloodwork (CBC, biochemical profile), and complete ophthalmic examination were considered normal for each horse. Mean preoperative IOPs for all eyes were 20.88 ± 4.32, 20.38 ± 2.77, and 18.00 ± 3.02 mmHg for days 0, 1, and 2, respectively (Fig. 7).

**In vivo acute tissue effects** Diode laser pulse duration of 3000 ms using 300, 380, and 460 mW (0.90, 1.14, and 1.38 J, respectively) resulted in grossly visible lesions in the
CB. Visible tissue effect scoring resulted in a mean of 2.90 ± [SD] 0.48, 3.61 ± 0.57, and 4.52 ± 0.56 at the 0.90, 1.14, and 1.38 J energy levels, respectively (Fig. 4). The mean visible score of 2.90 ± 0.48 at 0.90 J correlated with mild tissue whitening and contraction. The mean visible score of 3.61 ± 0.57 at 1.14 J correlated with moderate tis-

**Figure 6.** Light microscopy (40× magnification) of normal and endoscopic cyclophotocoagulation–treated ciliary body tissue *in vitro* (Phase I). (a) Untreated tissue showing normal pigmented and nonpigmented ciliary epithelium (CE) and fibromuscular stroma; (b) Tissue treated with 250 mW × 3000 ms (0.75 J) showing mild pigmented and nonpigmented CE damage, separation of epithelial bilayers, and mild fibromuscular stromal damage; (c) Tissue treated with 300 mW × 3000 ms (0.90 J) showing an increase in CE and stromal damage with an increase in energy level; (d) Tissue treated with 350 mW × 3000 ms (1.05 J) showing moderate CE loss and separation of epithelial and stromal layers; (e) Tissue treated with 400 mW × 3000 ms (1.20 J) showing an increase in CE damage, extracellular pigment granule dispersion, and disorganization of the stroma; (f) Tissue treated with 450 mW × 3000 ms (1.35 J) showing severe loss of nonpigmented CE, consolidation of the pigmented CE, and stromal damage; (g) Tissue treated with 500 mW × 3000 ms (1.50 J) showing complete loss of the nonpigmented CE and severe stromal damage.
sue whitening and contraction. The mean visible score of 4.52 ± 0.56 at 1.38 J correlated with moderate-to-severe tissue whitening and contraction. Occasional overtreatment, characterized by bubble formation and tissue ‘popping’, was observed at 1.38 J. The mean visible scores at all energy levels evaluated were significantly different ($P < 0.001$) with respect to one another.

**In vivo clinical evaluation** Clinical effects of the ECP-treated and sham-operated eyes are summarized in Table 2. Corneal changes included diffuse corneal edema in 3/4 (75%) ECP eyes and 3/4 (75%) sham eyes at day 1 and superficial corneal ulceration in 3/4 (75%) ECP eyes and 3/4 (75%) sham eyes (Fig. 8). All corneal ulcers were adjacent to a corneal incision and healed in normal fashion.

Anterior chamber findings at day 1 included fibrin formation in 2/4 (50%) ECP eyes and 2/4 (50%) sham eyes, pigment dispersion in 2/4 (50%) ECP eyes and 2/4 (50%) sham eyes, and aqueous flare in 4/4 (100%) ECP eyes and 4/4 (100%) sham eyes. Aqueous flare scores for all eyes ranged from trace to 2+ during the first postoperative week; however, no appreciable flare was detected after day 7 in any eye. Aqueous flare scores decreased in all eyes from day 1 through day 7. Mild hyphema was noted in 1/4 (25%) sham eyes and 0/4 (0%) ECP eyes at day 1 and resolved by day 2 without any complications.

Lens findings included cataract formation in 4/4 (100%) ECP eyes and 4/4 (100%) sham eyes (Fig. 8). The cataracts were characterized by incipient (<10%) to early immature (<25%) anterior capsular, subcapsular, and anterior cortical lens opacities. Anterior lens opacities were noted in all eyes as early as day 1, which slowly progressed during the study period. The anterior cataract noted during the immediate postoperative period in a single horse progressed to involve a significant equatorial and early posterior cataract component by day 28 in both the ECP-treated and sham-operated eyes. No differences were noted in the level of cataract formation between ECP-treated and sham-operated eyes or between different energy level treatment quadrants within individual ECP-treated eyes.

Immediate postoperative ocular hypertension was noted in 3/4 (75%) ECP eyes and 1/4 (25%) sham eyes at day 1. Ocular hypertension was defined as an IOP >30 mmHg.43 No additional medical or surgical treatments were pursued to reduce the IOP in these horses. A reduction in IOP from preoperative baseline was noted by day 4 in all eyes (ECP-treated and sham-operated) and this IOP reduction persisted until euthanasia in all horses (Fig. 7).

**In vivo histologic evaluation** Histologic evidence of tissue damage, specifically coagulation necrosis and tissue shrinkage in the CB, was observed at all energy levels evaluated (0.90, 1.14, and 1.38 J). Acute postoperative inflammation, characterized predominantly by neutrophilic cellular infiltrate and vascular congestion in the CB, appeared to be minimal at day 3 and more obvious by day 7 in all treated quadrants. The CB inflammation reduced by day 14 and resolved by day 28 in all quadrants. Thinning of the pigmented and nonpigmented CE, extracellular dispersion of pigment granules, contraction of the pigmented CE, and loss of the normal lacy architecture in the anterior CB were observed at all energy levels by day 3 (Fig. 9). The nonpigmented CE had areas of extensive damage and loss of architecture by day 3, which increased with energy level and time. The coagulation necrosis was focused on the anterior face of the CPs and extended from tip to base. Occasional coagulation necrosis extended into the iris base (Fig. 9). Pigment clumping and epithelial loss increased with time (Fig. 10). Attempts at regeneration of the CE,

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**Table 2.** Common clinical findings following endoscopic cyclophotoocoagulation

<table>
<thead>
<tr>
<th>Clinical effects</th>
<th>Days present</th>
<th>Endoscopic cyclophotoocoagulation-treated eyes (%)</th>
<th>Sham-operated eyes (%)</th>
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</thead>
<tbody>
<tr>
<td>Conjunctivitis</td>
<td>1–5</td>
<td>4/4 (100)</td>
<td>4/4 (100)</td>
</tr>
<tr>
<td>Corneal edema</td>
<td>1–4</td>
<td>4/4 (100)</td>
<td>4/4 (100)</td>
</tr>
<tr>
<td>Corneal ulceration</td>
<td>1–14</td>
<td>3/4 (75)</td>
<td>3/4 (75)</td>
</tr>
<tr>
<td>Pigment dispersion</td>
<td>1–3</td>
<td>2/4 (50)</td>
<td>2/4 (50)</td>
</tr>
<tr>
<td>Hyphema</td>
<td>1–2</td>
<td>0/4 (0)</td>
<td>1/4 (25)</td>
</tr>
<tr>
<td>Fibrin formation</td>
<td>1–3</td>
<td>2/4 (50)</td>
<td>2/4 (50)</td>
</tr>
<tr>
<td>Aqueous flare</td>
<td>1–5</td>
<td>4/4 (100)</td>
<td>4/4 (100)</td>
</tr>
<tr>
<td>Cataract formation</td>
<td>2–28</td>
<td>4/4 (100)</td>
<td>4/4 (100)</td>
</tr>
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</table>

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characterized by stretching of dysplastic epithelium at the margins of laser-induced epithelial damage, were not present at day 28 in the ECP-treated eyes. Other lesions at day 28 included dissolution of the CB fibromuscular stroma, replacement with fibrous tissue, and atrophy of the CPs (Fig. 10).

Histologic scoring resulted in a mean of 5.60 ± 0.83, 8.69 ± 1.42, and 10.58 ± 1.47 from a possible total of 12 for the 0.90, 1.14, and 1.38 J energy levels, respectively (Fig. 5). Milder histologic changes were observed at the lowest energy (0.90 J), 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 (<10% affected) relative to the other treated quadrants (Fig. 9). The histologic changes at 1.14 J included more widespread CB destruction, characterized by greater damage to the pigmented and nonpigmented CE, separation of the epithelial bilayers, and fibromuscular stromal damage. The normal structure of the CB was more affected (10–50%) at this energy level relative to the lower energy level (Fig. 9). The highest energy level evaluated (1.38 J) resulted in severe ablation of the pigmented and nonpigmented CE, greater pigment dispersion, and moderate fibromuscular stromal damage adjacent to the treated surface. The greatest loss of normal CB structure (>50%) was observed at this energy level (Fig. 9). The mean histologic scores at all energy levels evaluated were significantly different (P < 0.001) with respect to one another and the control.

The control quadrants (within ECP-treated eyes) possessed CPs with intact bilayers of normal nonpigmented and pigmented CE surrounding a homogenous stroma (Fig. 9). Markers of inflammation (cellular infiltrate, vascular congestion) were minimal to absent at all time points and, when evident, followed a similar course as the treated quadrants. The sham-operated eyes also possessed CPs with intact bilayers of normal nonpigmented and pigmented CE surrounding a homogenous stroma. Lens changes in both the ECP-treated and sham-operated eyes included early anterior subcapsular and cortical cataractous changes first noted at day 7. Corneal

![Figure 8. Color photographs of common clinical findings following endoscopic cyclophotocoagulation (ECP) and a sham operation. (a) Diffuse corneal edema in an ECP-treated eye, (b) Superficial corneal ulceration in an ECP-treated eye, (c) Immature anterior subcapsular/cortical cataract formation in an ECP-treated eye; (d) Diffuse corneal edema in a sham-operated eye, (e) Superficial corneal ulceration in a sham-operated eye, (f) Immature anterior subcapsular/cortical cataract formation in a sham-operated eye.](image-url)
changes in both the ECP-treated and sham-operated eyes included mild stromal edema and mild perilimbal inflammatory cellular infiltrate, which was most prominent at day 3 and resolved by day 28. No additional histologic lesions were identified in the complete globe sections.

Figure 9. Early (Day 3) postoperative light microscopy of normal and endoscopic photocoagulation–treated ciliary body (CB) tissue in vivo (Phase II). (a) 4× magnification of untreated tissue showing normal CB architecture; (b) 40× magnification of untreated tissue showing normal pigmented and nonpigmented ciliary epithelium (CE) and fibromuscular stroma; (c) 4× magnification of tissue treated with 300 mW × 3000 ms (0.90 J) showing mild loss of CB architecture at treated surface; (d) 40× magnification of tissue treated with 300 mW × 3000 ms (0.90 J) showing mild 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; (e) 4× magnification of tissue treated with 380 mW × 3000 ms (1.14 J) showing greater loss of normal CB structure at treated surface; (f) 40× magnification of tissue treated with 380 mW × 3000 ms (1.14 J) showing 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; (g) 4× magnification of tissue treated with 460 mW × 3000 ms (1.38 J) showing severe changes in the normal CB structure; (h) 40× magnification of tissue treated with 460 mW × 3000 ms (1.38 J) showing severe ablation of the pigmented and nonpigmented CE, greater pigment dispersion, and severe fibromuscular stromal damage adjacent to the treated surface.
DISCUSSION

Diode laser ECP has been used in clinical veterinary patients without any species-specific standardized protocol or evaluation of the histologic effects within the eye. A recent report evaluated an AC surgical approach and ECP treatment parameters in large animal cadaver eyes and provided information on the tissue effects of this treatment modality.30

Figure 10. Late (Day 28) postoperative light microscopy of normal and endoscopic photocoagulation–treated ciliary body (CB) tissue in vivo (Phase II). (a) 4× magnification of untreated tissue showing normal CB architecture; (b) 40× magnification of untreated tissue showing normal pigmented and nonpigmented ciliary epithelium (CE) and fibromuscular stroma; (c) 4× magnification of tissue treated with 300 mW × 3000 ms (0.90 J) showing loss of normal CB structure at treated surface; (d) 40× magnification of tissue treated with 300 mW × 3000 ms (0.90 J) showing moderate loss of pigmented and nonpigmented CE, pigment clumping, and consolidation of the anterior face of the ciliary process; (e) 4× magnification of tissue treated with 380 mW × 3000 ms (1.14 J) showing greater loss of normal CB structure at treated surface; (f) 40× magnification of tissue treated with 380 mW × 3000 ms (1.14 J) showing greater epithelial loss, pigment clumping, and tissue consolidation; (g) 4× magnification of tissue treated with 460 mW × 3000 ms (1.38 J) showing almost complete loss of normal pars plicata CB structure; (h) 40× magnification of tissue treated with 460 mW × 3000 ms (1.38 J) showing severe ablation of the CE, pigment clumping, and stromal damage.
Prior ECP studies utilized various surgical approaches, including clear corneal and limbal incisions in phakic, aphakic, or pseudophakic eyes, as well as a pars plana approach in aphakic and pseudophakic eyes. Several reports of ECP in veterinary patients involve concurrent removal of the lens, but the necessity of lens removal has not been determined and may not be the optimal treatment in glaucoma patients without cataract formation. A clear corneal approach in a phakic eye was chosen in this study to determine the feasibility in performing ECP without concurrent removal of the lens. In this study, the entire ciliary sulcus was accessible through multiple CCIs around the cornea, and a combination of the dorsolateral, dorsomedial, and ventrolateral CCI locations achieved the greatest angle of access to the ciliary sulcus. These findings are consistent with the previous study, and these CCI locations were used in the in vivo phase of this study.

Probe design plays an important role in effective ECP treatment. The probe utilized in this study is a 50-mm, 18-gauge Endo Optiks prototype developed for use in the large animal eye, specifically the equine. The prototype contains the same components as the commercially available small animal probe, but the extended fiber allows for access across the larger AC and ciliary sulcus. A curved prototype probe, as used in a previous study, was used during the in vitro phase, while a modified probe with an S-shaped curve that contours to the dimensions of the equine AC and minimizes contact with the anterior lens was utilized in the in vivo phase. The modified probe allowed for easy introduction into the equine AC and ciliary sulcus in this study.

Whitening and contraction of the CPs are the desired visible tissue effects during ECP treatment. Determining the visible tissue effects at specific ECP settings is necessary before developing species-specific protocols. The present study demonstrates that diode ECP can create reproducible lesions in vivo using energy levels of 0.90, 1.14, and 1.38 J. The visible tissue effects at the low (0.90 J) and medium (1.14 J) energy levels ranged from mild-to-moderate tissue whitening and contraction. Therefore, these settings are more appropriate in vivo, resulting in reproducible visible tissue effects without overtreatment. The highest energy level utilized (1.38 J) may be excessive, as evidenced by moderate-to-severe tissue whitening and contraction and occasional tissue ‘popping’ indicating overtreatment. This overtreatment may result in a greater inflammatory response, pigment dispersion, peripheral lens damage, or hypotony. Further studies are warranted to correlate this tissue response with these clinical findings.

Coagulation necrosis of the CE and CB stroma are the desired histologic changes following ECP and was observed at all energy levels in a dose-dependent manner in this study. All three ECP settings evaluated in the in vivo phase resulted in predictable histologic changes in the CB and have the potential to treat glaucoma, as evidenced by destruction of the aqueous-producing CE. Mild CE and stromal damage was observed at 0.90 J, while increasing epithelial and stromal damage was observed at 1.14 and 1.38 J. A larger percentage of the CB was also affected with an increase in energy level, indicating that energy level may be titrated to achieve different depths of tissue damage. Greater histologic changes were observed in the quadrants where visible overtreatment (popping) was observed during ECP. No direct histologic changes were attributed to the tissue overtreatment in these treatment quadrants. Additional studies are required to determine the effects of visible overtreatment at the time of lasering on clinical outcome and the percentage of CB damage necessary to achieve a desired IOP reduction. When correlating these histologic findings to the visible changes at the time of ECP, the energy range from 0.90 to 1.14 J may be appropriate choice in vivo, resulting in visible and histologic CE damage without visible overtreatment. The tissue effects of ECP in this study were similar to the effects reported in a previous study in large animal cadaver eyes. It is important to note that milder tissue effects were noted in the equine cadaver eye when compared to the bovine cadaver eye and resulted in the evaluation of higher energy levels in the equine eye. Furthermore, evaluation of the long-term effects of ECP on the CB did not show evidence of epithelial regeneration. This finding is consistent with previous studies showing a lack of epithelial regeneration following cycloablation and is important in the long-term success of ECP.

A goal of ECP is limiting damage to nontarget ocular structures through real-time visualization of targeted tissue. A significant finding in this study was the relatively localized laser-induced damage to the CB. No overt evidence of peripheral laser damage was identified in the control sections or in the adjacent cornea, iris, pars plana, or sclera. Additionally, no damage was observed in the posterior segment. Interestingly, only the anterior portion of the CPs was damaged with ECP treatment while the posterior portion remained relatively normal. Although not evaluated in this study, additional laser treatment of the CPs, such as second pass across the CP with the same laser energy, may allow for targeted treatment deeper in the CB. The posterior portion of the CPs may also be more effectively treated through a pars plana approach.

Endoscopic cyclophotocoagulation is an invasive procedure that requires surgical entry into the eye, manipulation of ocular tissues, and laser-induced tissue damage. Evaluation of clinical effects of ECP on the equine eye is also necessary before using this treatment modality in clinical glaucoma patients. Ocular inflammation is expected following a procedure of this nature and was evident on clinical and histologic evaluation. Aqueous flare and conjunctival inflammation were present in the ECP-treated eyes and quickly resolved during the first postoperative week. Interestingly, a similar degree of inflammation was noted in the sham-operated eyes; therefore, further conclusions are difficult to make as to the role of the laser treatment in formation of this inflammation. Surgical entry into the eye with subsequent breakdown of the blood-aqueous barrier was likely
the cause of the intraocular inflammation in treated and untreated eyes. However, laser-induced inflammation cannot be ruled out as a contributing factor in the ECP-treated eyes. It is important to note that the inflammation was relatively mild and transient. Additional clinical findings included fibrin formation in four eyes (two ECP-treated, two sham-operated) and mild hyphema in one sham-operated eye. The hyphema was noted intraoperatively, attributed to transient hypotony after surgical entry, and resolved by day 2 without any complications.

Cataracts were detected in all ECP-treated and sham-operated eyes. Anterior lens opacities were noted during the immediate postoperative period as linear striations in the anterior lens capsule and progressed over the course of the study to anterior capsular, subcapsular, and anterior cortical lens opacities. The anterior cataract noted during the immediate postoperative period in a single horse progressed to involve a significant equatorial and early posterior cataract component by day 28. The cataracts noted in the other horses may have progressed if there was a longer postoperative evaluation period. The cataracts were probably caused by anterior lens contact with surgical equipment during the procedure. While the endolaser probe was modified to avoid contact with the lens, the cannula was a commercially available design and made repeated contact with the axial anterior lens capsule during attempts to inflate the ciliary sulcus. The endolaser probe also made infrequent contact with the anterior lens capsule and may have contributed to cataract development. It is unlikely that the cataracts were laser-induced as the cataracts started in the anterior, axial lens and similar cataracts were noted in the sham-operated eyes. If the cataracts developed secondary to laser energy, the cataracts would be expected to start development in the peripheral lens (nearest the laser energy application) and cataracts would not be expected in the sham-operated eyes. Mild uveitis was documented in ECP-treated and sham-operated eyes, which may have potentially contributed to the progression of the noted cataracts. Based on this study’s results, modifications in instrumentation or surgical approach are necessary to avoid iatrogenic cataract formation. A modification in instrument design may include developing a viscoelastic cannula that conforms to the equine AC, similar to the ECP probe. Although concurrent lens removal may not be the optimal treatment in glaucoma patients without pre-existing cataract formation, postoperative cataract formation may be avoided using this technique. It is important to consider alternative options and potential complications prior to concurrent lens removal in the horse. Cataract formation has been reported as a complication following other CPC procedures but has been attributed to different mechanisms, including direct CPC-induced damage and secondary to uveitis.12,13,18,19,50,51

Although not the focus of this study, IOP was recorded during the preoperative and postoperative periods as part of a complete ophthalmic examination. Immediate postoperative ocular hypertension was noted in three ECP-treated eyes and one sham-operated eye at day 1. The IOP normalized by day 2, and a significant reduction in IOP from baseline was noted by day 4 in all eyes (ECP-treated and sham-operated). This IOP reduction persisted until euthanasia in all horses. The postoperative ocular hypertension (POH) may be related to laser-induced effects, as POH was more common in ECP-treated eyes than sham-operated eyes. Other possible causes include residual viscoelastic occluding the AH outflow pathways or transient collapse of the iridocorneal angle from iatrogenic anterior displacement of the iris.

Although TSCP is routinely used for the management of equine glaucoma and has been shown to lower IOP, numerous complications have been documented, including uveitis, hyphema, corneal ulceration, cataract formation, and retinal detachment.12,15,17,22 These complications may be attributed to laser damage to nontarget tissues as a result of non-uniformity of the equine CB anatomy and/or inaccurate probe placement from buphthalmos in equine glaucoma. In contrast to TSCP, real-time visualization during ECP allows a precise application of the laser energy, thus decreasing the risk of overtreatment or collateral tissue damage. This is evident in the present study by laser-induced damage localized to the pars plicata of the CB. In contrast to a study by Morreale et al., where variable damage to the CB was documented depending on laser energy and probe position, damage to the target CB was documented in all histologic sections in this study.17 In addition, the Morreale et al. study documented retinal detachment and intraocular hemorrhage in up to 20% and 24% histologic sections, respectively, while these complications were not experienced in this study.17 There were similarities in clinical effects between the current study and previous TSCP studies, including a reduction in IOP, corneal ulceration, anterior uveitis, and cataract formation.12,22

There are limitations in this study, particularly with the small number of animals. In addition, it is difficult to separate postoperative complications into laser-related complications and those that occurred as a result of surgical entry into the eye. For example, cataract formation as a result of instrument contact with the anterior lens may mask any cataract induction as a result of laser treatment. Although a reduction in IOP was documented in this study, further study is needed to determine the number of CPs that must be treated to achieve a particular reduction in IOP in equine glaucoma. A methylcellulose–based viscoelastic was used to inflate the eye and sulcus in the in vitro phase owing to relative availability, while a sodium hyaluronate–based viscoelastic was used in the in vivo phase. A prior study concluded that both dispersive and cohesive viscoelastics preserve the intended laser energy during ECP,33 so this difference likely did not influence the degree of histologic findings between phases. It is the current recommendation of Endo Optiks® to use a sodium hyaluronate–based viscoelastic during ECP because of its ability to expand the ciliary sulcus and relative ease of removal from the eye following ECP to limit the risk.
of POH (personal communication with Endo Optiks®). In addition, each ECP-treated eye was divided into four quadrants and received three different laser energy levels to minimize the number of animals used and maximize the number of tissue responses evaluated. This resulted in the same total energy in each eye; therefore, further studies may be necessary that involve treating individual eyes with different total energy levels to determine the effect of each setting on clinical effects. Finally, this study was performed in normal horse eyes, and differences may exist in glaucomatous horse eyes. There may be variation in tissue responses and surgical access to the ciliary sulcus, with a possibility of a greater or lesser AR with buphthalmic eyes commonly encountered in equine glaucoma.

Endoscopic cyclophotocoagulation treatment parameters currently being used in veterinary patients have been extrapolated from human literature, anecdotal reports, or personal observations. This study demonstrates a repeatable surgical approach and shows that destruction of the CB may be accomplished in normal equine eyes using diode laser ECP. ECP settings between 300 mW × 3000 ms (0.90 J) and 380 mW × 3000 ms (1.14 J) produce repeatable visible and histologic tissue effects without overtreatment and may be appropriate parameters in equine glaucoma patients. This study serves as a starting point for evidence-based evaluation of ECP as a treatment option for equine glaucoma. Further studies are necessary to determine the appropriate surgical approach for ECP treatment of glaucoma in horses, as well as to determine the ability for the parameters established in the present study to reduce IOP in the equine globe. In addition, further studies are necessary to evaluate modified surgical instruments in normal and glaucomatous equine eyes.

ACKNOWLEDGMENTS
The authors would like to acknowledge Jacklyn H. Salmon, Nathan Whitehurst, and Sandra Horton for technical assistance. This research was partially supported by an ACVO Vision for Animals Foundation grant. Paula Ender from Endo Optiks, Inc. for providing the integrated laser and endoscope and modified large animal endolaser probe used in this study.

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